scientific reports

OPEN

Association between relative fat mass and risk of gallstones in adults

Jianjun Wang[1,2,6], Wei He[3,6], Ruizi Shi[1,6], Yu Huang[1,6], Chuan Qin[1], Xi Chen[1], Xintao Zeng[1✉], Yongwei Huang[4✉] & Decai Wang[2,5✉]

Gallstones (GS) are closely associated with obesity. The body mass index (BMI) is the most commonly used index for evaluating obesity. However, BMI does not consider the distribution of adipose tissue and does not differentiate between differences in fat distribution by sex; therefore, BMI only provides a rough indication of overweight status. Relative fat mass (RFM) is an emerging, simple, and low-cost anthropometric index that reflects obesity (particularly abdominal obesity), and is associated with various obesity-related diseases. However, the relationship between the RFM and GS has not yet been explored. Therefore, this study aimed to evaluate the relationship between RFM and the risk of developing GS, the age of patients at the time of their first GS surgery, and to evaluate the predictive value of RFM for the risk of developing GS. Data from the National Health and Nutrition Examination Survey (2017–2020) were used. Logistic regression and dose-response curves were used to analyze the relationship between RFM levels and the prevalence of GS. Multiple linear regression and dose-response curves were used to analyze the relationship between RFM levels and patient age at the time of the first GS surgery. Subgroup analyses further explored the relationship between RFM and age, sex, race, hypertension, and diabetes mellitus. Receiver operating characteristic (ROC) curves were used to analyze the predictive ability of RFM for GS development. Overall, 7978 adults aged ≥ 20 years were included in this study, of whom 828 had a history of GS. After adjusting for potential confounders, each 1-unit increase in RFM was associated with a 9% increase in GS prevalence (odds ratio: 1.09, 95% confidence interval : 1.07, 1.11), with dose-response curves confirming a positive, nonlinear correlation. And the threshold for the effect of RFM on the prevalence of GS was 40.99. Subgroup analyses revealed that the positive correlation between RFM and GS prevalence remained stable in all populations despite subtle differences. The results of the ROC curves suggested that the predictive value of RFM for GS prevalence was superior to that of BMI. In addition, dose-response curves revealed a nonlinear relationship between RFM and patient age at the time of the first GS surgery, and the threshold for the effect of RFM on age at the first GS surgery was 30.99. RFM is closely associated with GS, and in general, higher levels of RFM are associated with a higher risk of GS. RFM is a better predictor of the risk of GS than BMI. Due to the nature of this cross-sectional study, we were unable to determine a causal relationship between the two.

Keywords Gallstones, Relative fat mass, Obesity, Cross-sectional study, Body mass index

Abbreviations
RFM   relative fat mass
GS   gallstone(s)
BMI   body mass index

[1]Department of Hepatobiliary Surgery, School of Medicine, Mianyang Central Hospital, University of Electronic Science and Technology of China, Mianyang 621000, China. [2]NHC Key Laboratory of Nuclear Technology Medical Transformation, School of Medicine, Mianyang Central Hospital, University of Electronic Science and Technology of China, Mianyang 621000, China. [3]Department of Stomatology, School of Medicine, Mianyang Central Hospital, University of Electronic Science and Technology of China, Mianyang 621000, China. [4]Department of Neurosurgery, School of Medicine, Mianyang Central Hospital, University of Electronic Science and Technology of China, Mianyang 621000, China. [5]Department of Urology, School of Medicine, Mianyang Central Hospital, University of Electronic Science and Technology of China, Mianyang 621000, China. [6]Jianjun Wang, Wei He, Ruizi Shi and Yu Huang contributed equally. ✉email: zengxintao@163.com; huangyongwei@sc-mch.cn; decaiwang_2020@163.com

MTTP	microsomal triglyceride transfer proteins
NHANES	National Health and Nutrition Examination Survey
CI	confidence interval
OR	odds ratio
ROC	receiver operating characteristic

Background

Gallstones (GS), common digestive diseases worldwide, are solid substances that form in the gallbladder or bile ducts, typically composed of cholesterol, bile salts, or other substances[1]. Epidemiological investigations have found that the prevalence of GS varies widely in different regions[1]. For example, the prevalence of GS is significantly higher in developed regions, such as the United States and Europe, than in Asia[2]. The annual healthcare cost associated with GS in the United States is approximately $6 billion[3]. Thus, GS places a heavy burden on public health and healthcare. Most patients with GS are asymptomatic, and a small percentage of patients may present with clinical symptoms, mainly in the form of sudden onset of epigastric or right upper abdominal pain[4]. Despite being benign, GS can cause several complications, such as acute cholecystitis, choledocholithiasis, acute cholangitis, and acute pancreatitis[5]. Additionally, GS is a significant risk factor for gallbladder cancer[1]. GS is usually treated surgically, with laparoscopic cholecystectomy being the preferred treatment option[5].

The pathogenesis of GS is complex, with numerous risk factors[6,7]. Among these, the role of obesity, particularly abdominal obesity, in stone formation has been the focus of GS etiology[8–10]. Body mass index (BMI) is the most commonly used obesity indicator. Previous studies have found that for every 5-unit increase in BMI, the incidence of GS increases by 63%[10]. However, BMI has significant limitations, as it does not reflect body fat distribution or differences in adiposity between sexes, making it a rough indicator of overweight rather than an accurate measure of body fat. In this context, Relative fat mass (RFM) has emerged as a novel indicator for assessing obesity, particularly abdominal obesity.

RFM is a recently proposed indicator of obesity (particularly abdominal obesity) in recent years[11]. RFM is an indicator based on the ratio of height to waist circumference, adjusted by sex, and effectively reflects both fat distribution and sex differences in fat content[11]. Thus, RFM not only considers the distribution of fat (height and waist circumference ratio), but also distinguishes differences in fat distribution and content by sex, which makes RFM more sensitive and accurate in the assessment of obesity and obesity-related diseases. Previous studies have shown that RFM is closely associated with obesity-related diseases, such as non-alcoholic fatty liver disease, metabolic syndrome, and cardiovascular disease[12–15]. However, the relationship between the RFM and GS remains unclear.

To address this gap, we hypothesize that RFM may be associated with an increased prevalence of GS and could be a valuable predictor for the age at first GS surgery. Therefore, the aim of our study was to investigate the association between RFM and GS prevalence in adults, assess its predictive value for GS risk, and explore its potential impact on the timing of surgical intervention for GS.

Methods

Source of participants

The participants were drawn from the National Health and Nutrition Examination Survey (NHANES), a survey program administered by the National Center for Health Statistics. The program is a valuable resource for understanding and tracking health trends and needs in the United States. The NHANES plays a crucial role in identifying health priorities, developing disease prevention strategies and public health policies, preventing chronic diseases, improving nutrition, and promoting healthy lifestyles. It uses a stratified multi-stage probability sampling approach (consisting of the following steps: stratification, primary sampling units, secondary sampling units, household sampling, and oversampling) to ensure that the sample is nationally representative. The NHANES consists of four main components: demographic, screening, laboratory, and questionnaire data. The NHANES is updated every 2 years and covers a population of approximately 10,000 people. The program was suspended in March 2020 owing to the COVID-19 pandemic. Since 2017, NHANES has collected information about GS in the population through questionnaires; therefore, we selected participants from this period (2017–March 2020) as the study population. Overall, 15,560 participants were included in the study period, of which 6328 were younger than 20 years of age and were excluded. Participants who lacked information on the GS (22) or RFM (1168) were excluded. In addition, 64 individuals were excluded due to a lack of information on covariates such as hypertension (10 individuals), diabetes (3 individuals), coronary heart disease (25 individuals), asthma (5 individuals), cancer (4 individuals), education (9 individuals), marital status (5 individuals), and smoking status (3 individuals). Finally, 7978 individuals were included in the study. Figure 1 shows the details of the selection criteria for the study population.

The data from the NHANES used in our study is cross-sectional in nature, and participants were not followed up continuously during the study period. The study period spans from 2017 to March 2020, and we selected data collected during this period for our analysis. This cross-sectional design enables us to explore the association between RFM and the risk of GS at a specific point in time, rather than over a longitudinal follow-up period.

Data collection

GS was assessed based on a questionnaire that focused on the question, "Have you ever been told that you have GS?" and "Age at the time of your first GS surgery." The questionnaire was only administered to adults aged ≥ 20 years; therefore, patients younger than 20 years were excluded from this study. Multiple covariates (demographic, examination, and laboratory data) were included in this study, with details provided in **Supplementary Table 1**.
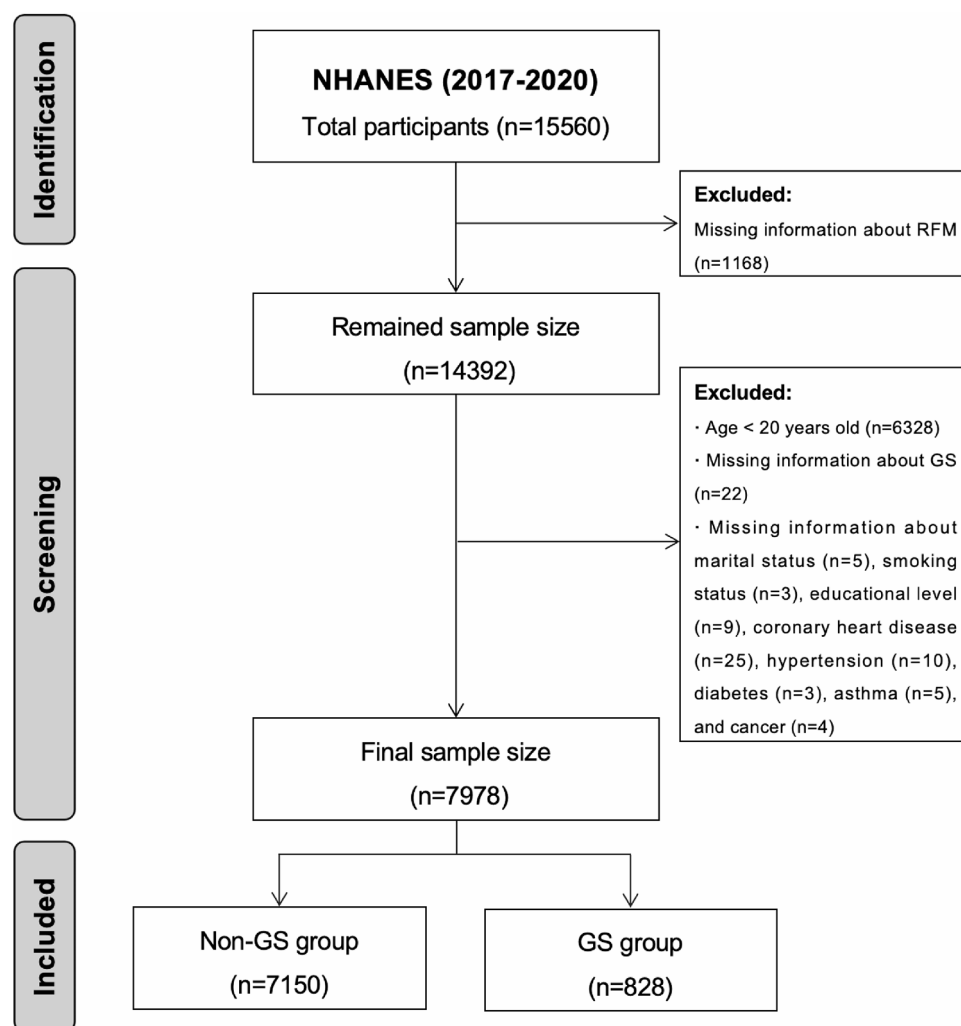
Fig. 1. Flowchart for participants from NHANES.

All participants underwent two 24-hour dietary recalls (these recalls were conducted on non-consecutive days within the same survey cycle); therefore, the average consumption of the two recalls was used in the analysis. The measurement procedures for all covariates are available on the NHANES website (www.cdc.gov/nchs/nhanes/). RFM was calculated as follows: RFM = 64-(20 × height/waist circumference)+(12 ×sex); sex equals 0 for men and 1 for women[16].

Ethics statement
All participants signed an informed consent form; therefore, the NHANES was exempt from additional licensing or ethical review.

Statistical methods
Categorical variables were expressed as numbers and percentages (%), and comparisons between groups were made using Pearson's $\chi2$ test or Fisher's exact test. Continuous variables are expressed as medians and interquartile ranges, and comparisons of continuous variables between groups were performed using a t-test or one-way ANOVA. Several multivariate logistic regression models were constructed to investigate the relationship between the RFM and GS prevalence. Multiple linear regression analyses were used to explore the relationship between RFM and patient age at the time of the first GS surgery. Model 1 was unadjusted, and Model 2 was adjusted for age, sex, race, and marital status. Model 3 was additionally adjusted for education level, physical activity, smoking status, alcohol consumption, diabetes mellitus, hypertension, asthma, cancer, coronary heart disease, total cholesterol, triglycerides, blood glucose, blood pressure, total cholesterol, triglycerides, high-density lipoprotein cholesterol, low-density lipoprotein cholesterol, alanine aminotransferase, aspartate aminotransferase, hemoglobin A1c, ferritin, poverty income ratio, total calories, total fat, and total sugar. To further investigate the relationship between RFM, GS prevalence, and age at which patients first underwent GS surgery, we used a generalized additive model with smooth curve fitting (penalized spline method). The use of generalized additive model enabled us to explore potential thresholds or non-linear trends that could have been

overlooked using simpler models. For nonlinear relationships, we used the logarithmic likelihood ratio test to calculate inflection points. Subgroup analyses were performed to assess the relationship between RFM and GS in various populations, including age, sex, race, hypertension, and diabetes. Receiver operating characteristic (ROC) curves were used to analyze the predictive ability of RFM for GS development. Statistical significance was defined as $P < 0.05$. All analyses were performed using the R software version 4.0.2 (R Foundation for Statistical Computing, Vienna, Austria), details are presented in the **Supplementary material**.

Results

Characteristics of participants

Overall, 7978 participants were finally included in this study, comprising 3900 males and 4078 females. Of these, 828 participants had a history of GS. RFM was significantly higher in the GS group compared with the non-GS group (43.80 [35.09–47.78] vs. 34.91 [29.31–42.72], $P < 0.001$). In the GS group, non-Hispanic white individuals were the most numerous (42.27%), followed by Mexican Americans (24.28%). Participants in the GS group were more likely to have hypertension, diabetes, asthma, coronary heart disease, and cancer than those in the non-GS group (All $P < 0.001$). In addition, the serum triglyceride and hemoglobin A1c levels were significantly higher in the GS group (All $P < 0.001$). Further details are provided in Table 1.

Logistic regression results between RFM and prevalence of GS

We observed a positive correlation between RFM and GS prevalence (Table 2). In Model 3, after adjusting for potential confounders, we found that for every 1-unit increase in RFM, GS prevalence increased by 9% (odds ratio (OR): 1.09, 95% confidence interval (CI): 1.07, 1.11). After converting RFM from a continuous variable to a categorical variable (tertiles) for sensitivity analyses, tertile 3 was associated with a significant 3.11-fold increase in GS prevalence (OR = 3.11, 95% CI: 2.20, 4.38) compared with the lowest RFM tertile (tertile 1).

Dose-response relationship and threshold effect analysis of RFM and GS prevalence

The relationship between the RFM and GS prevalence was further explored using additive generalized modeling and smoothed curve fitting. Figure 2; Table 3 show a positive (nonlinear) correlation between RFM and GS prevalence. Considering the effect of the saturation threshold between them, the likelihood natural ratio test determined the optimal RFM threshold to be 40.99.

Subgroup analysis

To assess whether the relationship between RFM and GS prevalence holds in different populations, we performed additional subgroup analyses. The results in Table 4 suggest that the positive correlation between RFM and GS prevalence remained stable in all populations, although there were subtle differences between populations. For example, for every 1-unit increase in RFM, the prevalence of GS in men would increase by 6% (OR = 1.06, 95% CI: 1.02, 1.09), whereas the prevalence of GS in women would increase by 11% (OR = 1.11, 95% CI: 1.09, 1.13). For patients with diabetes, each 1-unit increase in RFM was associated with a 6% increase in GS prevalence (OR = 1.06, 95% CI: 1.02, 1.11) and a 10% increase in the non-diabetic population (OR = 1.10, 95% CI: 1.08, 1.12). Additional details are presented in Table 4.

ROC curve analysis

We plotted the ROC curve of the RFM to assess its predictive value for GS prevalence (Fig. 3). It was found that RFM had a higher predictive value for the risk of GS prevalence (69.66% [95% CI: 67.78–71.53]) compared to BMI (63.96% [95% CI: 61.99–65.92]) and waist circumference (63.99% [95% CI: 62.08–65.90]). Figure 3 pairs of information are presented in Table 5.

Results of multivariate linear analysis between RFM and patients' age at first GS surgery

In Model 1, for every 1-unit increase in RFM, the patient's age at first GS surgery would be 0.45 years earlier ($\beta = -0.45$, 95% CI: −0.59, −0.31). When we adjusted for potential confounders, for each 1-unit increase in RFM, patients might be 0.19 years earlier at the time of their first GS surgery ($\beta = -0.19$, 95% CI: −0.40, 0.02), though this result did not reach statistical significance (Table 6).

Dose-response relationship and threshold effect analysis of RFM and patient's age at first GS surgery

To further investigate the relationship between RFM and patient age at first GS surgery, generalized additive modeling and smoothed curve fitting were performed. The results suggested a nonlinear relationship between RFM and patient age at the time of the first GS surgery (Fig. 4). The results in Table 7 demonstrated that the threshold for the effect of RFM on patient age at the first GS surgery was 30.99.

Discussion

This is the first study to elucidate the relationship between RFM and GS prevalence in adults, and patient age at the time of first GS surgery. The results showed that RFM was positively associated with GS prevalence. When RFM was converted from a continuous to a categorical variable, GS prevalence increased significantly as RFM increased. A positive correlation between RFM and GS prevalence was established in all the patient groups analyzed in this study. In addition, we observed a nonlinear relationship between RFM and the age at first GS surgery and found a significant threshold effect of RFM. Specifically, when RFM reaches 30.99, the age at first GS surgery is significantly younger, suggesting that a higher RFM may be associated with earlier surgical intervention. Moreover, the predictive value of RFM for GS prevalence was significantly higher than those

Characteristic	All (*n* = 7978)	Non-GS group (*n* = 7150)	GS group (*n* = 828)	*P*-value
RFM	35.62 (29.69–43.62)	34.91 (29.31–42.72)	43.80 (35.09–47.78)	< 0.001
Age (years)	51(36–64)	50(34–63)	60(46–70)	< 0.001
Gender				< 0.001
Male	3900 (48.88)	3661 (51.20)	239 (28.86)	
Female	4078 (51.12)	3489 (48.80)	589 (71.14)	
BMI	28.7 (24.8–33.6)	28.4 (24.6–33.2)	31.8 (27.7–37.6)	< 0.001
Waist Circumference	99.4 (89.0–111.5)	98.6 (88.2-11.04)	107.2 (97.1–118.5)	< 0.001
Race (%)				< 0.001
Non-Hispanic White	2771 (34.73)	2421 (33.86)	350 (42.27)	
Non-Hispanic Black	2126 (26.65)	1955 (27.34)	171 (20.65)	
Mexican American	1741 (21.82)	1540 (21.54)	201 (24.28)	
Other Race	1340 (16.8)	1234 (17.26)	106 (12.80)	
Physical Activity (%)				< 0.001
Never	2363 (29.62)	2063 (28.85)	300 (36.23)	
Moderate	2700 (33.84)	2400 (33.57)	300 (36.23)	
Vigorous	2915 (36.54)	2687 (37.58)	228 (27.54)	
Marital Status (%)				< 0.001
Cohabitation	4667 (58.50)	4175 (58.39)	492 (59.42)	
Solitude	1754 (21.99)	1524 (21.31)	230 (27.78)	
Never married	1557 (19.52)	1451 (20.29)	106 (12.80)	
Alcohol Use(%)				< 0.001
Never	681 (8.54)	612 (8.56)	69 (8.33)	
Ever	1484 (18.60)	1262 (17.65)	222 (26.81)	
Now	5433 (68.10)	4936 (69.03)	497 (60.02)	
Unclear	380 (4.76)	340 (4.76)	40 (4.83)	
Education Level (%)				0.38
Less than high school	1446 (18.12)	1305 (18.25)	141 (17.03)	
High school	1938 (24.29)	1722 (24.08)	216 (26.09)	
More than high school	4594 (57.58)	4123 (57.66)	471 (56.88)	
Hypertension (%)				< 0.001
No	5004 (62.72)	4616 (64.56)	388 (46.86)	
Yes	2974 (37.28)	2534 (35.44)	440 (53.14)	
Diabetes (%)				< 0.001
No	6792 (85.13)	6176 (86.38)	616 (74.40)	
Yes	1186 (14.87)	974 (13.62)	212 (25.60)	
Asthma (%)				< 0.001
No	6717 (84.19)	6057 (84.71)	660 (79.71)	
Yes	1261 (15.81)	1093 (15.29)	168 (20.29)	
Coronary Heart Disease (%)				< 0.001
No	7636 (95.71)	6879 (96.21)	757 (91.43)	
Yes	342 (4.29)	271 (3.79)	71 (8.58)	
Cancers (%)				< 0.001
No	7158 (89.72)	6476 (90.57)	682 (82.37)	
Yes	820 (10.28)	674 (9.43)	146 (17.63)	
Smoking Status (%)				< 0.001
Never	4650 (58.29)	4204 (58.80)	446 (53.86)	
Ever	1872 (23.46)	1622 (22.69)	250 (30.19)	
Now	1456 (18.25)	1324 (18.52)	132 (15.94)	
PIR				0.03
< 1.3	1928 (24.17)	1733 (24.24)	195 (23.55)	
≥ 1.3–< 3.5	2724 (34.14)	2405 (33.64)	319 (38.53)	
≥ 3.5	2274 (28.5)	2055 (28.74)	219 (26.45)	
Unclear	1052 (13.19)	957 (13.38)	95 (11.47)	
Total Sugar Intake	90.16 (54.8–137.22)	90.61 (54.74–137.84)	87.28 (55.08–131.9)	0.34
Total Kcal Intake	1969 (1431–2647)	1992 (1450–2670)	1781 (1342–2365)	< 0.001
Total Fat Intake	78.05 (52.78–111.63)	79.11 (53.03–112.74)	72.99 (50.84–102.07)	< 0.001
Continued				

Characteristic	All (*n* = 7978)	Non-GS group (*n* = 7150)	GS group (*n* = 828)	*P*-value
Total Water Intake	1920 (718.2–3480)	1920 (720–3480)	1920 (630–3522)	0.76
TG (mmol/L)	1.28 (0.89–1.86)	1.25 (0.88–1.84)	1.46 (1.03–1.98)	< 0.001
TC (mmol/L)	4.73 (4.09–5.46)	4.73 (4.09–5.48)	4.68 (4.03–5.41)	0.12
HDL-C (mmol/L)	1.32 (1.09–1.6)	1.32 (1.09–1.6)	1.29 (1.09–1.55)	0.24
LDL-C (mmol/L)	2.78 (2.22–3.44)	2.79 (2.22–3.44)	2.69 (2.12–3.44)	0.07
ALT (U/L)	18 (13–26)	18 (13–26)	17 (13–25)	0.59
AST (U/L)	19 (16–24)	19 (16–24)	19 (15–23)	0.02
HbA1c (%)	5.6 (5.3–6.0)	5.6 (5.3–5.9)	5.7 (5.4–6.2)	< 0.001
Ferritin (ng/ml)	109 (51.6–202)	110 (52.2–204)	101 (45.1–189)	0.01

Table 1. Baseline characteristics of participants. RFM, relative fat mass; GS, gallstone; BMI, body mass index; PIR, poverty income ratio; TG, triglyceride; TC, total cholesterol; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; ALT, alanine aminotransferase; AST, aspartate aminotransferase; HbA1c, haemoglobin A1c.

Characteristic	Model 1 OR (95%CI)	Model 2 OR (95%CI)	Model 3 OR (95%CI)
RFM	1.09 (1.08–1.10)	1.11 (1.09–1.13)	1.09 (1.07–1.11)
Categories			
Tertile 1	1	1	1
Tertile 2	1.88 (1.49–2.36)	1.68 (1.30–2.17)	1.37 (1.05–1.78)
Tertile 3	4.87 (3.95–6.00)	4.28 (3.07–5.96)	3.11 (2.20–4.38)

Table 2. Results of logistic regression analysis between RFM and prevalence of gallstone. Model 1 = no covariates were adjusted. Model 2 = Model 1 + age, gender, race, and marital status were adjusted. Model 3 = Model 2 + education level, physical activity, smoked status, alcohol use, diabetes, hypertension, asthma, cancers, coronary heart disease, total cholesterol, triglyceride, high-density lipoprotein cholesterol, low-density lipoprotein cholesterol, alanine aminotransferase, aspartate aminotransferase, haemoglobin A1c, ferritin, poverty income ratio, total kcal, total fat, and total sugar were adjusted.

of BMI and waist circumference. Therefore, this study provides strong evidence that RFM can be used as a predictive tool for identifying individuals at high risk of GS.

The pathogenesis of GS is complex and involves several factors. For example, mutations in certain genes (e.g., *ABCG5/ABCG8*) increase the risk of GS, leading to a certain familial predisposition to the condition[17]. The risk of developing GS varies across countries and regions. Generally, the incidence of GS is significantly higher in developed regions, such as Europe and the United States, than in Asia[18]. In addition, the risk of GS in women is usually higher than that in men[4,5], which is mainly influenced by high estrogen levels in women. In this study, the prevalence of GS was higher in women than in men (71.14% vs. 28.86%; *P* < 0.001). Estrogen not only increases HMG-CoA reductase activity in the liver, promoting cholesterol synthesis and secretion[19,20] but also inhibits the synthesis of bile acids[21], leading to the formation of cholesterol crystals and stones in supersaturated bile. Age is also an important factor in the development of GS. In this study, the age was significantly higher in the GS group than in the non-GS group (60 [46–70] vs. 50 [34–63], *P* < 0.001). This was consistent with the results of previous studies[22,23]. In old age, gallbladder motility is weakened, and emptying ability decreases, leading to cholestasis[24]. The composition of bile changes with age, particularly when the ratio of phospholipids to bile acids decreases, leading to decreased cholesterol solubility and increased risk of cholesterol crystallization[22,23].

Obesity, particularly abdominal obesity, is significantly associated with an increased incidence of GS[25,26]. Therefore, the role of obesity in the stone formation has been the focus of GS etiology research[8–10]. However, the specific mechanisms underlying obesity-induced GS formation have not yet been fully elucidated. Existing studies suggest that obesity can promote stone formation by affecting several aspects, such as cholesterol synthesis and secretion, bile composition, gallbladder function, and inflammation.

Abnormal cholesterol metabolism in patients with obesity is a key factor in GS formation. Studies have shown that increased hepatic synthesis of cholesterol in obese individuals leads to the overproduction of cholesterol in bile, which puts bile in a state of cholesterol supersaturation[25,26]. When cholesterol is supersaturated in bile, it tends to form cholesterol crystals, which is an early process in GS formation[25,26]. Additionally, a decreased bile acid-to-lecithin ratio is associated with obesity. Bile acids and lecithin play key roles in maintaining cholesterol in a soluble state, and reductions in both further promote the precipitation of cholesterol crystals[27]. In addition, increased fasting gallbladder volume and abnormal postprandial gallbladder emptying in patients with obesity lead to cholestasis, which may be associated with the abnormal secretion of cholecystokinin (CCK)[28], a phenomenon that was confirmed in mice with a knockout of the CCK gene[29]. Microsomal triglyceride transfer proteins (MTTP) play important roles in lipid metabolism by acting as lipid transport proteins. Thus, MTTP mutations increase the risk of stone formation by affecting cholesterol transport and reabsorption in
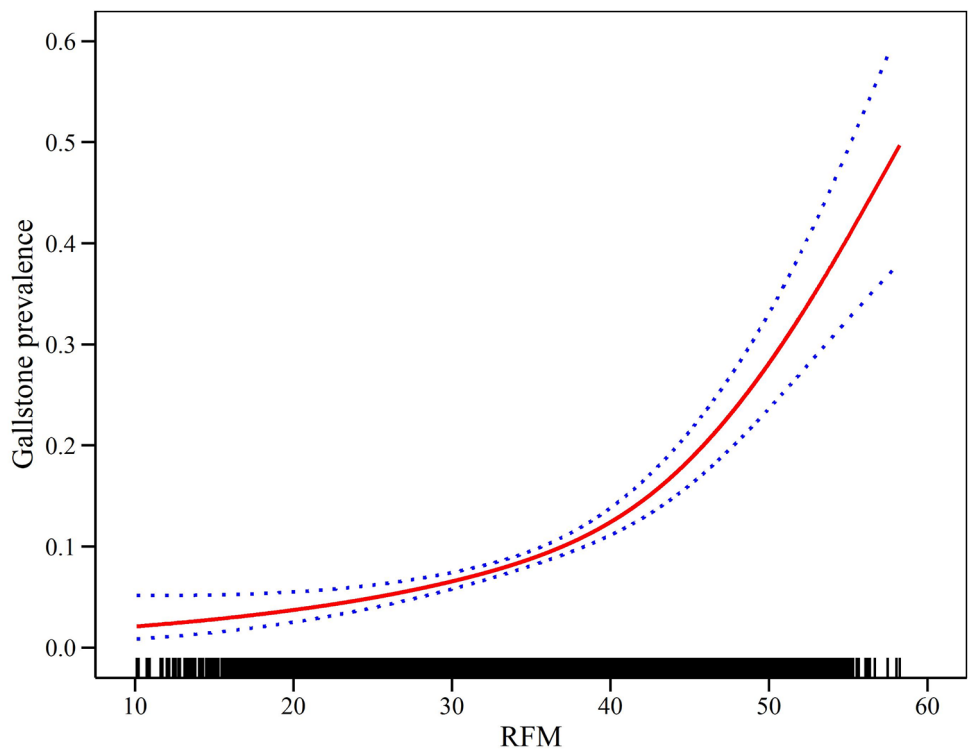
Fig. 2. Density dose-response relationship between RFM with gallstones prevalence. The area between the upper and lower dashed lines is represented as 95% CI. Each point shows the magnitude of the RFM and is connected to form a continuous line. Adjusted for all covariates except effect modifier.

RFM	ULR Test OR (95%CI)	PLR Test OR (95%CI)	LRT Test P-value
< 40.99	1.09 (1.07–1.11)	1.06 (1.03–1.08)	0.002
>= 40.99		1.13 (1.10–1.16)	

Table 3. Two-piecewise linear regression and logarithmic likelihood ratio test explained the threshold effect analysis of RFM with gallstone prevalence. ULR, univariate linear regression; PLR, piecewise linear regression; LRT, logarithmic likelihood ratio test, statistically significant: $P < 0.05$.

the intestine[30]. Moreover, activation of the ileal Fxr-Fgf15 pathway in an obese rat model inhibits cholesterol 7α-hydroxylase (CYP7A1) expression, which affects endogenous cholesterol conversion and disrupts the homeostasis of bile constituents and increases the potential for stone formation[31]. Therefore, obesity can induce and promote stone formation by affecting bile synthesis, excretion, and reabsorption.

In addition, patients with obesity are often accompanied by insulin resistance and metabolic syndrome, which are important risk factors for GS formation[32–34]. Insulin resistance increases hepatic cholesterol synthesis and decreases bile acid synthesis, further contributing to cholesterol supersaturation[32]. Metabolic syndrome is also characterized by hypertriglyceridemia and low high-density lipoproteinemia, which are not only related to bile composition, but may also increase the risk of stone formation by altering gallbladder function (e.g., delayed gallbladder emptying)[35]. Obesity is also closely associated with a systemic low-grade chronic inflammatory state, particularly in patients with obesity, where adipose tissue overproduces pro-inflammatory factors (e.g., TNF-α, IL-6), which not only trigger systemic metabolic disorders, but also have a direct effect on the gallbladder, resulting in chronic inflammation and fibrosis in the gallbladder wall[36,37]. These inflammatory responses may further weaken the contractile function of the gallbladder, increasing the risk of bile retention[38,39]. Recent studies have also shown that obesity may indirectly contribute to GS formation by affecting the gut flora[40]. Individuals with obesity experience significant changes in the gut flora, particularly an increase in bile salt-hydrolyzing bacteria, which break down bile salts and reduce the reabsorption of bile acids in the gut, thereby affecting the circulation and concentration of bile acids, and further exacerbating cholesterol supersaturation in the bile[41,42]. Additionally, intestinal dysbiosis may affect bile secretion via the enterohepatic axis, increasing the risk of stone formation[41,43]. In conclusion, obesity contributes to the development of GS through a combination of mechanisms, and an in-depth understanding of these mechanisms could contribute to better prevention and treatment of obesity-associated GS.

Characteristic	Model 1 OR (95%CI)	Model 2 OR (95%CI)	Model 3 OR (95%CI)
Stratified by age (years)			
20–39	1.13 (1.10–1.16)	1.12 (1.09–1.16)	1.11 (1.07–1.15)
40–59	1.10 (1.08–1.12)	1.12 (1.09–1.15)	1.10 (1.07–1.14)
60–80	1.06 (1.05–1.08)	1.08 (1.05–1.11)	1.06 (1.03–1.09)
Stratified by gender			
Male	1.09 (1.06–1.12)	1.08 (1.04–1.11)	1.06 (1.02–1.09)
Female	1.12 (1.10–1.14)	1.12 (1.10–1.14)	1.11 (1.09–1.13)
Stratified by race			
Non-Hispanic White	1.10 (1.08–1.12)	1.12 (1.09–1.14)	1.10 (1.08–1.13)
Non-Hispanic Black	1.08 (1.06–1.10)	1.09 (1.06–1.13)	1.08 (1.04–1.12)
Mexican American	1.10 (1.08–1.12)	1.08 (1.04–1.13)	1.08 (1.04–1.12)
Other Race	1.08 (1.05–1.11)	1.14 (1.09–1.19)	1.12 (1.06–1.17)
Stratified by hypertension			
No	1.10 (1.08–1.11)	1.10 (1.08–1.13)	1.10 (1.07–1.12)
Yes	1.07 (1.06–1.09)	1.10 (1.07–1.12)	1.08 (1.06–1.11)
Stratified by diabetes			
No	1.09 (1.08–1.10)	1.10 (1.08–1.12)	1.10 (1.08–1.12)
Yes	1.07 (1.05–1.09)	1.08 (1.04–1.12)	1.06 (1.02–1.11)

Table 4. Subgroup analysis to evaluate the relationship between RFM and gallstone prevalence in different populations. Model 1 = no covariates were adjusted. Model 2 = Model 1 + age, gender, race, and marital status were adjusted. Model 3 = Model 2 + education level, physical activity, smoked status, alcohol use, diabetes, hypertension, asthma, cancers, coronary heart disease, total cholesterol, triglyceride, high-density lipoprotein cholesterol, low-density lipoprotein cholesterol, alanine aminotransferase, aspartate aminotransferase, haemoglobin A1c, ferritin, poverty income ratio, total kcal, total fat, and total sugar were adjusted.
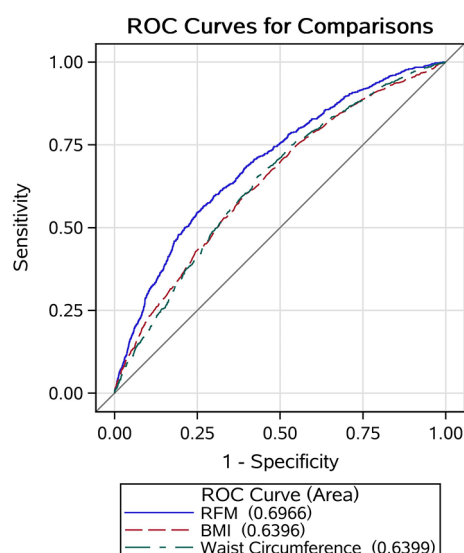

Fig. 3. ROC curves for predicting the risk of gallstones.

Variables	AUC (%)	95% CI (%)	Threshold	Sensitivity	Septicity
RFM	69.66	67.78–71.53	42.131	0.732	0.564
BMI	63.96	61.99–65.92	30.150	0.607	0.600
Waist Circumference	63.99	62.08–65.90	101.450	0.568	0.655

Table 5. Information of ROC curves in Fig. 3. ROC: receiver operating characteristic; RFM, relative fat mass; BMI, body mass index.

Characteristic	Model 1β(95%CI)	Model 2β(95%CI)	Model 3β(95%CI)
RFM	−0.45 (−0.59, −0.31)	−0.11 (−0.33, 0.11)	−0.19 (−0.40, 0.02)

Table 6. Relationship between RFM and patient's age at first gallstone surgery. Model 1 = no covariates were adjusted. Model 2 = Model 1 + age, gender, race, and marital status were adjusted. Model 3 = Model 2 + education level, physical activity, smoked status, alcohol use, diabetes, hypertension, asthma, cancers, coronary heart disease, total cholesterol, triglyceride, high-density lipoprotein cholesterol, low-density lipoprotein cholesterol, alanine aminotransferase, aspartate aminotransferase, haemoglobin A1c, ferritin, poverty income ratio, total kcal, total fat, and total sugar were adjusted.
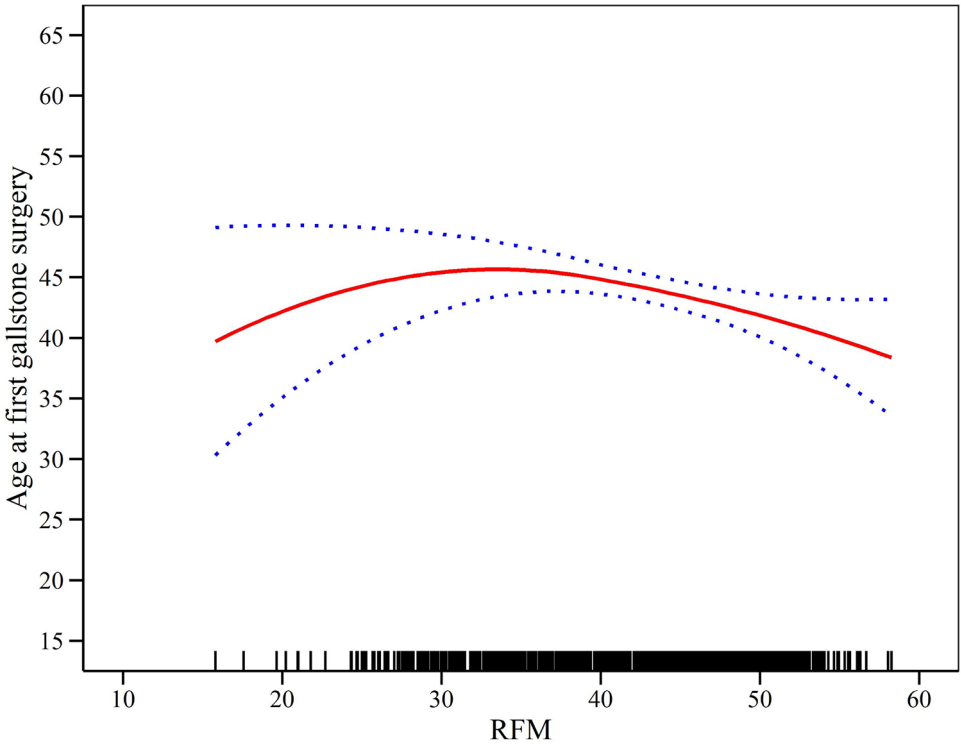
Fig. 4. Density dose–response relationship between RFM and age at first gallstone surgery. The area between the upper and lower dashed lines is represented as the 95% CI. Each point shows the magnitude of RFM and is connected to form a continuous line. Adjusted for all covariates except the effect modifier.

RFM	ULR Test β(95%CI)	PLR Test β(95%CI)	LRT Test P-value
< 30.99	−0.19 (−0.40, 0.02)	0.80 (0.07, 1.54)	0.005
>= 30.99		−0.33 (−0.56, −0.10)	

Table 7. Two-piecewise linear regression and logarithmic likelihood ratio test explained the threshold effect analysis of RFM with patient's age at first gallstone surgery. ULR, univariate linear regression; PLR, piecewise linear regression; LRT, logarithmic likelihood ratio test, statistically significant: $P < 0.05$.

RFM, an obesity indicator that integrates waist circumference, height, and sex, effectively reflect the distribution of adipose tissue and obesity (particularly abdominal obesity)[11]. BMI is the most commonly used indicator for evaluating obesity; however, the calculation of BMI is based only on weight and height and does not consider the distribution of adipose tissue in the body. Therefore, BMI can only roughly reflect whether the weight is overweight, while RFM is calculated as the ratio of height to waist circumference, which can more directly reflect the body fat content, making RFM have higher sensitivity and accuracy in the assessment of obesity. In addition, BMI does not differentiate between the sexes; therefore, BMI may have some limitations in the assessment of obesity in different sexes. Gender adjustment of the RFM makes it more applicable and accurate for different sexes. Abdominal fat (particularly visceral fat) is closely associated with various metabolic

diseases, and BMI is only a total body mass index that does not reflect the local fat distribution. RFM can be used to assess abdominal obesity more accurately by introducing waist circumference into the formula. Recent studies have found that RFM is closely associated with obesity-related diseases, such as non-alcoholic fatty liver disease, metabolic syndrome, and cardiovascular disease[12–15]. Our study also found that RFM was closely associated with GS, confirming the significance of RFM in predicting the risk of GS.

Our study had several strengths. First, we elucidated for the first time in the general adult population the association of RFM with the prevalence of GS and the age of patients at the time of their first GS surgery. Second, the design of stratified multistage probability sampling ensured the diversity and representativeness of the samples. Therefore, our findings reflect the general health status of adults. Third, we adjusted for confounders that could potentially affect the results to ensure that our conclusions are applicable to a more generalized population. Finally, our study confirmed the superior predictive value of RFM over BMI for the risk of developing GS.

However, this study has some limitations. First, because this was a cross-sectional study, we were unable to determine a causal relationship between RFM and GS. Second, the diagnosis of GS and the patient's age at the time of the first GS surgery were determined using a questionnaire; thus, recall (recollection) bias may be present. Finally, although we adjusted for many potential confounders, we were unable to eliminate the effects of other unmeasured confounders. Despite these limitations, the findings of this study are significant. As a novel validated surrogate for obesity, RFM provides a novel method to assess the risk of GS prevalence.

Based on our findings, it is important to highlight the potential clinical applications of RFM as an adjunctive tool for screening individuals at high risk for GS. Given that RFM has shown a significant association with GS prevalence, it could complement traditional measures such as BMI and waist circumference in identifying high-risk populations. Moreover, the global applicability of RFM as a preventive tool warrants further investigation. Future studies should aim to validate its effectiveness across diverse populations with varying characteristics, and explore additional factors, such as genetic susceptibility and dietary habits, which may influence the relationship between RFM and GS risk. Longitudinal studies are also essential to establish causal relationships and better understand the temporal dynamics of RFM in the development of GS.

Conclusion
In conclusion, this study is the first to examine the relationship between RFM and GS prevalence and patient age at the time of the first GS surgery and to explore the value of RFM in predicting the risk of developing GS. In the future, a multicenter prospective cohort study is necessary to confirm the potential of RFM as an independent predictor of GS occurrence.

Data availability
The corresponding author(s) will provide the raw data supporting the conclusions of this paper without reservation.

References
1. Wang, X. et al. Global epidemiology of gallstones in the 21st century: A systematic review and Meta-Analysis. *Clin. Gastroenterol. Hepatol.* **22** (8), 1586–1595. https://doi.org/10.1016/j.cgh.2024.01.051 (2024).
2. Stinton, L. M., Myers, R. P. & Shaffer, E. A. Epidemiology of gallstones. Gastroenterol Clin North Am. 39(2):157–69, vii. (2010). https://doi.org/10.1016/j.gtc.2010.02.003
3. Sandler, R. S. et al. The burden of selected digestive diseases in the united States. *Gastroenterology* **122** (5), 1500–1511. https://doi.org/10.1053/gast.2002.32978 (2002).
4. Madden, A. M., Smeeton, N. C., Culkin, A. & Trivedi, D. Modified dietary fat intake for treatment of gallstone disease in people of any age. *Cochrane Database Syst. Rev.* (2(2), CD012608. https://doi.org/10.1002/14651858.CD012608.pub3 (2024).
5. Patel, H. & Jepsen, J. Gallstone disease: common questions and answers. *Am. Fam Physician*. **109** (6), 518–524 (2024).
6. Unalp-Arida, A. & Ruhl, C. E. Increasing gallstone disease prevalence and associations with gallbladder and biliary tract mortality in the US. *Hepatology* **77** (6), 1882–1895. https://doi.org/10.1097/HEP.0000000000000264 (2023).
7. Chen, L. et al. Insights into modifiable risk factors of cholelithiasis: A Mendelian randomization study. *Hepatology* **75** (4), 785–796. https://doi.org/10.1002/hep.32183 (2022).
8. Zhang, M. et al. Independent association of general and central adiposity with risk of gallstone disease: observational and genetic analyses. *Front. Endocrinol. (Lausanne)*. **15**, 1367229. https://doi.org/10.3389/fendo.2024.1367229 (2024).
9. Li, J. et al. Abdominal obesity mediates the causal relationship between depression and the risk of gallstone disease: retrospective cohort study and Mendelian randomization analyses. *J. Psychosom. Res.* **174**, 111474. https://doi.org/10.1016/j.jpsychores.2023.111474 (2023).
10. Aune, D., Norat, T. & Vatten, L. J. Body mass index, abdominal fatness and the risk of gallbladder disease. *Eur. J. Epidemiol.* **30** (9), 1009–1019. https://doi.org/10.1007/s10654-015-0081-y (2015).
11. Woolcott, O. O. & Seuring, T. Temporal trends in obesity defined by the relative fat mass (RFM) index among adults in the united States from 1999 to 2020: a population-based study. *BMJ Open.* **13** (8), e071295. https://doi.org/10.1136/bmjopen-2022-071295 (2023).
12. Cao, C. et al. The nonlinear connection between relative fat mass and non-alcoholic fatty liver disease in the Japanese population: an analysis based on data from a cross-sectional study. *Diabetol. Metab. Syndr.* **16** (1), 236. https://doi.org/10.1186/s13098-024-01472-z (2024).
13. Zhao, L., Cao, R. & Zhang, S. Association between relative fat mass and periodontitis: results from NHANES 2009–2014. *Sci. Rep.* **14** (1), 18251. https://doi.org/10.1038/s41598-024-69048-5 (2024).
14. Wang, D. et al. Association between two novel anthropometric measures and type 2 diabetes in a Chinese population. *Diabetes Obes. Metab.* **26** (8), 3238–3247. https://doi.org/10.1111/dom.15651 (2024).
15. Wang, J. et al. Sex differences in the associations between relative fat mass and all-cause and cardiovascular mortality: A population-based prospective cohort study. *Nutr. Metab. Cardiovasc. Dis.* **34** (3), 738–754. https://doi.org/10.1016/j.numecd.2023.10.034 (2024).

16. Woolcott, O. O. & Bergman, R. N. Relative fat mass (RFM) as a new estimator of whole-body fat percentage A cross-sectional study in American adult individuals. *Sci. Rep.* **8** (1), 10980. https://doi.org/10.1038/s41598-018-29362-1 (2018).
17. Castro-Torres, I. G. et al. Future therapeutic targets for the treatment and prevention of cholesterol gallstones. *Eur. J. Pharmacol.* **765**, 366–374. https://doi.org/10.1016/j.ejphar.2015.08.045 (2015).
18. Fujita, N. et al. Evidence-based clinical practice guidelines for cholelithiasis 2021. *J. Gastroenterol.* **58** (9), 801–833. https://doi.org/10.1007/s00535-023-02014-6 (2023).
19. Jackson, S. S., Graubard, B. I., Gabbi, C. & Koshiol, J. Association with menopausal hormone therapy and asymptomatic gallstones in US women in the third National health and nutrition examination study. *Sci. Rep.* **14** (1), 191. https://doi.org/10.1038/s41598-023-50509-2 (2024).
20. Wang, H. H. et al. Activation of Estrogen receptor G Protein-Coupled receptor 30 enhances cholesterol cholelithogenesis in female mice. *Hepatology* **72** (6), 2077–2089. https://doi.org/10.1002/hep.31212 (2020).
21. de Bari, O., Wang, T. Y., Liu, M., Portincasa, P. & Wang, D. Q. Estrogen induces two distinct cholesterol crystallization pathways by activating ERα and GPR30 in female mice. *J. Lipid Res.* **56** (9), 1691–1700. https://doi.org/10.1194/jlr.M059121 (2015).
22. Shaffer, E. A. Gallstone disease: epidemiology of gallbladder stone disease. *Best Pract. Res. Clin. Gastroenterol.* **20** (6), 981–996. https://doi.org/10.1016/j.bpg.2006.05.004 (2006).
23. Wang, H. H., Portincasa, P. & Wang, D. Q. Molecular pathophysiology and physical chemistry of cholesterol gallstones. *Front. Biosci.* **13**, 401–423. https://doi.org/10.2741/2688 (2008).
24. Misciagna, G. et al. Epidemiology of cholelithiasis in Southern Italy. Part II: risk factors. *Eur. J. Gastroenterol. Hepatol.* **8** (6), 585–593. https://doi.org/10.1097/00042737-199606000-00017 (1996).
25. Ke, B., Sun, Y., Dai, X., Gui, Y. & Chen, S. Relationship between weight-adjusted waist circumference index and prevalence of gallstones in U.S. Adults: a study based on the NHANES 2017–2020. Front endocrinol (Lausanne). **14**:1276465. (2023). https://doi.org/10.3389/fendo.2023.1276465
26. Cruz-Monserrate, Z., Conwell, D. L. & Krishna, S. G. The impact of obesity on gallstone disease, acute pancreatitis, and pancreatic cancer. *Gastroenterol. Clin. North. Am.* **45** (4), 625–637. https://doi.org/10.1016/j.gtc.2016.07.010 (2016).
27. Lammert, F. et al. *Gallstones Nat. Rev. Dis. Primers* **2**:16024. doi: https://doi.org/10.1038/nrdp.2016.24 (2016).
28. Di Ciaula, A., Wang, D. Q. & Portincasa, P. Gallbladder and gastric motility in obese newborns, pre-adolescents and adults. *J. Gastroenterol. Hepatol.* **27** (8), 1298–1305. https://doi.org/10.1111/j.1440-1746.2012.07149.x (2012).
29. Wang, H. H., Liu, M., Portincasa, P., Tso, P. & Wang, D. Q. Lack of endogenous cholecystokinin promotes cholelithogenesis in mice. *Neurogastroenterol Motil.* **28** (3), 364–375. https://doi.org/10.1111/nmo.12734 (2016).
30. Dai, D. et al. Association of MTTP gene variants with pediatric NAFLD: A candidate-gene-based analysis of single nucleotide variations in obese children. *PLoS One.* **12** (9), e0185396. https://doi.org/10.1371/journal.pone.0185396 (2017).
31. Duan, Y. et al. Hepatic cholesterol accumulation ascribed to the activation of ileum Fxr-Fgf15 pathway inhibiting hepatic Cyp7a1 in high-fat diet-induced obesity rats. *Life Sci.* **232**, 116638. https://doi.org/10.1016/j.lfs.2019.116638 (2019).
32. Cortés, V. A., Barrera, F. & Nervi, F. Pathophysiological connections between gallstone disease, insulin resistance, and obesity. *Obes. Rev.* **21** (4), e12983. https://doi.org/10.1111/obr.12983 (2020).
33. Parra-Landazury, N. M., Cordova-Gallardo, J. & Méndez-Sánchez, N. *Obes. Gallstones Visc. Med.* **37**(5):394–402. doi: https://doi.org/10.1159/000515545 (2021).
34. Di Ciaula, A., Wang, D. Q. & Portincasa, P. Cholesterol cholelithiasis: part of a systemic metabolic disease, prone to primary prevention. *Expert Rev. Gastroenterol. Hepatol.* **13** (2), 157–171. https://doi.org/10.1080/17474124.2019.1549988 (2019).
35. Chen, J., Liu, Z. T., Lyu, J. T. & Jiang, G. P. Impact of metabolic disorders on gallstone disease and perioperative recovery after laparoscopic cholecystectomy. Hepatobiliary Pancreat Dis Int. S1499-3872(24)00106-1. (2024). https://doi.org/10.1016/j.hbpd.2024.08.001
36. Wang, H. H., Portincasa, P., Mendez-Sanchez, N., Uribe, M. & Wang, D. Q. Effect of Ezetimibe on the prevention and dissolution of cholesterol gallstones. *Gastroenterology* **134** (7), 2101–2110. https://doi.org/10.1053/j.gastro.2008.03.011 (2008).
37. Maurer, K. J., Carey, M. C. & Fox, J. G. Roles of infection, inflammation, and the immune system in cholesterol gallstone formation. *Gastroenterology* **136** (2), 425–440. https://doi.org/10.1053/j.gastro.2008.12.031 (2009).
38. Van Erpecum, K. J. Pathogenesis of cholesterol and pigment gallstones: an update. *Clin. Res. Hepatol. Gastroenterol.* **35** (4), 281–287. https://doi.org/10.1016/j.clinre.2011.01.009 (2011).
39. Nakeeb, A. et al. Gallstones: genetics versus environment. *Ann. Surg.* **235** (6), 842–849. https://doi.org/10.1097/00000658-200206000-00012 (2002).
40. Pi, Y. et al. Interactions between gut microbiota and natural bioactive polysaccharides in metabolic diseases. *Rev. Nutrients.* **16** (17), 2838. https://doi.org/10.3390/nu16172838 (2024).
41. Molinero, N., Ruiz, L., Sánchez, B., Margolles, A. & Delgado, S. Intestinal bacteria interplay with bile and cholesterol metabolism: implications on host physiology. *Front. Physiol.* **10**, 185. https://doi.org/10.3389/fphys.2019.00185 (2019).
42. Acevedo-Román, A., Pagán-Zayas, N., Velázquez-Rivera, L. I., Torres-Ventura, A. C. & Godoy-Vitorino, F. Insights into gut dysbiosis: inflammatory diseases, obesity, and restoration approaches. *Int. J. Mol. Sci.* **25** (17), 9715. https://doi.org/10.3390/ijms25179715 (2024).
43. Liu, X., Qi, X., Han, R., Mao, T. & Tian, Z. Gut microbiota causally affects cholelithiasis: a two-sample Mendelian randomization study. *Front. Cell. Infect. Microbiol.* **13**, 1253447. https://doi.org/10.3389/fcimb.2023.1253447 (2023).

Acknowledgements

Author contributions

Funding

Declarations

Competing interests
The authors declare no competing interests.

Ethics approval and consent to participate
NHANES are publicly available, and all participants have provided informed consent. The database adheres to protocols for de-identification and anonymity, thus obviating the requirement for investigators to duplicate ethical approval or informed consent procedures.

Additional information
Supplementary Information The online version contains supplementary material available at https://doi.org/10.1038/s41598-025-93963-w.

Correspondence and requests for materials should be addressed to X.Z., Y.H. or D.W.

Reprints and permissions information is available at www.nature.com/reprints.

Publisher's note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.