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Association between metabolic dysfunction associated steatotic liver disease and gallstones in the US population using propensity score matching

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The novel diagnostic term Metabolic Dysfunction-Associated Steatotic Liver Disease (MASLD) requires at least one cardiovascular risk factor for diagnosis. While the relationship between gallstones and Non-Alcoholic Fatty Liver Disease (NAFLD) has been debated, the association between MASLD and gallstones remains unclear. This cross-sectional study aimed to explore this relationship using National Health and Nutrition Examination Survey (NHANES) data from 2017 to 2020. Participants were stratified into two groups based on MASLD diagnosis, and propensity score matching (PSM) was employed to reduce biases. Of 15,560 participants, 7922 met the inclusion criteria, with 2697 (34.0%) diagnosed with MASLD. Gallstone prevalence was higher in the MASLD group (14.2%) compared to the non-MASLD group (8.5%). After PSM, 4536 participants were analyzed, revealing a significant association between MASLD and gallstones (OR = 1.30, 95% CI 1.09–1.56, $P = 0.003$). This association remained robust across crude and adjusted analyses, with subgroup and sensitivity analyses further supporting the findings. In conclusion, MASLD is significantly associated with an increased risk of gallstones in the US population. These findings highlight the need to consider this relationship in clinical strategies for prevention and management of gallstone disease.

Keywords Metabolic-associated steatotic liver disease, Gallstones, National Health and Nutrition Examination Survey, Propensity score matching, Cross-sectional study

The landscape of liver disease nomenclature has witnessed a significant evolution with the introduction of 'Metabolic-Associated Fatty Liver Disease (MASLD)' in this year's Delphi consensus[1]. This new term represents a paradigm shift in the understanding of Fatty Liver Disease (FLD), emphasizing the pivotal role of systemic metabolic dysfunction in liver disease. The global prevalence of MASLD among adults is approximately 30%, while in Japan, the prevalence has been reported to be 28%[2,3]. The adoption of MASLD, with its stringent diagnostic criteria that necessitate the presence of specific metabolic risk factors, has refined the scope of diagnosis, thereby excluding idiopathic cases[4,5]. his development is critical, as recent findings from the consensus highlight the extensive prevalence of metabolic dysfunction in the U.S. population, affecting over 91.2% of individuals, including 68.7% of those with normal weight[6]. This data brings to light the pervasive nature of metabolic dysfunction, implicating it not only in MASLD but also as a contributing factor in a range of disorders, such as obesity, diabetes, and cardiovascular diseases. The introduction of MASLD thus marks a pivotal moment in understanding the intricate relationship between metabolic health and liver disease.

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Simultaneously, gallstone disease, specifically cholelithiasis, emerges as a prominent global health concern[7]. Its rising prevalence, currently affecting 5–25% of adults in the United States and Europe, coupled with the substantial economic burden of over 700,000 annual cholecystectomies in the U.S., signals an urgent need for understanding its etiology and risk factors[8,9]. Gallstones are also recognized as a significant risk factor for the development of gallbladder cancer, particularly in the context of asymptomatic static gallstones, often referred to as the silent "killer" within the body[10,11]. Notably, data from the National Health and Nutrition Examination Survey (NHANES) between 1988 and 1994 and March 2017–2020 indicate a rise in the prevalence of gallstone disease from 7.4 to 13.9%, and gallbladder surgeries from 6.0–11.6%[12]. The rising incidence of gallstone disease, documented by NHANES, and its link to gallbladder cancer, particularly in cases of asymptomatic static gallstones, further underscore the severity of this condition.

A critical intersection of these two domains is observed in the association between NAFLD and gallstone disease. A meta-analysis from 2005 to 2019 reveals a 1.71-fold increased risk of gallstone disease in NAFLD patients, pointing to an intricate relationship yet to be fully understood, especially within the U.S. context[13]. The varying findings from international studies, including those from China, South Korea, and Turkey, emphasize the need for focused research in the American demographic to elucidate this association more clearly[14–18].

The shared risk factors between MASLD and gallstone disease, such as obesity, insulin resistance, and metabolic syndrome, underscore a common metabolic foundation linking these two conditions[19–21]. The high comorbidity of MASLD and gallstone disease presents challenges for clinical management, particularly in patients with metabolic abnormalities. Investigating this association is clinically relevant, as it may facilitate the early identification of high-risk patients and enable targeted interventions, such as lifestyle modifications and lipid control, to prevent the occurrence of gallstone disease. This study utilizes NHANES data to examine the relationship between MASLD and gallstone disease. The findings aim to provide insights that may inform clinical practice and public health strategies for managing these increasingly prevalent conditions.

Results

Baseline characteristics of participants

In this study of 7,922 participants, baseline characteristics were meticulously documented, focusing on individuals aged between 20 and 80 years. The average age in this group was 50.7 years, with a balanced representation of gender, evidenced by females comprising 50.6% of the participants. 34.0% of the subjects were diagnosed with MASLD. Further details on the baseline characteristics of the participants before Propensity Score Matching (PSM) are available in Supplementary Table 1.

Before the application of PSM, significant statistical disparities were observed between the two groups in aspects such as gender, age, race, physical activity levels, smoking status, CRP levels, ALT, AST, ALP, Albumin, and LSM. Following PSM, a precise pairing process was executed, resulting in 2,268 matched pairs of participants. This meticulous matching ensured that the standardized differences for all covariates remained below the 10% threshold. Post-PSM analysis revealed no statistically significant differences between the groups for key liver function indicators, including ALT, AST, ALP, albumin, and LSM. However, the MASLD group had a slightly higher proportion of females, individuals with higher educational attainment, and those reporting no physical activity (shown in Table 1).

Association between MASLD and gallstones

In the initial unadjusted model, participants diagnosed with MASLD exhibited a substantially increased risk of gallstone development compared to those without MASLD, as indicated by an odds ratio (OR) of 1.78 (95% Confidence Interval [CI]: 1.54–2.06, $P<0.001$). This association was further examined through Multivariable Regression Analyses, accounting for a range of potential confounders. Even after these rigorous adjustments, the association remained statistically significant, with an OR of 1.49 (95% CI: 1.27–1.75, $P<0.001$), as detailed in Table 2.

Upon employing PSM, both the PSM model (OR: 1.30, 95% CI: 1.09–1.56, $P=0.003$) and Doubly robust analysis model (OR: 1.49, 95% CI: 1.27–1.75, $P<0.001$) consistently indicated an increased gallstones in the MASLD group (Table 2). Further insights were gained from a univaria logisitc regression analysis, as shown in Supplementary Table S2. Subsequent application of an extended multivariate model to the post-PSM data revealed an unadjusted OR of 1.30 (95% CI: 1.09–1.56). Importantly , even after comprehensive adjustments for all confounding factors, the OR remained significant at 1.39 (95% CI: 1.16–1.68), reinforcing the robustness and validity of the observed association between MASLD and increased gallstone risk, as shown in Table 3.

Subgroup analysis

Subgroup analyses were conducted to confirm the robustness of the association between MASLD and gallstone prevalence, as illustrated in Fig. 1. The results demonstrated that MASLD was significantly associated with an increased prevalence of gallstones across various demographic and clinical subgroups.

In participants aged 20 to 60 years, the association was particularly notable, with an odds ratio (OR) of 1.42 (95% confidence interval [CI]: 1.07–1.87), indicating a higher risk of gallstones among younger individuals with MASLD. Similarly, the relationship was evident in women (OR = 1.50; 95% CI: 1.19–1.88) and in participants with liver fibrosis (OR = 1.76; 95% CI: 1.24–2.48). Interestingly, a significant association was also observed in individuals without hypertension (OR = 1.59; 95% CI: 1.19–2.11). In contrast, the strength of the association appeared attenuated in specific subgroups, such as men, participants without liver fibrosis, and those with hypertension. Despite these variations in effect size, the overall association between MASLD and gallstone prevalence remained consistent and statistically significant across subgroups.

Variable	Unmatched patients			Propensity score matched patients		
	Without MASLD ($n=5225$)	With MASLD ($n=2697$)	SMD	Without MASLD ($n=2268$)	With MASLD ($n=2268$)	SMD
Age (years)	49.38 (17.90)	53.38 (15.79)	0.237	55.15 (17.12)	53.27 (15.79)	0.114
Sex, female	2777 (53.1)	1232 (45.7)	0.15	1049 (46.3)	1094 (48.2)	0.04
Race			0.268			0.066
Non-Hispanic white	1746 (33.4)	961 (35.6)		826 (36.4)	812 (35.8)	
Non-Hispanic black	1536 (29.4)	573 (21.2)		475 (20.9)	529 (23.3)	
Mexican American	486 (9.3)	451 (16.7)		350 (15.4)	314 (13.8)	
Others	1457 (27.9)	712 (26.4)		617 (27.2)	613 (27.0)	
Education			0.112			0.045
Less than high school	351 (6.7)	256 (9.5)		223 (9.8)	197 (8.7)	
High school	1817 (34.8)	969 (35.9)		827 (36.5)	814 (35.9)	
More than high school	3057 (58.5)	1472 (54.6)		1218 (53.7)	1257 (55.4)	
PIR	2.65 (1.64)	2.58 (1.59)	0.043	2.61 (1.61)	2.60 (1.60)	0.001
Energy (kcal)	2057.35 (884.35)	2019.49 (808.03)	0.045	2005.27 (817.98)	2033.75 (822.02)	0.035
Physical activity	1471 (28.2)	461 (17.1)	0.267	357 (15.7)	418 (18.4)	0.072
Smoking (%)			0.144			0.031
Never	3053 (58.4)	1551 (57.5)		1293 (57.0)	1310 (57.8)	
Former	1138 (21.8)	725 (26.9)		620 (27.3)	590 (26.0)	
Current	1034 (19.8)	421 (15.6)		355 (15.7)	368 (16.2)	
CRP(mg/L)	3.40 (8.54)	5.43 (9.88)	0.22	4.82 (12.41)	4.89 (8.60)	0.007
ALT(U/L)	20.18 (18.23)	26.40 (18.51)	0.339	24.19 (20.45)	24.21 (15.65)	0.002
AST(U/L)	21.46 (14.38)	22.67 (13.49)	0.087	22.16 (14.00)	21.85 (11.29)	0.024
Albumin (g/dL)	4.08 (0.33)	4.03 (0.33)	0.148	4.03 (0.34)	4.04 (0.33)	0.027
ALP(IU/L)	75.56 (25.51)	82.38 (25.81)	0.266	82.29 (29.39)	80.69 (23.89)	0.06
LSM (kpa)	5.39 (4.20)	7.30 (6.49)	0.348	5.99 (4.90)	6.64 (5.23)	0.128

Table 1. Demographics and baseline characteristics of patients before and after propensity score matching. PIR, family poverty income ratio; ALT, Alanine Aminotransferase; AST, Aspartate Aminotransferase; ALP, Alkaline Phosphatase; LSM, liver stiffness measurements.

Analysis	OR (95% CI)	*P* value
Crude analysis	1.78 (1.54 ~ 2.06)	< 0.001
Multivariable analyses *a	1.49 (1.27 ~ 1.75)	< 0.001
Adjusted for propensity Score *b	1.37 (1.17 ~ 1.61)	< 0.001
Propensity score with matching *c	1.3 (1.09 ~ 1.56)	0.003
Doubly robust analysis	1.49 (1.27 ~ 1.75)	< 0.001

Table 2. Associations between MASLD and gallstones. OR, odds ratio; CI, confidence interval. *a Shown is the odds ratio from the multivariable regression model adjusted for all covariates in Table 1. *b Shown is a multivariable logistic regression model adjusted for all covariates after propensity score matching. *c Shown is the odds ratio from a multivariable logistic regression model with the same strata and covariates after propensity score matching. The analysis included 4,536 patients (2,268 diagnosed with MASLD and 2,268 without MASLD).

Sensitivity analyses

Following the exclusion of participants with incomplete data, a sensitivity analysis was conducted on a complete dataset comprising 5,909 individuals. This analysis integrated Multivariable Regression and PSM, with detailed results presented in Supplementary Table 3. The outcomes of these sensitivity analyses provided robust evidence affirming that the exclusion of incomplete data did not undermine the integrity or validity of the study's findings. Crucially, all results from these analyses were in alignment with the primary outcomes, further substantiating the significant association between Metabolic Associated Fatty Liver Disease (MASLD) and an elevated prevalence of gallstones. These findings underscore the reliability of the study's conclusions and reinforce the imperative nature of the relationship observed between MASLD and gallstone prevalence.

Variable	Crude	Adjusted model OR (95% CI)				
	OR (95% CI)	Model 1	Model 2	Model 3	Model 4	Model 5
Without MASLD	1(Ref)	1(Ref)	1(Ref)	1(Ref)	1(Ref)	1(Ref)
With MASLD	1.3 (1.09 ~ 1.56)	1.37 (1.14 ~ 1.65)	1.38 (1.15 ~ 1.66)	1.38 (1.15 ~ 1.66)	1.39 (1.16 ~ 1.67)	1.39 (1.16 ~ 1.68)

Table 3. Multivariable regression analyses in matched cohort. Ref, reference; OR, odds ratio; CI, confidence interval. Model 1:Adjusted for age and gender. Model 2:Adjusted for age, gender, race, education and PIR. Model 3:Adjusted for age, gender, race, education, PIR, energy and physical activity. Model 4:Adjusted for age, gender, race, education, PIR, energy, physical activity, CRP and Smoking. Model 5: Adjusted for age, gender, race, education, PIR, energy, physical activity, CRP, Smoking, AST, ALT, ALP, Albumin and LSM. Abbreviations: PIR, family poverty income ratio; ALT, Alanine Aminotransferase; AST, Aspartate Aminotransferase; ALP, Alkaline Phosphatase; LSM, liver stiffness measurements.

Discussion

This study provides a comprehensive analysis of the association between MASLD and gallstones using NHANES data from 2017 to 2020. By employing the Propensity Score Matching (PSM) method to minimize potential biases, our research offers robust evidence of this relationship in a nationally representative US population.

The study demonstrates a significant positive association between MASLD and gallstone incidence. Subgroup analyses revealed that this association was more pronounced in specific populations, including females, individuals under 60 years of age, those with lower household income, reduced physical activity, higher liver stiffness measurement (LSM) values, and non-hypertensive individuals. These findings provide valuable insights into the role of MASLD in increasing gallstone risk, particularly within these vulnerable subgroups.

Gallstones are a prevalent digestive disorder, influenced by a variety of factors including infection, genetics, lifestyle choices, and environmental conditions. Notably, their development is closely linked to metabolic dysfunctions such as insulin resistance, type 2 diabetes, and obesity. The increasing global prevalence of gallstones underscores their significance as a public health concern[22,23]. Against this backdrop, gallstones are widely recognized as a disease intricately linked to metabolic dysfunction[24]. Metabolically Associated Fatty Liver Disease (MASLD), which includes hepatic steatosis and metabolic abnormalities, has emerged as a key player in understanding gallstone formation. This condition reflects a broader spectrum of metabolic disturbances than NAFLD, making it particularly relevant in the study of gallstones. While metabolic syndrome and gallstone disease are recognized as significant health issues, the precise causal relationship between them remains to be fully elucidated.

Research has consistently identified metabolic syndrome as a major risk factor for gallstone development. Key factors include obesity, altered lipid profiles, and elevated blood glucose levels.
[25–27]. The link between obesity and increased gallstone risk is particularly pronounced, given the metabolic changes associated with obesity, such as increased bile secretion and altered lipid metabolism[28]. Beyond general obesity, recent studies have highlighted the critical role of body composition, particularly visceral fat accumulation, in metabolic dysfunction and gallstone formation, even in individuals with normal BMI[29]. This underscores the importance of considering body composition when evaluating gallstone risk in MASLD patients. Previous observational studies further emphasize the tight connection between metabolic syndrome (MetS) and gallstone disease (GSD). A recent Mendelian study provides explicit evidence that MetS significantly increases the incidence of gallstone formation, particularly among MetS patients with abdominal obesity. It emphasizes that controlling and treating MetS can effectively mitigate the risk of gallstone formation[30].

There is ongoing debate in the literature regarding the relationship between NAFLD and gallstones. Some studies, like those by Chang et al., suggest an increased risk of gallstones in individuals with NAFLD, particularly among specific groups like the elderly and females[31]. However, other research, including a retrospective study in China, has found no significant difference in gallstone prevalence between NAFLD and non-NAFLD patients[32,33]. A meta-analysis encompassing studies on gallstone occurrence among individuals with and without NAFLD revealed a 1.71-fold increased risk in NAFLD patients, based on seven hospital-based studies and one population-based study conducted in China, with a lack of evaluation in the American population[13]. Our study, focusing on U.S. outpatients, adds to existing knowledge by adjusting for key factors, including age, gender, race, income, lifestyle, and liver function. Subgroup analysis reveals higher gallstone risk in non-Hispanic Black individuals, lower-income groups, those with liver fibrosis, and non-hypertensive patients. Proactive management of metabolic risk factors, such as obesity, dyslipidemia, and insulin resistance, may not only improve liver health but also reduce gallstone-related morbidity, particularly in high-risk subpopulations. Notably, recent studies have demonstrated that improving lipid profiles, specifically reducing LDL-c levels, can lower the risk of gallstone formation by mitigating cholesterol supersaturation in bile, a key factor in gallstone pathogenesis[34]. This highlights the clinical importance of lipid management as a preventive strategy for gallstone disease in MASLD patients.

Our study has several notable strengths and limitations that warrant consideration. Firstly, it represents the first comprehensive analysis of the correlation between MASLD and gallstones within the American population. We utilized NHANES data, which is collected through standardized questionnaires and computer-assisted personal interviews (CAPI) under rigorous quality control measures, ensuring the reliability of self-reported gallstone diagnosis. Additionally, we applied precise diagnostic criteria for MASLD, ensuring clear metabolic associations. This contrasts with the lack of standardized diagnostic criteria for NAFLD, as highlighted in Cole et al.'s study,

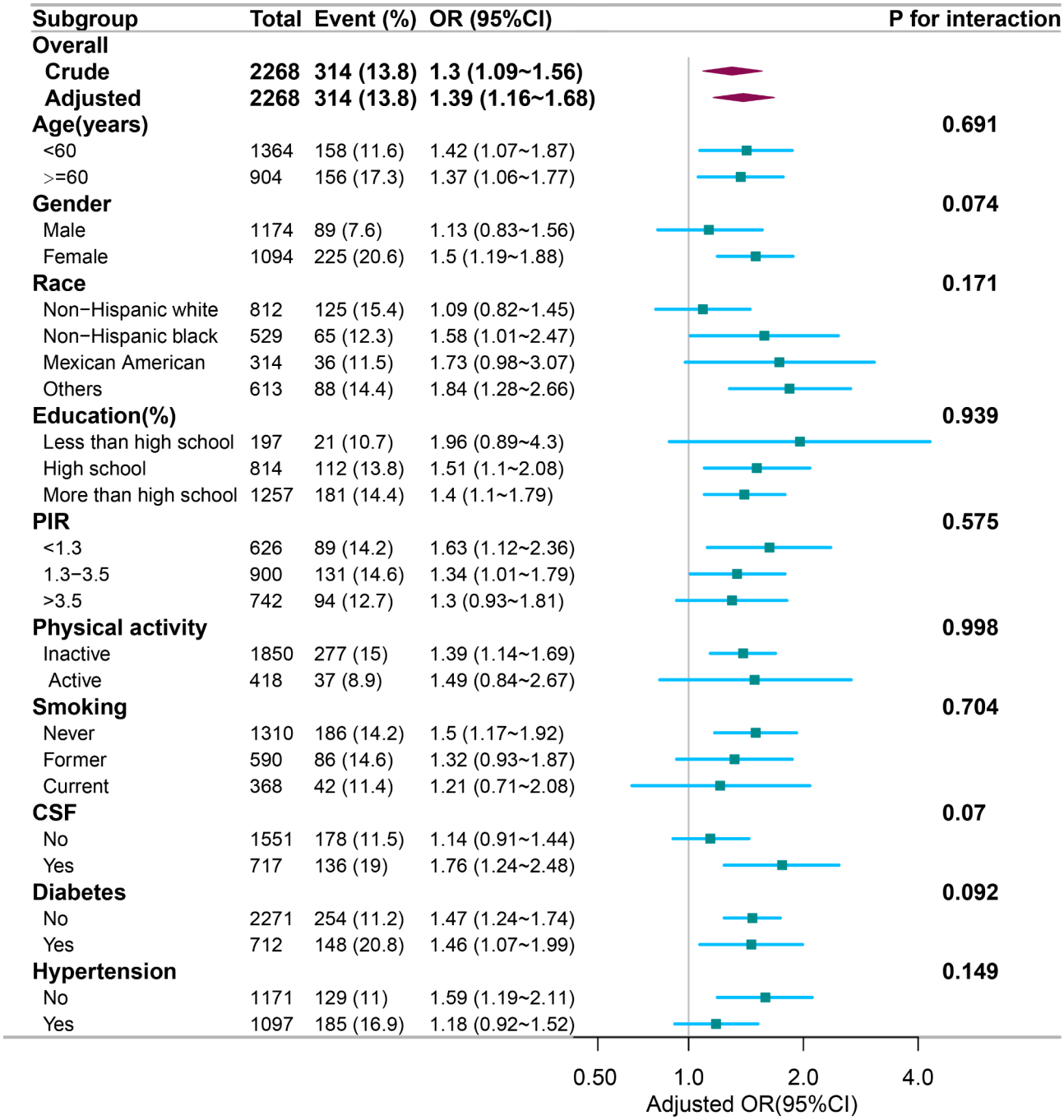

Fig. 1. The association between MASLD and Gallstones in subgroups. PIR, Family poverty income ratio, Categorized into the following 3 levels based on the family poverty income ratio: low income (< 1.3), medium income (> 1.3 to 3.5), and high income (> 3.5). CSF, clinically significant fibrosis (CSF) was identified using liver stiffness measurement.
(LSM) ≥ 6.5 kPa .

which limits generalizability due to varying diagnostic methodologies[14]. However, several limitations must be acknowledged. The cross-sectional design limits our ability to infer causality between MASLD and gallstones. Future longitudinal studies are needed to confirm this association and explore underlying mechanisms. Secondly, residual confounding factors, such as underlying medical conditions, may exist. Although we adjusted for some potential confounders and used Inverse Probability of PSM to minimize bias, certain metabolic indicators integral to the diagnosis of MASLD (e.g., hepatic steatosis and metabolic factors) were not included as covariates due to multicollinearity concerns. To address this, we conducted subgroup analyses for diabetes, hypertension, and liver fibrosis, and performed sensitivity analyses excluding individuals with missing covariate data to ensure robustness. Thirdly, the diagnosis of gallstones relied on self-reported data, introducing the potential for recall

bias. While NHANES employs rigorous quality control measures, including standardized questionnaires and well-trained interviewers, self-reported data may still underestimate the true prevalence of gallstones. This underestimation could lead to a lower observed odds ratio for the association between MASLD and gallstones. Future studies incorporating imaging-based or clinically verified diagnoses alongside self-reported data would further enhance the robustness and validity of these findings.

Conclusion

The findings from this cross-sectional study indicate a positive association between MASLD and gallstones in the U.S. outpatient adult population. These two conditions are closely interrelated and necessitate integrated management as comorbidities.

Methods

Data sources

The National Health and Nutrition Examination Survey (NHANES), administered by the National Center for Health Statistics (NCHS), is a comprehensive survey designed to assess the health and nutritional status of the noninstitutionalized population in the United States. Approval for the NHANES study protocol was obtained from the NCHS research ethics review board, and participants provided written informed consent during enrollment. An exemption from the requirement for informed consent was granted due to the utilization of publicly available deidentified data. This exemption ensures the protection of participant privacy and is in line with established ethical standards. Additionally, the research conducted at the Affiliated Wuxi Fifth Hospital of Jiangnan University received an exemption from the institutional review board.

The study strictly adhered to the reporting guidelines outlined in the Strengthening the Reporting of Observational Studies in Epidemiology (STROBE). This commitment to rigorous reporting standards enhances the transparency and credibility of the research findings.

Study design and population

Our investigation relies on data acquired from participants during the NHANES survey cycles spanning 2017 to 2020. Initially, 15,560 participants were screened from the NHANES cycles during this period. Following the exclusion of 5,862 individuals due to missing data on Liver Ultrasound Transient Elastography and 1,776 participants with incomplete information on gallstones, the final analysis encompassed 7,922 participants. Within this group, 2,983 were diagnosed with MASLD (Fig. 2).

Diagnosis of MASLD and gallstones

In our study, the diagnostic approach for MASLD adheres to the standards set forth by the Delphi consensus[1]. Definition of MASLD: Firstly, evidence of hepatic steatosis is required, which is determined by a Controlled Attenuation Parameter (CAP) measurement exceeding 285 dB/m[35]. Secondly, MASLD diagnosis requires hepatic steatosis and at least one metabolic dysfunction criterion. Thirdly, MASLD diagnosis excluded individuals with viral hepatitis, autoimmune, hereditary, drug-induced liver diseases, or excessive alcohol intake ($\geq$30 g/day for men, $\geq$20 g/day for women) (supplementary Fig. 1). Additionally, the occurrence of gallstones was determined based on affirmative responses to the question, "Have you ever been diagnosed with gallstones by a doctor?" in the National Health and Nutrition Examination Survey (NHANES) questionnaire, with gallstone incidence being an important outcome variable in this study.

Data extraction

In the context of the NHANES 2017–2020 cycle, exclusive data on Liver Ultrasound Transient Elastography was analyzed. To mitigate potential confounding factors, descriptive statistics were examined, augmented by prior knowledge within our cohort[36]. The refined model incorporated a range of covariates, including age, gender, race, educational attainment, family poverty income ratio, physical activity levels, smoking habits, CRP, and hepatic enzyme levels (Alanine Aminotransferase, Aspartate Aminotransferase, Alkaline Phosphatase, Albumin), along with liver stiffness measurement (LSM).

Racial and ethnic classifications were derived from self-reported survey responses, categorized into Non-Hispanic White, Non-Hispanic Black, Mexican American, and Others (inclusive of non-Hispanic multiracial individuals). Educational levels were stratified into three categories: high school or less, some college, and college graduate or higher. Family income levels were segmented into low ($\leq$1.3), medium ($>$1.3 to $\leq$3.5), and high ($>$3.5) brackets, based on the family poverty income ratio, aligning with the reporting conventions of US government departments. Physical activity (PA) was defined as participation in vigorous-intensity sports, fitness, or recreational activities on a typical week, exclusive of occupational and transportation activities. Smoking status was delineated into three groups: Never Smoked, Former Smoker, and Current Smoker, ascertained through lifetime cigarette consumption and current smoking patterns. Heavy drinking was characterized by the consumption of four or more alcoholic beverages on most days within a given period. Dietary factors, specifically energy intake, were accounted for through two 24-hour dietary recalls, with the mean intake serving as a covariate. Participants' chronic disease profiles, specifically hypertension and diabetes, were evaluated based on self-reported doctor diagnoses and medication usage. Clinically significant fibrosis (CSF) was identified by LSM values equal to or exceeding 6.5 kPa, demonstrating a sensitivity of 66% and a specificity of 80%[37].

Statistical analysis

In this analysis, categorical variables were expressed as percentages. Continuous variables adhering to a normal distribution were presented as means with standard deviations (SD). For non-normally distributed variables, the

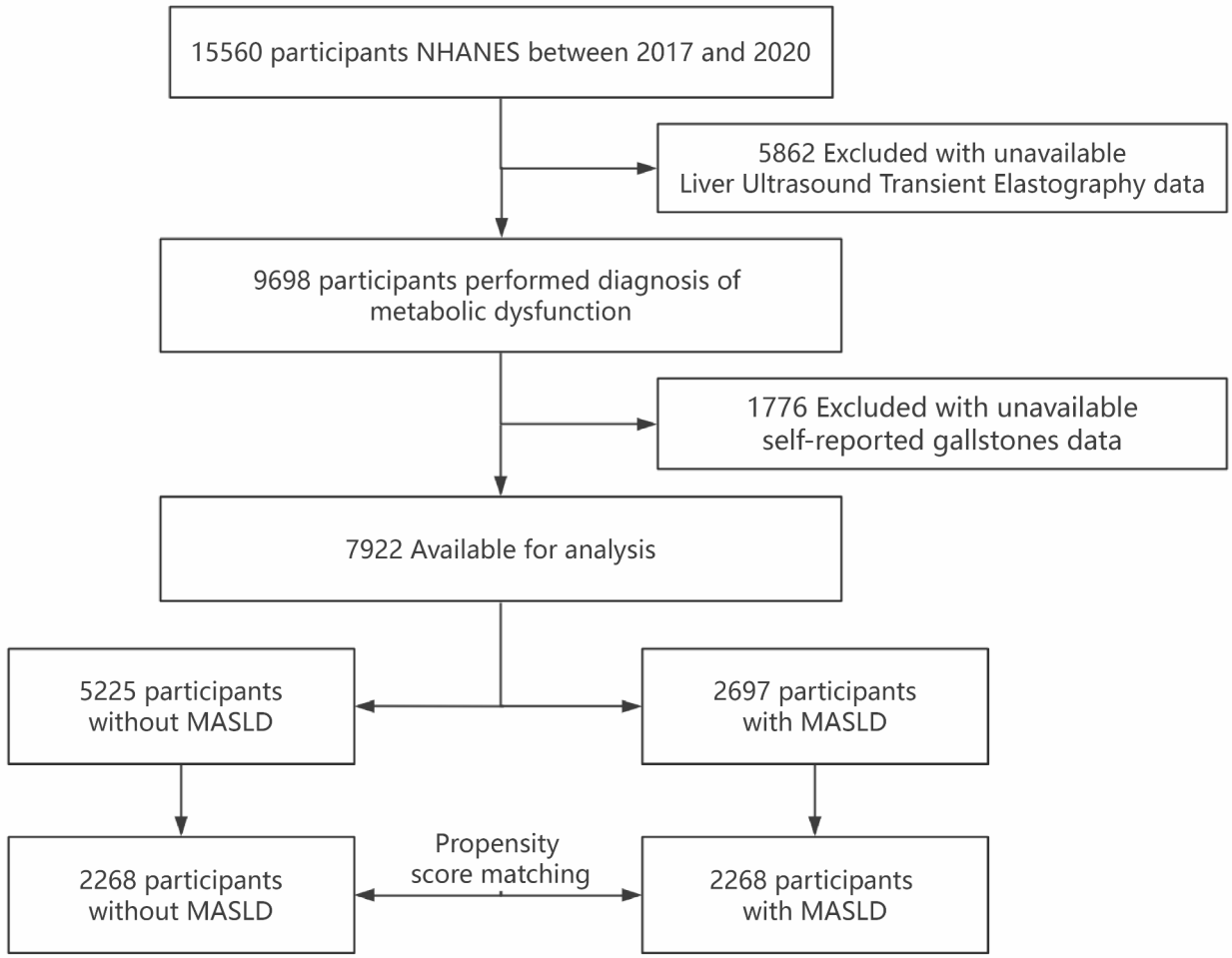

Fig. 2. Flow diagram of the screening and enrollment of study participants.

median and interquartile range (IQR) were utilized for description. To elucidate the association between MASLD and gallstones, logistic regression models were applied, yielding odds ratios (ORs) and 95% confidence intervals (CIs). The presence of multicollinearity within the models was assessed using the variance inflation factor (VIF) method, with a VIF value of 5 or greater indicating significant multicollinearity. Analytical robustness was further enhanced by the application of t-tests for continuous data and $\chi 2$ tests for categorical variables.

Addressing the challenge of missing data, a robust statistical methodology was employed. This involved multiple imputation with five iterations using the chained equations approach, as facilitated by the R 'mice' package. This strategy was selected to augment the analytical power and reduce potential biases that may arise from incomplete data sets.

To enhance the integrity of the research and minimize potential biases, Propensity Score Matching (PSM) and logistic regression analysis were employed, following the methodology outlined by Shen et al. (2019)[38]. This approach incorporated a comprehensive set of matching variables, including sex, age, race, education level, poverty income ratio (PIR), energy intake, physical activity levels, smoking status, and key biochemical parameters such as C-reactive protein (CRP), alanine aminotransferase (ALT), aspartate aminotransferase (AST), albumin, alkaline phosphatase (ALP), and liver stiffness measurement (LSM). A stringent matching criterion with a caliper width of 0.2, equivalent to a 10% standard deviation, was utilized to ensure balanced distribution between the cohorts. Logistic regression models were pivotal in computing propensity scores, odds ratios (ORs), and 95% confidence intervals (CIs) for each assessed parameter, thereby bolstering the study's reliability and interpretability.

A series of sensitivity analyses were implemented to ensure the robustness of the research findings. Initially, five adjusted models were developed. The first model accounted for age and gender. The second model expanded upon this by including race, education, and PIR. The third model encompassed all variables from the second model, with an additional adjustment for physical activity. The fourth model included all factors from the third model, with further adjustments for smoking habits and CRP levels. The fifth and final model incorporated all variables from the fourth model, with additional adjustments for biochemical markers, namely ALT, AST, ALP, Albumin, and LSM. Acknowledging the risk of overadjustment and collinearity, diabetes and hypertension were

deliberately excluded from the covariates. However, to assess their potential impact on the relationship between MASLD and gallstones, specific subgroup analyses were conducted for these conditions.

Further stratification in logistic regression models was applied for interaction and subgroup analyses, focusing on age groups, gender, race, education, PIR, physical activity, smoking status, and LSM. In an effort to consolidate the integrity of the results, additional sensitivity analyses were performed. To address the potential bias introduced by missing data, records with incomplete covariate information were excluded from the analysis.

Statistical analyses were carried out using R version 3.3.2 (http://www.R-project.org, R Foundation) and Free Statistics software version 1.7, with statistical significance defined as a two-tailed p-value < 0.05. Detailed information about the study variables can be accessed on the NHANES website at www.cdc.gov/nchs/nhanes/.

Data availability

The data used in this study are sourced from the National Health and Nutrition Examination Survey (NHANES) (https://www.cdc.gov/nchs/nhanes/index.htm). The NHANES dataset is publicly available and can be accessed through its official website. We confirm that all relevant data have been transparently used in accordance with the study design, and any use of the data complies with the terms and conditions of the original data source.

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Author contributions
Y.Y.Z. and M.L.Z. contributed to study design, data analysis and interpretation, and drafting the original manuscript and revisions.J.J.Z. and C.B.C. contributed to study design, data interpretation, and manuscript review and editing.D.Y. and F.T. contributed to data interpretation, and manuscript review and editing.J.L. and B.H. contributed to study design, and manuscript review and editing. Y.H.Y. and B.H.L. contributed to study conceptualisation and design, data interpretation, and manuscript review and editing.

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Declarations

Competing interests
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