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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. suffer 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]. The 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]. The etiology of gallstone formation remains incompletely elucidated, possibly arising from the intricate interplay of various factors, including genetics, environmental influences, and lifestyle [11]. Surgical removal of gallbladder is currently the only solution for gallstones disease. Given the large population affected by gallbladder stones, finding efficient 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]. The 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 influences the formation of bilirubin coordination polymers [17]. The 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 deficiency and excess have significant 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 affects the homeostasis of bile flow 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]. The previous research focused on the incidence of gallstones in individuals suffering from iron-deficiency anemia [25]. However, few studies investigated the relationship of iron with gallstones in adults. The 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. The dataset was sourced from the National Health and Nutrition Examination Survey (NHANES).

Methods

Survey description

The data in the study were all sourced from NHANES. The 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. These 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. This survey includes health interviews and measurements at health screening centers, ensuring that health-related aspects are assessed. The 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. The analysis comprised 15,560 people in total, of whom information regarding gallstones ($n=9210$) was available. Those 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, $n=5$). The 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. The 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: The initial dietary recall interview was conducted in the MEC, followed by a second interview conducted via telephone 3 to 10

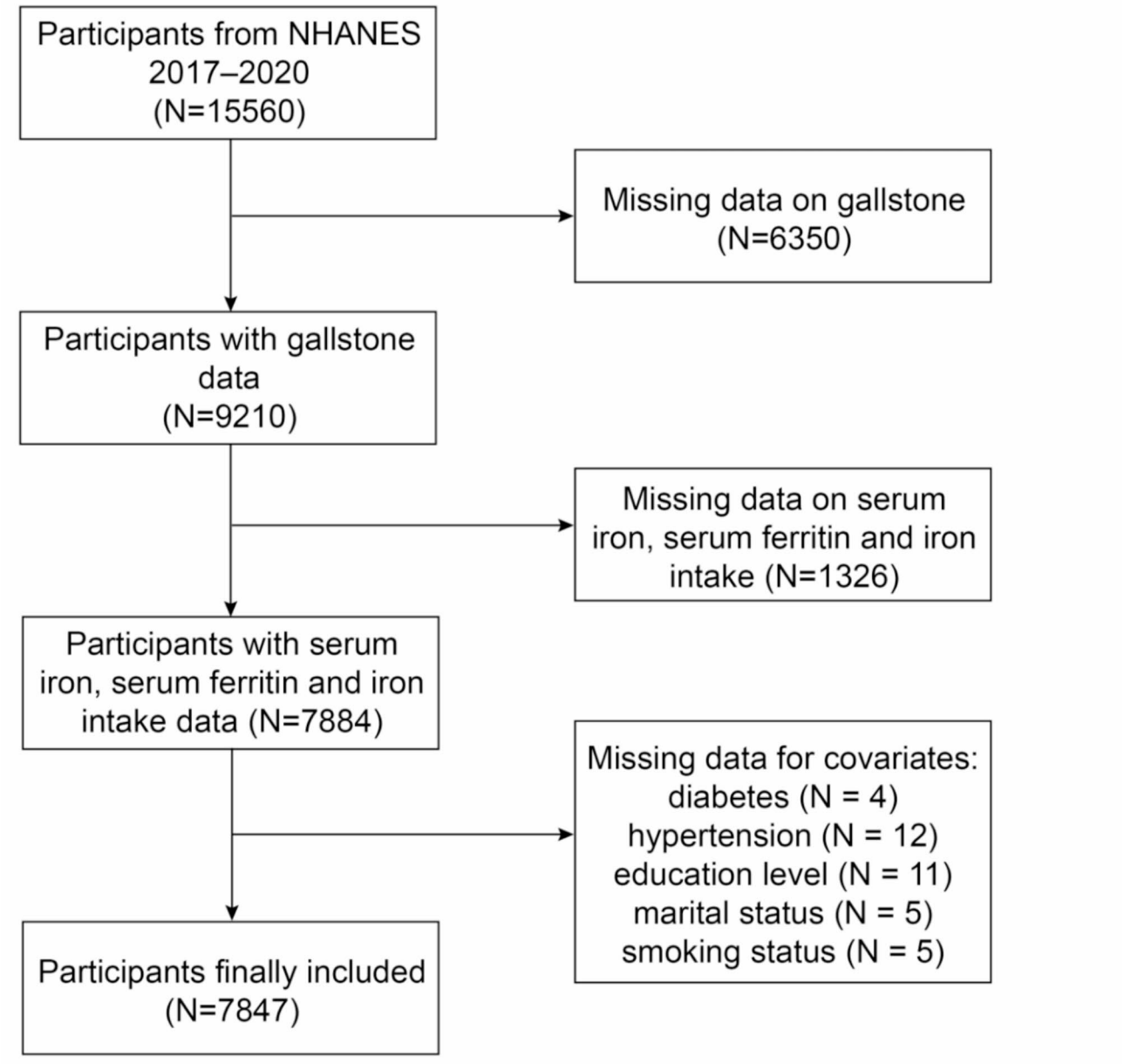

Fig. 1 Flowchart of the sample selection from NHANES 2017–2020

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

Outcome definitions

The presence of gallstones was defined as the outcome variable. Participants underwent a questionnaire survey to determine their gallstones status. The 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. Those 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 classified as Mexican-American, non-Hispanic white, non-Hispanic black, and other races. The history of diabetes or hypertension was ascertained through a questionnaire survey. The 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 specific to each participant for both the first and second 24-hour periods. Dietary data were converted into categorical variables, using the 50th percentile of the sample size as the cut-off point to classify them into "low" and "high" groups. Missing data in the covariates were labeled as "unclear".

The specific 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. Three 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 different gender, age, race, and diabetes status. $P<0.05$ was considered statistically significant.

Results

Baseline characteristics of participants

The 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 significantly lower in patients with gallstones than in controls ($P=0.002$). The level of serum ferritin is not significantly associated with the presence of gallstones ($P=0.386$). In addition, between the two groups of individuals with and without gallstones, there were no significant differences 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 significant 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 significant linear association between serum iron and prevalence of gallstones was presented in the smoothing curve (Fig. 2).

Subgroup analysis

To determine potential influencing 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 significant 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). This negative relationship between serum iron and gallstones was stronger in the non-diabetes participants. Furthermore, we showed that stratifications such as gender, age, race, and diabetes did not significantly 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 affected when considering the effects of covariates. Across the board, we found a linear relationship between serum iron and gallstones, as indicated by the fitted 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

Table 1 (continued)

Characteristic	Non-stone formers (*n* = 7002)	Stone formers (*n* = 845)	*P*-value
Lower (< 220.4 g)	38.87	42.96	
Higher (≥ 220.4 g)	39.55	37.51	
Unclear	21.58	19.53	
Serum ferritin (μg/L)	159.95 ± 186.97	153.95 ± 213.94	0.386
Iron intake (mg)	13.4 ± 6.05	12.74 ± 5.46	0.002
Serum iron (μmol/L)	15.44 ± 6.48	13.98 ± 5.69	< 0.001

Mean ± SD for continuous variables: *P* value was calculated by weighted linear regression model

N% for categorical variables: *P* value was calculated by weighted chi-square test

PIR, income-to-poverty ratio; BMI, body mass index

Table 2 Logistic regression analysis between serum iron, serum ferritin and iron intake with gallstones prevalence

	Model 1 OR (95% CI)	Model 2 OR (95% CI)	Model 3 OR (95% CI)
Serum iron level (μmol/L)			
Continuous	0.961 (0.949–0.973)	0.969 (0.956–0.982)	0.979 (0.965–0.992)
Categories			
Tertile 1 (< 12.2)	1.0	1.0	1.0
Tertile 2 (≥ 12.2, < 17.2)	0.803 (0.680–0.949)	0.817 (0.687–0.972)	0.881 (0.738–1.052)
Tertile 3 (≥ 17.2)	0.566 (0.472–0.679)	0.636 (0.526–0.769)	0.763 (0.628–0.929)
***P*for trend**	0.951 (0.936–0.966)	0.961 (0.945–0.977)	0.977 (0.960–0.994)
Serum ferritin level (μg/L)	1.000 (0.999–1.000)	1.000 (1.000–1.001)	1.000 (1.000–1.001)
Iron intake level (mg)	0.980 (0.967–0.993)	0.998 (0.985–1.012)	0.999 (0.982–1.016)

Model 1: no covariates were adjusted

Model 2: gender, age, race, marital status, income-to-poverty ratio, and education level were adjusted

Model 3: 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

curves. The results of interaction tests for each subgroup indicated that within-group differences were not statistically significant, suggesting that the relationship between serum iron and gallstones was stable across various populations. This is the first big sample cross-sectional investigation on gallstones and iron measurements that we are aware of. These findings enhanced our understanding of the association between iron and gallstones.

The results of our study on the association between serum iron levels and the risk of gallstones align with findings from previous research studies [25–28]. In a controlled trial conducted in Turkey, a comparison of gallbladder status between patients with iron-deficiency anemia and non-anemic subjects revealed impaired gallbladder motility in patients with iron-deficiency anemia and an increased risk of gallstone formation [25]. The mechanism underlying the negative correlation between serum iron and gallstone formation remains unclear; however, several mechanisms have been proposed. In the context of hepatic lipid metabolism, the equilibrium between cholesterol and bile salt excretion within bile serves as a preventive mechanism against the formation of biliary crystals [29]. Iron deficiency has been shown to decrease cholesterol-7a-hydroxylase activity, resulting in a decrease in bile salt secretion and favoring cholesterol crystal formation [30]. A study conducted in rats revealed that iron deficiency promotes cholesterol supersaturation in bile, which may be linked to increased biliary cholesterol secretion via upregulation of the Abcg5/8 transporters [22]. Additionally, approximately one-third of patients with cholesterol gallstones exhibit an enlarged gallbladder and delayed emptying [31]. A previous study found that iron deficiency reduced gallbladder neuronal nitric oxide synthase, resulting in to biliary stasis [32], thus potentially elevating the risk of gallstone formation. As gallbladder motility disorders typically precede the onset of gallstones, they are considered a risk factor for the formation of gallstones [33, 34]. Beyond these proposed mechanisms, the interplay between iron metabolism and gallstone formation is complex and multifactorial. Factors such as oxidative stress, inflammation, and alterations in lipid metabolism may also contribute to the observed associations between serum iron levels and gallstone risk [35, 36]. Additionally, further research is warranted to elucidate the precise mechanisms linking iron status to gallstone formation and to explore potential

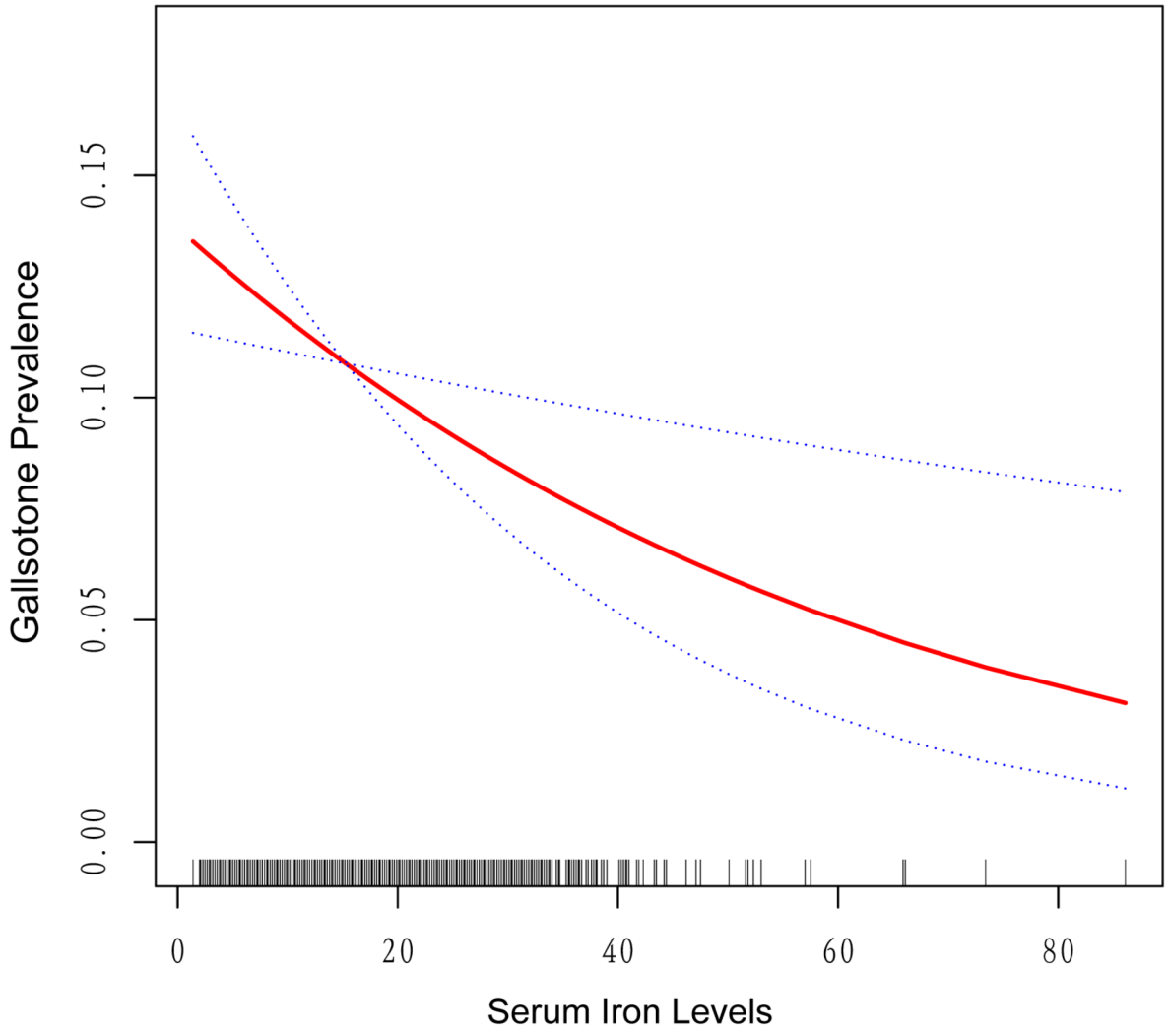

Fig. 2 Density dose-response relationship between serum iron with gallstone prevalence. 95% confidence interval (CI) is displayed for the region between the upper and lower dashed lines. All covariates were adjusted

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

It is noteworthy that in current research, we observed a significant difference in the relationship of serum iron with gallstones between genders. There 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 significant difference [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 differences 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-deficiency anemia compared to males [41, 42]. Given this susceptibility, the effects of iron deficiency on gallstone formation can be more noticeable in females. The mechanisms underlying gender differences 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
Stratified 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)	
Stratified 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)	
Stratified 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)	
Stratified 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 fiber, along with the consumption of plant-based proteins, and vitamin C supplementation, has been shown to offer protective benefits [44]. Although there is limited research on the impact of trace elements on gallstones, it holds significant clinical relevance. A large-scale cohort study indicated that magnesium intake has an independent protective effect 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 fiber, while nuts supply non-heme iron along with monounsaturated fat. Regrettably, our study did not differentiate between sources of dietary iron, which may have affected the results. Certain foods, such as red meat, are rich in both iron and saturated fats, making it difficult to exclude the influence of fat intake on the findings. 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. Therefore, diet could potentially play a role in the primary prevention of gallstones.

The intestinal iron concentration, altered by dietary iron intake, may impact the availability of bacterial iron, subsequently influencing 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 significant 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 sulfide exhibited excellent antibacterial activity and had the effect of increasing cholesterol solubility in the gallbladder [51]. This suggests that iron-based interventions could potentially offer a novel approach to gallstone prevention, highlighting the need for further investigation into the mechanisms by which iron affects 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-effective to manage the condition early with dietary modifications. This 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 sufficient for us to compare population differences 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. Thirdly, single measurements of serum iron and iron intake do not reflect long-term iron metabolism.

Conclusion

The present study revealed that elevated serum iron was associated with a decreased prevalence of gallstones. We realized that iron deficiency may affect 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 influenced by confounding factors and needs to be confirmed by further research. However, to confirm 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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