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Association between serum iron and gallstones in US adults: a cross-sectional study

Si-Hua Wen[1], Xin Tang[2], Tao Tang[1] and Zheng-Rong Ye[3*]

Abstract

Background Gallstones are a common digestive disorder that threatens human health. Iron deficiency may be related to the formation of gallstones, but there is limited current epidemiological research. The objective of this study was to investigate the relationship between iron status and gallstones.

Methods The datasets from the National Health and Nutrition Examination Survey (NHANES) 2017–2020 were used in a cross-sectional investigation. Gallstones were determined by using the 2007–2010 NHANES questionnaire. Multivariate linear regression models were used to examine the association between serum iron, serum ferritin and iron intake with the risk for gallstones. Subgroup analysis based on gender, age, race, and diabetes were performed. Fitted smoothing curves were used to describe the linear relationship.

Results The research involved 7847 participants aged 20 and above, among whom 845 were identified as having gallstones. Participants with higher serum iron levels tended to have a lower gallstones prevalence. A negative relationship between serum iron and gallstones prevalence was observed (OR = 0.979, 95% CI:0.965–0.992). The group with the highest serum iron tertile had a 23.7% lower risk of gallstones compared to the lowest tertile (OR = 0.763, 95% CI:0.628–0.929). Gallstone prevalence was inversely correlated with iron intake in model 1. The negative association between serum iron and gallstones remained stable in stratifications, including gender, age, race, and diabetes.

Conclusions Elevated serum iron was associated with a decreased prevalence of gallstones. However, to confirm the impact of long-term iron metabolism on gallstone formation, additional prospective research is necessary.

Keywords Iron, Gallstones, Trace element, Cross-sectional study, National Health and Nutrition Examination Survey

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Introduction

Gallstones are considered one of the most common digestive system disorders [1]. Approximately 25% of gallstone patients develop biliary colic or more severe complications [2], contributing to a growing economic burden year by year [3]. Large-scale epidemiological studies have been conducted, revealing that around 10–14% of adults in the U.S. su er from gallstones [4, 5]. Gallstones are solid particles formed in the gallbladder, primarily composed of cholesterol, bilirubin, calcium carbonate, calcium phosphate, and trace elements [6, 7]. e onset of gallstones is associated with various factors, with several risk factors including aging, female gender, diabetes, rapid weight loss, metabolic syndrome, and gallbladder stasis [8–10]. e etiology of gallstone formation remains incompletely elucidated, possibly arising from the intricate interplay of various factors, including genetics, environmental in uences, and lifestyle [11]. Surgical removal of gallbladder is currently the only solution for gallstones disease. Given the large population a ected by gallbladder stones, nding e cient non-surgical treatment methods makes sense.

Iron, a trace element in the human body, is recognized as a crucial nutrient for maintaining human health. It serves as an indispensable cofactor in multiple critical cellular processes, including cellular respiration, immune response, lipid metabolism, gene regulation, and DNA synthesis [12, 13]. Excess of iron may induce certain diseases, such as cognitive impairments, atherosclerosis, and diabetes [14–16]. e binding of metal ions with bile salts and bilirubin plays a crucial role in the formation of gallstones. Fe^{3+} as one of the paramagnetic centers in pigment gallstones in uences the formation of bilirubin coordination polymers [17]. e main sources of iron in the body are the recycling of damaged erythrocytes and dietary iron [18]. From a pathophysiological point of view, both iron de ciency and excess have signi cant consequences [19, 20]. Heme iron from red meat has high bioavailability and is an important source of dietary iron; however, this high-iron diet tends to increase the risk of gallstones in men [21]. Iron a ects the homeostasis of bile ow and bile components by altering the activity of hepatic enzymes that regulate cholesterol and bile salt levels [22]. It is clear that decreased iron levels in the liver are linked to chronic liver diseases connected to cholestasis [23, 24]. e previous research focused on the incidence of gallstones in individuals su ering from iron-de ciency anemia [25]. However, few studies investigated the relationship of iron with gallstones in adults.

e objective of this study was to investigate the correlations of serum iron, serum ferritin, and iron intake with gallstones in a sample representative of the entire nation.

e dataset was sourced from the National Health and Nutrition Examination Survey (NHANES).

Methods

Survey description

e data in the study were all sourced from NHANES.

e sample for the survey is selected to represent the U.S. population of all ages. Annually, 5000 participants distributed across various states nationwide were selected for health surveys. ese individuals are spread across various states nationwide, with 15 states being visited each year. Due to the COVID-19 pandemic, the survey was temporarily suspended in early 2020. is survey includes health interviews and measurements at health screening centers, ensuring that health-related aspects are assessed. e survey is conducted annually as part of routine procedures, and the data obtained are continuous over time. We included data from 2017 to 2020 because it was during this period that the survey on gallstones was conducted. All NHANES study protocols were approved by the Ethics Review Board. All survey participants in this study were over 20 years old and had signed written informed consent.

Study population

We collected data from 2017 to 2020 and included participants who answered whether they had gallstones.

e analysis comprised 15,560 people in total, of whom information regarding gallstones (n=9210) was available.

ose participants with missing serum iron, serum ferritin and iron intake data were excluded (n=1326). Missing data for covariates were also excluded (total, n=37; diabetes, n=4; hypertension, n=12; education level, n=11; marital status, n=5; smoking status, n=5). e study eventually included 7847 participants. (Fig. 1).

Measurement of serum iron, serum ferritin and iron intake

In this study, serum iron, serum ferritin and iron intake were analyzed as continuous variables. Participants ultimately included in the study were invited to the Mobile Examination Center (MEC) for measurements. Blood specimen collection was performed by professional medical personnel and stored in an environment at -30 °C. Subsequently, all samples were tested for serum iron (μmol/L) concentration using the Roche method.

e concentration of ferritin(μg/L) was measured by the Roche/Hitachi immunoturbidity assay. Detailed information on specimen processing methods is presented in the NHANES 2017–2020 Laboratory Procedures Manuals (LPMs). A comprehensive description of the entire operational process is accessible at https://wwwn.cdc.gov/Nchs/Nhanes/2017-2018/P_FETIB.htm. Dietary iron intake data were obtained from questionnaires. Participants had two 24-hour dietary records: e initial dietary recall interview was conducted in the MEC, followed by a second interview conducted via telephone 3 to 10

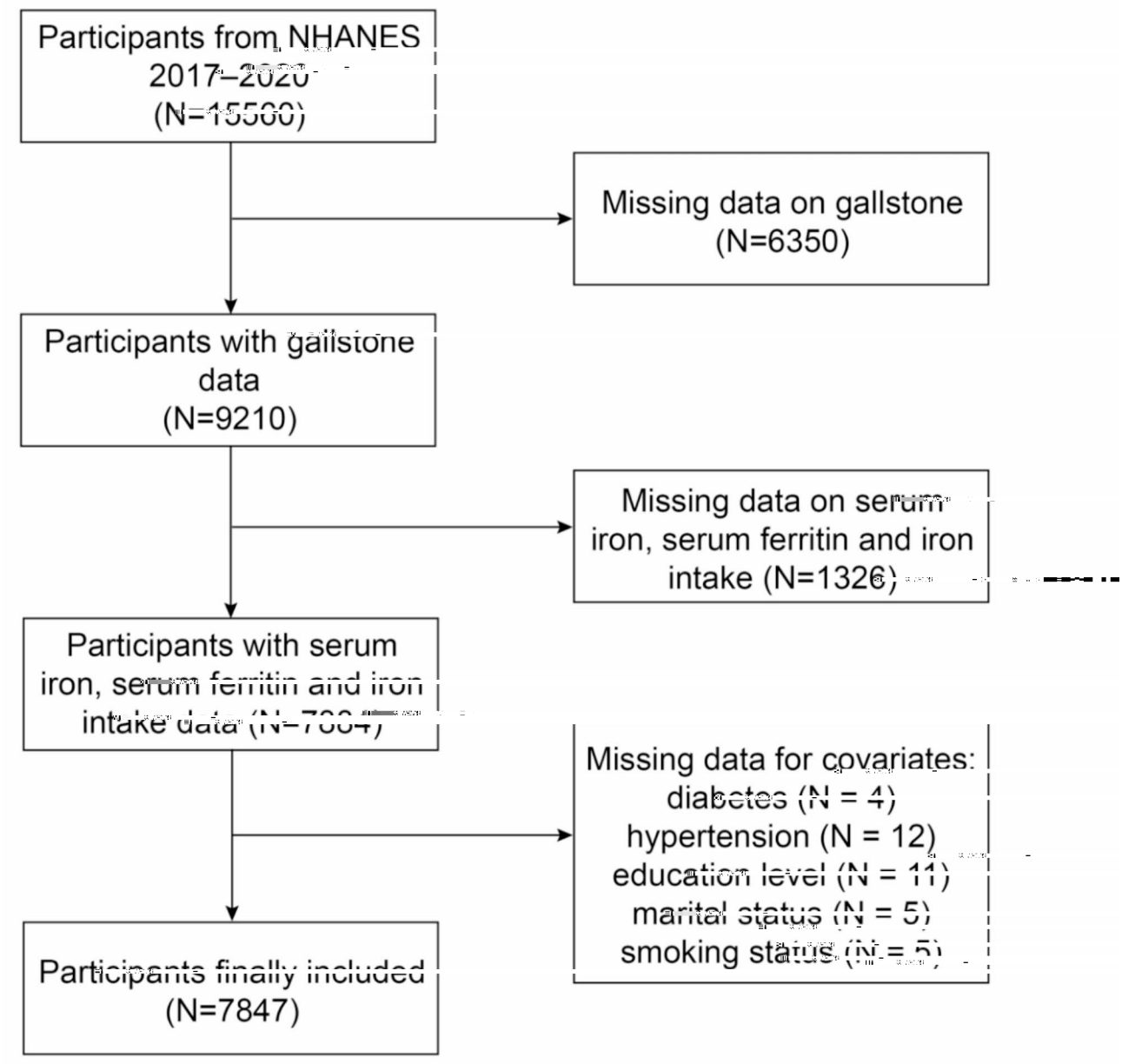

Fig. 1 Flowchart of the sample selection from NHANES 2017–2020

days later. We calculated the average of the two 24-hour records as the inclusion data to reduce bias.

Outcome de□nitions

e presence of gallstones was de ned as the outcome variable. Participants underwent a questionnaire survey to determine their gallstones status. e questionnaire survey was performed by trained interviewers using a computer-assisted interviewing system. Based on their prior visits to a physician or other healthcare provider, participants answered the question about whether they had gallstones. ose who refused to answer or responded with "uncertain" were also considered as having missing data.

Covariates

In this study, the authors included several potential confounders as covariates, including gender, age, race, education level, marital status, income-to-poverty ratio (PIR), body mass index (BMI), alcohol consumption, hypertension, diabetes, smoking status, and dietary intake information. Race was classi ed as Mexican-American, non-Hispanic white, non-Hispanic black, and other races. e history of diabetes or hypertension was ascertained through a questionnaire survey. e marital status

category comprised cohabitation and solitude. Participants who consumed alcohol at least once monthly were categorized as drinkers, whereas a person was considered a smoker if they had ever smoked 100 cigarettes or more. BMI data were collected as a continuous variable and later transformed into a categorical variable with three groups. Dietary data were taken from a 24-hour dietary questionnaire and comprised energy (kcal), sugar (g), fat (g), carbohydrate (g), protein (g), and water (g). We computed the mean nutrient intake speci c to each participant for both the rst and second 24-hour periods. Dietary data were converted into categorical variables, using the 50th percentile of the sample size as the cut-o point to classify them into "low" and "high" groups. Missing data in the covariates were labeled as "unclear".

e speci c measurements of these variables are publicly available at NHANES.

Statistical analysis

Statistical analysis was performed using EmpowerStats 4.0. Continuous variables are presented as mean with standard deviation (SD), while categorical variables are represented as proportions. Considering several potential confounders, multiple logistic regression models were used to study the relationship between serum iron, serum ferritin and iron intake with the risk for gallstones. Serum iron levels were converted from a continuous variable to a categorical variable (tertile), and a trend test was used to examine the linear association trend. ree models were used to construct the multivariate test: Model 1 did not include any variables; Model 2 included adjustments for gender, age, race, marital status, PIR, and educational attainment; and Model 3 included adjustments for all covariates. Fitted smoothing curves were performed to examine linear relationship. Subgroup analysis was utilized to test the association between serum iron and gallstones in patients with di erent gender, age, race, and diabetes status. $P<0.05$ was considered statistically signi cant.

Results

Baseline characteristics of participants

e study included a total of 7,847 participants. Table 1 shown the baseline characteristics of the participants. 845 participants in the study reported with gallstones. Serum iron level was 13.98 (8.29, 19.67) in the group of participants with gallstones, compared to 15.44 (8.96, 21.92) in the group without gallstones ($P<0.001$). Dietary iron intake was signi cantly lower in patients with gallstones than in controls ($P=0.002$). e level of serum ferritin is not signi cantly associated with the presence of gallstones ($P=0.386$). In addition, between the two groups of individuals with and without gallstones, there were no signi cant di erences in PIR, education level, marital status, carbohydrate intake and sugar intake ($P>0.05$).

Associations of serum iron, serum ferritin and iron intake with gallstones prevalence

Table 2 shows the association of serum iron, serum ferritin and iron intake with gallstones prevalence. Iron intake was negatively associated with the presence of gallstones in Model 1. A higher serum iron level was associated with decreased prevalence of gallstones in Model 1 (OR=0.961, 95% CI:0.949–0.973). Similarly, a consistent relationship between serum iron and gallstones remained in the fully adjusted model (OR=0.979, 95% CI:0.965–0.992). In model 3 that considered multiple confounders, an increase of one unit in serum iron corresponded to a 2.1% decrease in the likelihood of gallstones. After adjusting for covariates, neither dietary iron intake nor serum ferritin showed a signi cant association with the prevalence of gallstones (all $P>0.05$). Additionally, we found a tendency indicating that groups with higher serum iron levels had a lower risk of gallstones as we transformed serum iron to a trichotomies variable for sensitivity analysis (OR=0.763, 95% CI:0.628–0.929). Overall, a signi cant linear association between serum iron and prevalence of gallstones was presented in the smoothing curve (Fig. 2).

Subgroup analysis

To determine potential in uencing factors between serum iron and gallstones, we conducted subgroup analyses, with groups categorized by gender, race, and diabetes status. Table 3 presents the results of subgroup studies conducted to evaluate the impact of serum iron status on gallstones in various groups. A signi cant relationship between serum iron and the risk of gallstones was detected in females, Non-Hispanic White, and non-diabetes participants (OR=0.983, 0.978 and 0.975, respectively). is negative relationship between serum iron and gallstones was stronger in the non-diabetes participants. Furthermore, we showed that strati cations such as gender, age, race, and diabetes did not signi cantly alter the negative correlation between serum iron and the likelihood of gallstones (P for interaction >0.05).

Discussion

In the cross-sectional study involving 7847 participants, we observed a negative correlation between serum iron levels and the incidence of gallstones (OR=0.979, 95% CI:0.965–0.992, in Model 3). While iron intake was potentially negatively associated with the prevalence of gallstones, the stability of this correlation was a ected when considering the e ects of covariates. Across the board, we found a linear relationship between serum iron and gallstones, as indicated by the tted smoothing

Table 1 Baseline characteristics of participants

Characteristic	Non-stone formers ($n=7002$)	Stone formers ($n=845$)	*P*-value
Age (years)	50.03 ± 17.44	58.46 ± 15.8	< 0.001
Gender (%)			< 0.001
Male	50.76	28.52	
Female	49.24	71.48	
Race (%)			< 0.001
Mexican American	11.94	13.49	
Other Race	27.58	24.38	
Non-Hispanic White	34.27	42.84	
Non- Hispanic Black	26.21	19.29	
Education lever (%)			0.781
Less than high school	18.72	18.46	
High school	23.99	25.09	
More than high school	57.29	56.45	
Marital status (%)			0.508
Cohabitation	42.02	40.83	
Solitude	57.98	59.17	
PIR	2.61 ± 1.52	2.56 ± 1.47	0.347
BMI (kg/m2)	29.67 ± 7.2	33.34 ± 8.57	< 0.001
Alcohol consumption (%)			< 0.001
Yes	40.79	51.6	
No	45.17	32.78	
Unclear	14.04	15.62	
Smoked (%)			0.014
Yes	58.6	54.2	
No	41.4	45.8	
Diabetic (%)			< 0.001
Yes	82.92	71	
No	17.08	29	
Hypertension (%)			< 0.001
Yes	63.51	45.09	
No	36.49	54.91	
Total Energy (%)			< 0.001
Lower (< 2019.5 kcal)	38.49	46.15	
Higher (≥ 2019.5 kcal)	39.93	34.32	
Unclear	21.58	19.53	
Total Sugar (%)			0.296
Lower (< 93.5 g)	39.33	39.17	
Higher (≥ 93.5 g)	39.09	41.3	
Unclear	21.58	19.53	
Total Fat (%)			0.018
Lower (< 77 g)	38.77	43.79	
Higher (≥ 77 g)	39.65	36.68	
Unclear	21.58	19.53	
Total Water (%)			0.010
Lower (< 2527.8 g)	38.73	44.14	
Higher (≥ 2527.8 g)	39.69	36.33	
Unclear	21.58	19.53	
Total Protein (%)			< 0.001
Lower (< 72.48 g)	38.35	47.34	
Higher (≥ 72.48 g)	40.07	33.13	
Unclear	21.58	19.53	
Total Carbohydrate (%)			0.065

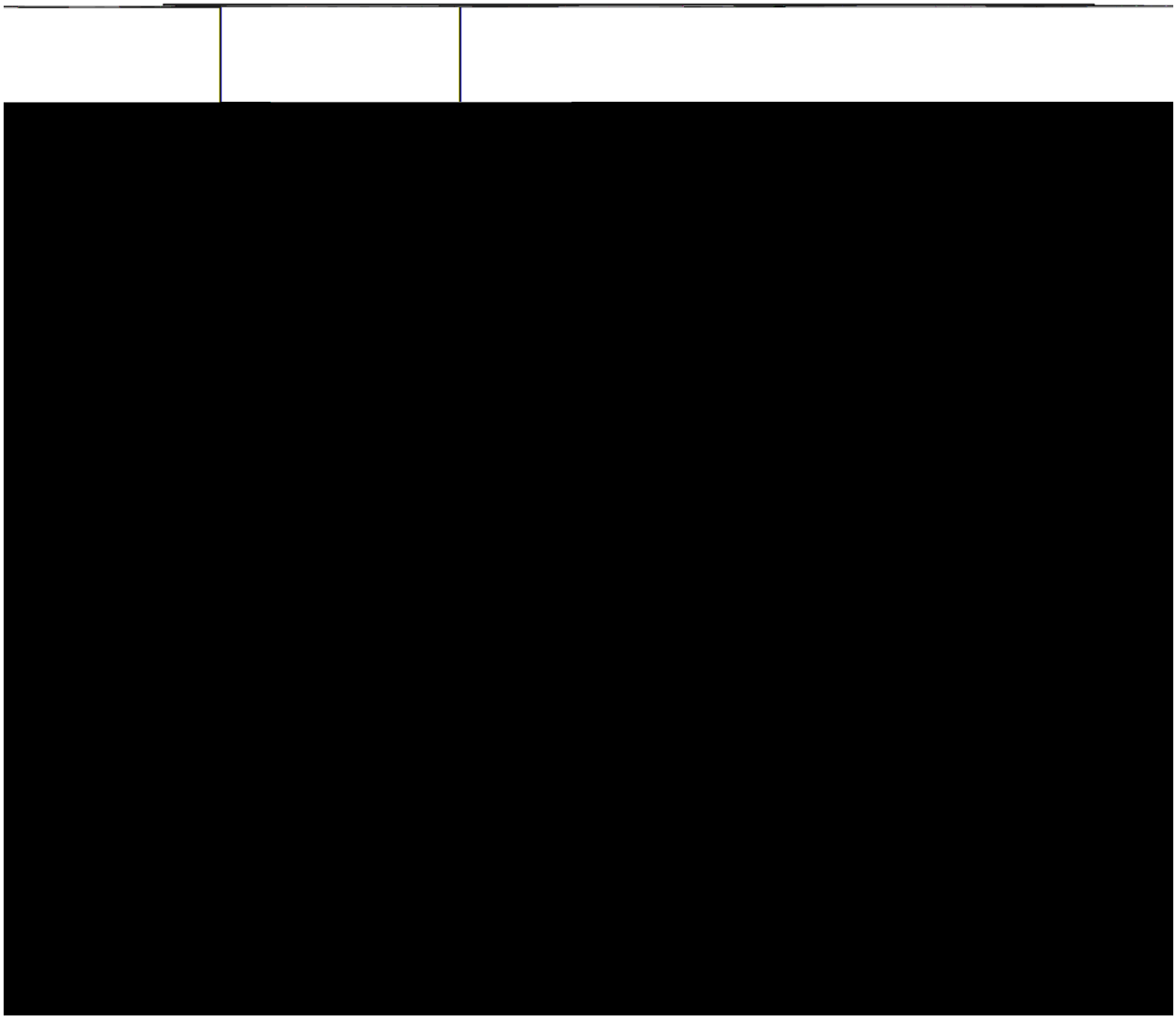

Fig. 2 Density dose-response relationship between serum iron with gallstone prevalence. 95% con□dence interval (CI) is displayed for the region between the upper and lower dashed lines. All covariates were adjusted

therapeutic interventions targeting iron metabolism for the prevention and management of gallstone disease.

It is noteworthy that in current research, we observed a signi cant di erence in the relationship of serum iron with gallstones between genders. ere is a negative correlation between serum iron and gallstones among females, but not among males. In a previous study, it was observed that female patients with cholesterol gallstones revealed lower serum iron levels compared to the control group, while male patients exhibited no signi cant di erence [37]. Results from previous studies indicate a higher incidence of gallstones in females [4, 38], which is consistent with the demographic characteristics we have demonstrated. In general, this is attributed to di erences in sex hormone levels. Estrogen promotes the excretion of cholesterol in bile by upregulating the expression of the 3-hydroxy-3-methylglutaryl (HMG)-CoA gene, thus promoting the formation of cholesterol crystals [39, 40]. Premenopausal women are more likely to experience iron-de ciency anemia compared to males [41, 42]. Given this susceptibility, the e ects of iron de ciency on gallstone formation can be more noticeable in females. e mechanisms underlying gender di erences merit further investigation.

Dietary factors are one of the contributing mechanisms to the pathogenesis of gallstone disease. Excessive intake

Table 3 Subgroup analysis between serum iron with gallstone prevalence

Characteristic	Model 1 OR (95% CI)	Model 2 OR (95% CI)	Model 3 OR (95% CI)	*P* for interaction
Strati□ed by gender				0.7398
Male	0.966 (0.944–0.988)	0.968 (0.946–0.990)	0.978 (0.956–1.001)	
Female	0.980 (0.966–0.995)	0.969 (0.954–0.985)	0.983 (0.967–0.999)	
Strati□ed by age (years)				0.8433
< 40	0.946 (0.919–0.975)	0.962 (0.934–0.990)	0.976 (0.947–1.005)	
≥ 40, < 60	0.961 (0.940–0.982)	0.974 (0.953–0.995)	0.986 (0.965–1.008)	
≥ 60	0.961 (0.943–0.980)	0.967 (0.948–0.986)	0.981 (0.961–1.001)	
Strati□ed by race				0.6822
Mexican American	0.942 (0.912–0.973)	0.959 (0.928–0.991)	0.968 (0.935–1.001)	
Other Race	0.966 (0.943–0.990)	0.977 (0.953–1.001)	0.988 (0.964–1.013)	
Non-Hispanic White	0.953 (0.934–0.972)	0.964 (0.944–0.984)	0.978 (0.957–0.999)	
Non- Hispanic Black	0.964 (0.935–0.992)	0.976 (0.947–1.007)	0.992 (0.962–1.022)	
Strati□ed by diabetes				0.1122
Yes	0.981 (0.957–1.006)	0.991 (0.966–1.016)	0.980 (0.967–1.025)	
No	0.959 (0.946–0.973)	0.966 (0.952–0.981)	0.975 (0.960–0.991)	

Gender, age, race, education level, marital status, income-to-poverty ratio, body mass index, alcohol consumption, hypertension, diabetes, smoking status, energy intake, fat intake, sugar intake, carbohydrate intake, protein intake, and water intake were adjusted

of certain essential nutrients, including energy, fructose, and saturated fat, has been proposed to promote the development of gallstones [43]. In contrast, a high intake of monounsaturated fats and ber, along with the consumption of plant-based proteins, and vitamin C supplementation, has been shown to o er protective bene ts [44]. Although there is limited research on the impact of trace elements on gallstones, it holds signi cant clinical relevance. A large-scale cohort study indicated that magnesium intake has an independent protective e ect against gallstone disease [45]. A previous study indicated that dietary iron supplementation may potentially prevent the formation of gallstones [46]. However, results from a cohort study involving 44,758 American males showed an association between higher heme iron intake and an elevated risk of gallstones [21]. A typical westernized diet, characterized by high calorie and red meat consumption, is often accompanied by higher levels of saturated fat and cholesterol intake, which may increase the risk of gallstones [47]. On the other hand, the iron content of non-heme iron foods should not be underestimated. Spinach and legumes are rich in iron and also provide dietary ber, while nuts supply non-heme iron along with monounsaturated fat. Regrettably, our study did not di erentiate between sources of dietary iron, which may have a ected the results. Certain foods, such as red meat, are rich in both iron and saturated fats, making it di cult to exclude the in uence of fat intake on the ndings. In our present study, those with gallstones consumed substantially less dietary iron than the control group ($P=0.002$); however, this association became unstable when accounting for confounding factors. erefore, diet could potentially play a role in the primary prevention of gallstones.

e intestinal iron concentration, altered by dietary iron intake, may impact the availability of bacterial iron, subsequently in uencing the growth of symbiotic and pathogenic microorganisms [48]. Alteration of indigenous gut microbiota is a risk factor for gallstone formation [49]. Compared to healthy individuals, patients with gallstones show signi cant changes in the composition of their gut microbiota, characterized by an overgrowth of the phylum *Proteobacteria* [50]. Given the biological mechanisms connecting iron to gallstone formation, there is growing interest in exploring the potential of iron supplementation as a preventive strategy for gallstones. Recent research supports this idea. A study conducted in a lithogenic diet-induced rat model of cholelithiasis indicated that the supernatant of nanoiron sul de exhibited excellent antibacterial activity and had the e ect of increasing cholesterol solubility in the gallbladder [51].

is suggests that iron-based interventions could potentially o er a novel approach to gallstone prevention, highlighting the need for further investigation into the mechanisms by which iron a ects gallstone formation and the role of gut microbiota in this process.

In terms of lowering the prevalence of gallbladder stones, the study is instructive due to the sample size included in the study. It is more cost-e ective to manage the condition early with dietary modi cations. is study encompassed a multiracial and diverse dietary population of adults in the United States, and the results of analysis are nationally representative. In addition, the sample size was su cient for us to compare population di erences in subgroup analysis. However, some limitations in this study cannot be ignored. Firstly, causality could not be established because the study was a cross-sectional analysis. Additionally, the nutritional composition of common foods is intricate, making it challenging

to completely exclude the potential interference of various nutrients on the results, therefore the conclusion should be interpreted cautiously. irdly, single measurements of serum iron and iron intake do not re ect long-term iron metabolism.

Conclusion

e present study revealed that elevated serum iron was associated with a decreased prevalence of gallstones. We realized that iron de ciency may a ect liver cholesterol metabolism, contributing to the formation of gallstones. Furthermore, we found a negative association between dietary iron and gallstone prevalence, but this result is in uenced by confounding factors and needs to be con rmed by further research. However, to con rm the impact of long-term iron metabolism on gallstone formation, additional prospective research is necessary.

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Author contributions
SW and ZY designed the research. SW and XT collected, analyzed the data, and drafted the manuscript. SW and TT revised the manuscript. All authors contributed to the article and approved the submitted version.

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Data availability
All data in this study is available from NHANES database (www.cdc.gov/nchs/nhanes).

Declarations

Ethics approval and consent to participate
The studies involving human participants were reviewed and approved by the National Center for Health Statistics Institutional Research Ethics Review Board. The patients/participants provided their written informed consent to participate in this study. All our methods followed the guidelines of the Helsinki Declaration. And secondary analysis does not require additional institutional review committee approval.

Competing interests
The authors declare no competing interests.

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