Association between barium exposure and non-alcoholic fatty liver disease in U.S. adults

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Abstract

Background and aims: **There are very limited studies that have investigated the influence of environmental barium exposure on non-alcoholic fatty liver disease (NAFLD) in the general adult population. The purpose of the present paper was to examine any correlation between urinary barium levels (UBLs) and the risk of NAFLD.**

Patients and methods: **A total of 4,556 participants aged ≥ 20 years were recruited from the National Health and Nutritional Survey. NAFLD was defined as the U.S. fatty liver index (USFLI) ≥ 30 in the absence of other chronic liver disease. Multivariate logistic regression was conducted to inspect the correlation between UBLs and the risk of NAFLD.**

Results: **The outcome of adjusting covariates revealed a positive correlation between the natural log -transformed UBLs (Ln-UBLs) and the risk of NAFLD (OR: 1.24, 95%CI: 1.12-1.37, P<0.001). After dividing Ln-UBLs into quartiles, the participants in the highest quartile exhibited a 1.65-fold (95% CI: 1.26-2.15) increased likelihood of having NAFLD in contrast with the bottom quartile in the full model, and a distinct trend across the quartiles could be found (P for trend<0.001). Moreover, in the interaction analyses, it was further observed that the association between Ln-UBLs and NAFLD was modified by gender and was noticeably more pronounced in males (P for interaction =0.003).**

Conclusions: **Our findings provided evidence of a positive correlation between UBLs and the prevalence of NAFLD. Furthermore, this association changed across gender and was more pronounced in males. Nevertheless, our finding requires further confirmation with prospective cohort studies in the future.** (Acta gastroenterol. belg., **2023,** 86, **298-305**).

Keywords: non-alcoholic fatty liver disease, NHANES, barium.

Introduction

Non-alcoholic fatty liver disease (NAFLD) is attributable to over-accumulation of hepatic lipids in individuals without any habit of alcohol over-consumption or other causative factors for secondary hepatic steatosis (1). In recent decades, NAFLD has rapidly become a major contributor to liver diseases globally, which affects more than one-third of the population. In the U.S., the prevalence of NAFLD is estimated to be around 25% (2) and is expected to rise to more than 100 million by 2030 (3). This rapid rise is particularly concerning as NAFLD not only frequently progresses into secondary complications including liver cirrhosis and hepatocellular carcinoma but also is the second leading reason for liver transplantation in the U.S. (4). In addition, NAFLD tightly correlates to major extrahepatic diseases, like colorectal cancer, cardiovascular disease, and chronic kidney disease, therefore elevating the risk of these diseases (5,6,7). The pathogenesis of NAFLD remains poorly elucidated till date. Lifestyle modifications and genetic factors are well-recognized risk factors for NAFLD (8). However, these traditional risk factors do not account for the sharp increase in prevalence of NAFLD. Recent investigations have revealed that environmental exposures can significantly contribute to the development and progression of NAFLD (9,10).

Barium, an alkaline earth metal, is the 14th most common element on the Earth's crust with the density of around 500 ppm. Barium compounds are applied in a wide spectrum of industrial processes, such as production of oil and drilling muds and manufacture of plastics, paints, rubber, cosmetics, and pharmaceuticals (11,12). In recent years, the public has developed an increased awareness about the potential environmental and the health hazards caused from exposure to barium as a growing use of barium-containing products in our daily life. It is estimated that in the U.S. alone, more than 150,000 individuals are constantly exposed to barium at a level higher than the limit of 2 mg/L proposed by the Environmental Protection Agency (13). In the general population, exposure to barium occurs primarily through the contaminated food and drinking water, however in rarer occasions, this could result from air or skin contact in the workplace that contains barium (14-15). Barium exposure has been reported to be toxic to humans. For instance, individuals with acute barium poisoning suffer from respiratory and cardiac arrest (16,17). Prolonged barium exposure has the potential to cause muscular weakness (18), cardiovascular malfunction (19), and congenital defects in neonates (20-21). Nevertheless, the specific effects of barium on human health are not well characterized.

Of note, there were a few quantitative analyses of the relationship between barium exposure and NAFLD. Specifically, a prior study exhibited that barium could accumulate in the liver and cause inflammation, further contributing to the development of NAFLD (22).

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Ananda et al. also reported that patients with barium poisoning experienced mild fatty changes in the liver (23). Moreover, recent studies have demonstrated that chronic barium exposure was linked to metabolic disorders, including insulin resistance and obesity (24-26), which can induce multiple comorbidities, including NAFLD (27). Additionally, urinary barium can be used to evaluate the degree of barium exposure in humans through various routes, which has been used in biological monitoring research (28,29). Thus, it is warranted to discuss the potential effect of barium on NAFLD.

Hence, this study investigated the correlation between urinary barium levels (UBLs) and the risk of NAFLD in a large cross-sectional population of adults in the U.S. with the use of data from the National Health and Nutrition Examination Survey (NHANES) between 2005 and 2016.

Materials and Methods

Study design and population

The nutritional and health status of the general U.S. population can be well-represented through the NHANES survey which provides a comprehensive information using a multistage and complex probability sampling design. The prior approval of the protocols used in the present paper was granted by the National Center for Health Statistics of the Centers for Disease Control and Prevention Institutional Review Board, and written informed consent was obtained from every participant. The data from the six distinct cycles of the NHANES between 2005 and 2016 were pooled for this study.

The detailed information about the survey, examination, and laboratory procedures is accessible on the internet (www.cdc.gov/nchs/nhanes/). A total of 60,936 participants were initially identified but only the participants aged 20 years and over were recruited. In addition, our study further excluded 20,087 participants due to availability of incomplete information for the calculation of the United States fatty liver index (USFLI), 9,242 participants with missing urinary barium data, 88 participants with positive hepatitis B surface antigen and/or hepatitis C virus antibodies, 117 participants with a history of heavy alcohol consumption (over 10 g per day for women and over 20 g per day for men), and 90 pregnant participants. Ultimately, 4,556 eligible participants were included in this study. The workflow of the study design has been demonstrated in figure 1.

Measurement of urinary barium levels

The targeted independent variable in our study was *urinary barium levels* (UBLs). In the 2005-2016

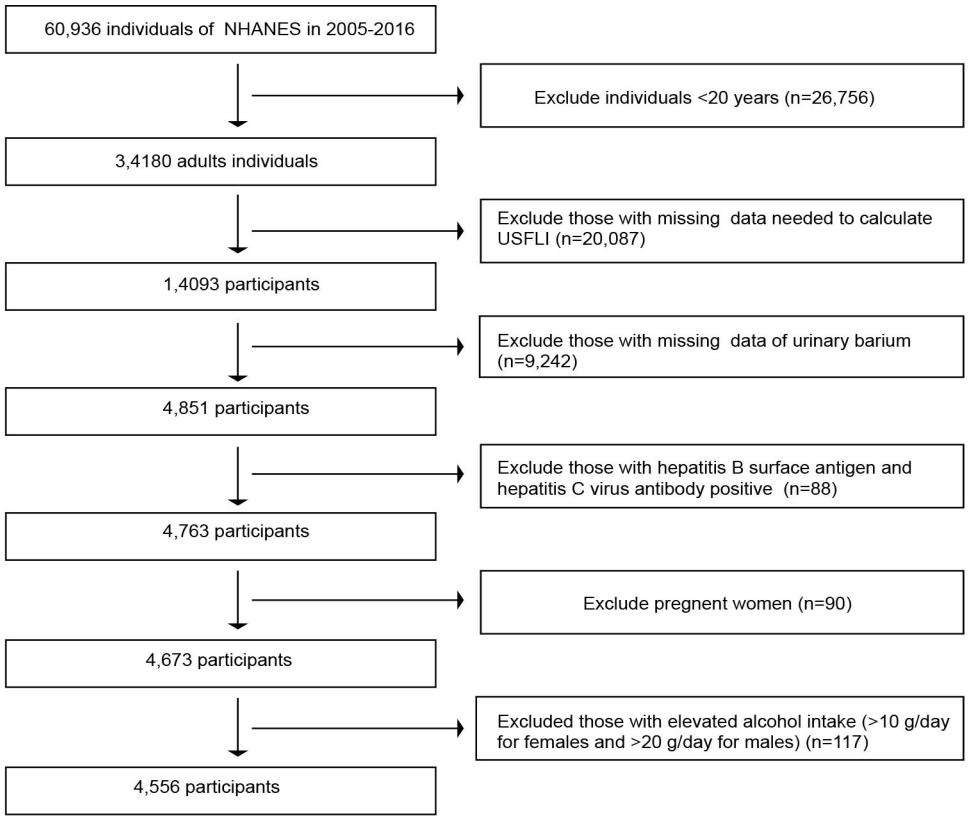

Figure 1.— Flow chart of the designed study.

NHANES, the samples of urine were processed, stored, and transported to the Centers for Disease Control and Prevention in Atlanta for analysis. The inductively coupled plasma-mass spectrometry was used for the quantification of UBLs. The NHANES Laboratory/ Medical Technologists Procedures Manual contains the detailed procedure for the measurement. The limit of detection (LOD) for urinary barium varied based on NHANES cycles, from 0.06 μg/L to 0.12 μg/L. The percentage of the participants with urinary measures at or above the LOD in the present study was 94.6%, and those below the LOD were substituted with the square root of the detection limit divided by 2 according to NHANES analysis guidelines.

Definition of NAFLD

NAFLD is defined based on the U.S. fatty liver index (USFLI). USFLI is a non-invasive diagnostic index which has been extensively used for the detection of liver diseases (30,31,32). This index incorporates race, age, waist circumference, γ-glutamyltransferase, fasting insulin and glucose levels. The USFLI is calculated as described below: USFLI = $(e^{-0.8073 * \text{non-Hispanic black} + 0.3458 * \text{Mexican American} + 0.0093 * \text{age} + 0.6151 * \log_e(\text{GGT}) + 0.0249 * \text{waist circumference} + 1.1792 * \log_e(\text{insulin}) + 0.8242 * \log_e(\text{glucose}) - 14.7812})/(1 + e^{-0.8073 * \text{non-Hispanic black} + 0.3458 * \text{Mexican American} + 0.0093 * \text{age} + 0.6151 * \log_e(\text{GGT}) + 0.0249 * \text{waist circumference} + 1.1792 * \log_e(\text{insulin}) + 0.8242 * \log_e(\text{glucose}) - 14.7812}) * 100$. The subjects with USFLI score of ≥ 30 could be assumed to have NAFLD (30).

Covariates

The different covariates based on known confounders from the prior literature and clinical experience were included. The various socio-demographic variables of sex, age, race/ethnicity, the family poverty-to-income ratio (PIR) and education level were obtained based on the questionnaires. Race/ethnicity was categorized into four distinct groups as Non-Hispanic White, Non-Hispanic Black, Mexican American, and Other Race. Family PIR was calculated as the annual household income divided by the federal poverty level and categorized into poverty (PIR≤1) and above the poverty threshold (PIR>1). In addition, educational attainment was recorded as below/above high school level or high school level. The smoking status was grouped into self-reported never smokers (smoking less than 100 cigarettes), former smokers (smoking more than 100 cigarettes in the past but not smoking at present), and current smokers.

Other demographic data including height, weight, waist circumference and blood pressures were recorded in the mobile examination center by trained doctors under a standard procedure. Body mass index (BMI)≥30 was regarded as obesity according to the CDC guideline. The laboratory covariates included triglycerides, total cholesterol, low-density lipoprotein (LDL)-cholesterol and direct high-density lipoprotein (HDL)-cholesterol. The urinary creatinine level was also included as a potential covariate to account for urinary dilution (33). Triglycerides, total cholesterol, LDL-cholesterol and direct HDL-cholesterol were measured enzymatically. Urinary creatinine was analyzed using a Jafférate reaction measured with Beckman Synchron CX3 clinical analyzer and Roche/Hitachi Modular P chemistry analyzer.

A diagnosis of diabetes mellitus (DM) was made when the fasting blood glucose level ≥126 mg/dl, or glycated hemoglobin levels ≥ 6.5%. The participants who self-reported to have DM and those currently taking anti-diabetic medications were also categorized as DM patients. In addition, history of hypertension was established based on the self-reported medical history of the past diagnosis, current use of prescription drugs for hypertension, a diastolic blood pressure ≥ 80 mmHg and/ or systolic blood pressure ≥ 140 mmHg.

Statistical analysis

The continuous variables were expressed as mean ± standard deviation (SD) or median (interquartile ranges) when their data were skewed distribution. The categorical variables were summarized as frequency (percentage). The Mann-Whitney (skewed distribution), independent *t* (normal distribution), and Pearson chi-squared (categorical variables) test was performed for comparisons between individuals with or without NAFLD, as appropriate.

Considering the highly skewed distribution of UBLs, the natural log transformed urinary barium levels (Ln-UBLs) were employed for the further analysis. The multiple logistic regression models were applied to inspect the possible correlation between UBLs and NAFLD. Model I left unadjusted and Model II was adjusted for age, gender, race, education level, family PIR; Model III was further adjusted for BMI, hypertension, DM, total cholesterol, triglyceride, LDL-cholesterol, direct HDL-cholesterol, smoking status, urinary creatinine. Moreover, a generalized additive model with a spline smoothing function was employed to characterize the shape of the correlation between UBLs and NAFLD.

The stratified and interaction analyses were also conducted based on age groups (20-44, 44-60, ≥60 years), sex, race, family PIR, education level, smoking status, BMI, hypertension, and DM. Statistical package R (version 3.6.3) and EmpowerStats software (X&Y Solutions Inc., Boston, MA) were adopted for all the conducted analyses. A two-tailed P value less than 0.05 represented a statistical significance.

Results

Basic characteristics of the participants

According to the exclusion and inclusion criteria, 4,556 participants were enrolled eventually for detailed

Table 1. — **Basic characteristics of the study population**

Characteristics	Participants without NAFLD	Participants with NAFLD	P-value
Number of participants	3022	1534	
Age[Mean ± SD] (year)	47.71 ± 17.79	52.79 ± 16.62	<0.001
Age (n, %)			<0.001
<40	1140 (37.72%)	383 (24.97%)	
>=40, <60	1006 (33.29%)	539 (35.14%)	
>=60	876 (28.99%)	612 (39.90%)	
Gender (n, %)			<0.001
Male	1412 (46.72%)	868 (56.58%)	
Female	1610 (53.28%)	666 (43.42%)	
Race (n, %)			<0.001
Mexican American	351 (11.61%)	362 (23.60%)	
Non-Hispanic White	1336 (44.21%)	686 (44.72%)	
Non-Hispanic Black	708 (23.43%)	180 (11.73%)	
Other Race	627 (20.75%)	306 (19.95%)	
Education level (n, %)			<0.001
Less than high school	687 (22.73%)	492 (32.07%)	
High school	679 (22.47%)	357 (23.27%)	
Above high school	1656 (54.80%)	685 (44.65%)	
Family PIR (n, %)			0.071
PIR<1	579 (19.16%)	319 (20.80%)	
PIR>=1	2218 (73.40%)	1079 (70.34%)	
Missing	225 (7.45%)	136 (8.87%)	
Smoking status (n, %)			<0.001
Current	752 (24.88%)	343 (22.36%)	
Former	605 (20.02%)	451 (29.40%)	
Never	1665 (55.10%)	740 (48.24%)	
BMI[Mean ± SD] (kg/m^2)	26.41 ± 5.07	33.79 ± 6.78	<0.001
BMI (n, %)			<0.001
<30	2416 (79.95%)	496 (32.33%)	
>=30	606 (20.05%)	1038 (67.67%)	
Waist circumference [Mean ± SD] (cm)	92.18 ± 12.30	112.20 ± 14.52	<0.001
Hypertension (n, %)			<0.001
No	2015 (66.68%)	699 (45.57%)	
Yes	1007 (33.32%)	835 (54.43%)	
Diabetes mellitus (n, %)			<0.001
No	2718 (89.94%)	1025 (66.82%)	
Yes	304 (10.06%)	509 (33.18%)	
Total cholesterol [Mean ± SD] (mg/dL)	192.31 ± 41.66	196.65 ± 42.31	<0.001
Direct HDL-Cholesterol [Mean ± SD] (mg/dL)	57.28 ± 16.09	46.45 ± 12.34	<0.001
Triglycerides[Median (IQR)] (mg/dL)	89.00 (64.00-124.00)	144.00(103.00-206.00)	<0.001
LDL-cholesterol [Mean ± SD] (mg/dL)	113.98 ± 35.27	116.26 ± 34.66	0.038
Urinary creatinine [Mean ± SD] (mg/dL)	118.31 ± 70.82	126.82 ± 73.46	<0.001
Barium[Median (IQR)] (ug/L)	1.04 (0.54-1.93)	1.17 (0.62-2.31)	<0.001
Fasting glucose[Mean ± SD] (mg/dL)	100.27 ± 22.71	123.73 ± 48.06	<0.001
Fasting insulin[Median (IQR)] (pmol/L)	43.68 (29.54-61.19)	112.74 (81.44-157.80)	<0.001
GGT [Median (IQR)] (U/L)	17.00 (13.00-23.00)	28.00 (20.00-44.00)	<0.001

NAFLD: Nonalcoholic Fatty Liver Disease; PIR: poverty-to-income ratio; BMI: Body Mass Index; IQR: interquartile range; GGT: γ-glutamyltransferase.

data analysis in our study. Table 1 demonstrates the comparison of the various basic characteristics of participants with and without NAFLD, which showed significant differences between the groups, with the exception of the family PIR. The subjects affected with NAFLD tended to be older, were more likely to be males, and mainly belonged to Mexican American ethnicity. There was a greater prevalence among patients with

Table 2. — **The association between urinary barium levels and NAFLD**

	Model I OR(95%CI) P value	Model II OR(95%CI) P value	Model III OR(95%CI) P value
Ln-UBLs (ug/L)	1.17 (1.09, 1.24) <0.001	1.23 (1.15, 1.32) <0.001	1.24 (1.12, 1.37) <0.001
Q1(0.04,0.55)	Ref.	Ref.	Ref.
Q2(0.56,1.07)	1.06 (0.89, 1.27) 0.523	1.12 (0.93, 1.35) 0.226	1.23 (0.96, 1.57) 0.101
Q3(1.08,2.01)	1.12 (0.94, 1.34) 0.202	1.27 (1.05, 1.53) 0.012	1.23 (0.95, 1.58) <0.001
Q4(2.02-72.24)	1.44 (1.21, 1.72) <0.001	1.65 (1.37, 1.99) <0.001	1.65 (1.26, 2.15) <0.001
P for trend	<0.001	<0.001	<0.001

Model I: Non-adjusted; Model II: adjusted for age, gender, race, education level, family PIR; Model III: adjusted for age, gender, race, education level, family PIR, BMI, hypertension, DM, smoke status, triglyceride, total cholesterol, LDL-cholesterol, Direct HDL-Cholesterol, urinary creatinine; Ln-UBLs: natural log transformed urinary barium levels; NAFLD: Non-alcoholic Fatty Liver Disease; BMI: Body Mass Index; OR: odds ratio; 95%CI: 95% confidence interval.

NAFLD to be former smokers and have hypertension as well as DM. They had less than an education level of above high school; had lower direct HDL-cholesterol but higher waist circumference, BMI, serum triglycerides, total cholesterol, LDL-cholesterol, urinary creatinine, urinary barium, γ-glutamyltransferase, fasting insulin and fasting glucose.

Association of UBLs with NAFLD

Three multivariable regression models were generated to evaluate the independent influence of Ln-UBLs on the risk of NAFLD. As shown in table 2, an elevated risk of NAFLD was substantially correlated with the increased UBLs in the unadjusted (Model I) (OR: 1.17, 95%CI: 1.09-1.24, $P < 0.001$), minimally adjusted (Model II) (OR: 1.23, 95%CI: 1.15-1.32, $P < 0.001$), and fully adjusted models (Model III) (OR: 1.24, 95%CI: 1.12-1.37, $P < 0.001$). After Ln-UBLs were divided into quartiles, the participants in the highest quartiles had a 1.65-fold (95% CI: 1.26-2.15) increased probability of having NAFLD in comparison to the bottom quartile in the full model, and a distinct trend across the quartiles could be found (P for trend<0.001). Since UBLs were a continuous variable, a smoothing function was further used to characterize the shape of the relationship between UBLs and the risk of NAFLD. As depicted in figure 2, the correlation between Ln-UBLs and the risk of NAFLD was linear and no threshold or saturation effect was observed.

Stratified analysis by potential modifiers

Stratified analyses were further performed with logistic regression to identify whether the correlation of Ln-UBLs with NAFLD was robust in a specific population. All potential confounders were adjusted in the models, except for the confounders utilized for the stratification itself. The data demonstrated concurrent associations in age, race, family PIR, education level, smoking status, BMI, hypertension, and DM subgroups ($P > 0.05$). However, the interaction was significant in gender subgroups ($P = 0.003$), indicating that gender markedly modified the correlation between Ln-UBLs and the risk of NAFLD. The impact of Ln-UBLs on the risk of NAFLD was more significant in males (OR = 1.40) than in females (OR = 1.06).

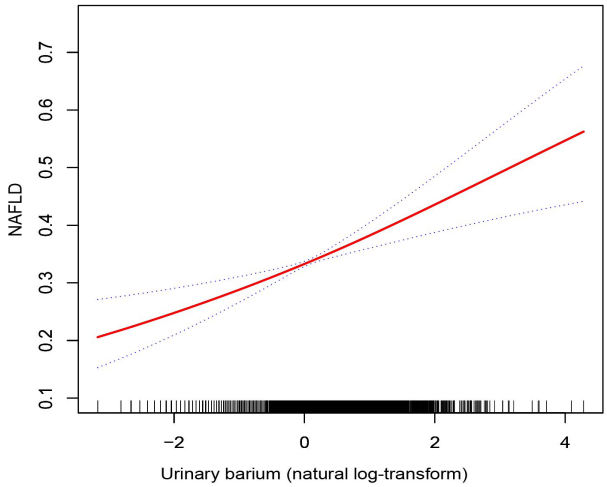

Figure 2. — The relationship between urinary barium levels (natural log -transformed) and the risk of NAFLD. A linear relationship between urinary barium levels and risk of NAFLD was observed after adjusting for age, gender, race, education level, family PIR, BMI, hypertension, DM, smoking status, triglyceride, total cholesterol, LDL-cholesterol, direct HDL-cholesterol, urinary creatinine; NAFLD: Non-alcoholic Fatty Liver Disease.

Discussion

This research provided fresh insight into the correlation between barium levels and NAFLD based on the data from the NHANES between 2005 and 2016. Our results unraveled that a positive correlation was observed between Ln-UBLs and the prevalence of NAFLD in the U.S. population and was influenced by gender. Specifically, higher Ln-UBLs were correlated with the elevated prevalence of NAFLD in males, whereas this correlation was weaker and insignificant in females. To the best of our knowledge, our study is the first to explore these associations in a nationally representative sample. These findings, although preliminary, may raise more attentions on the potential adverse effects of barium,

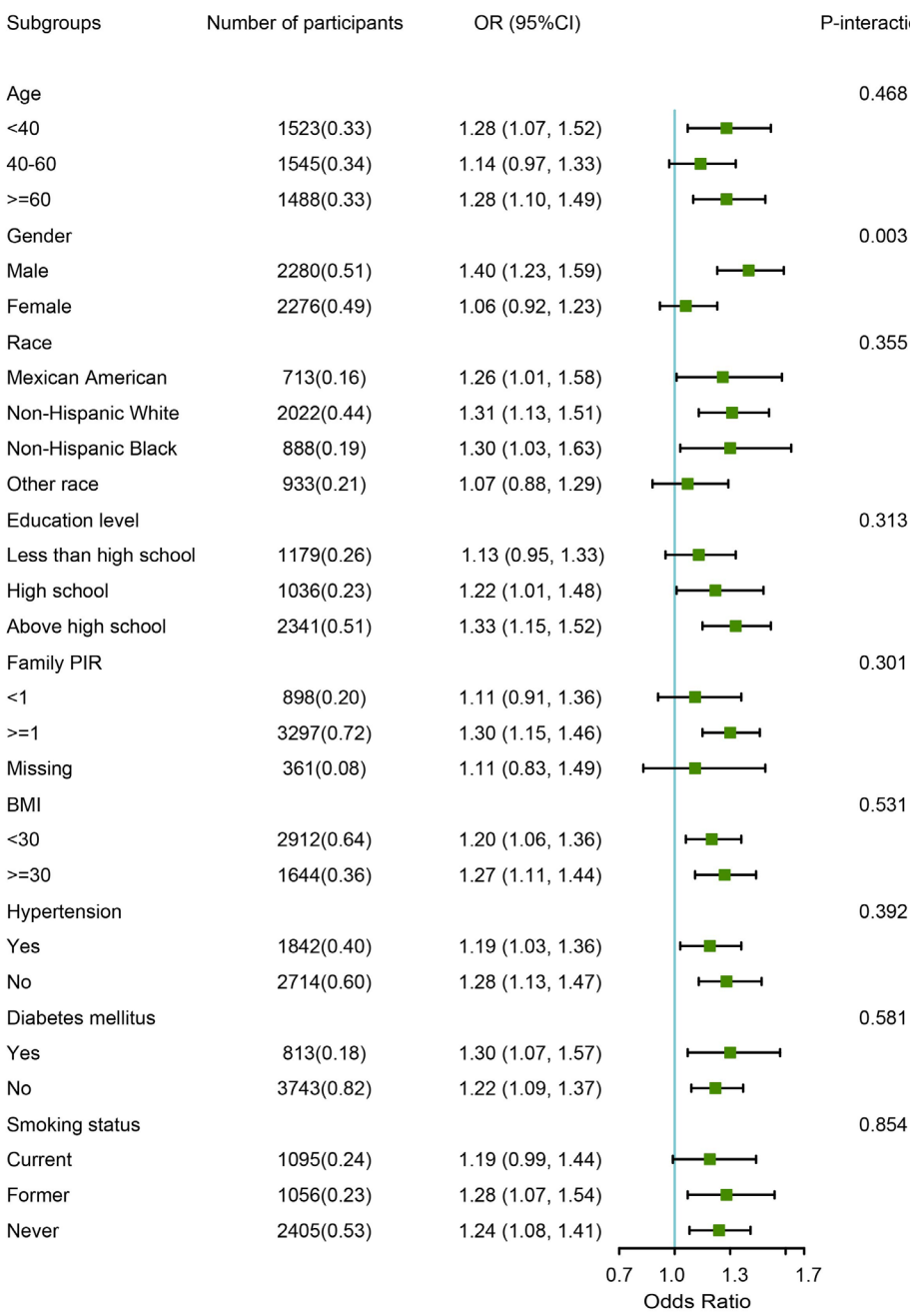

Figure 3. — Forest plots of the association between urinary barium levels and risk of NAFLD in the various subgroups. Adjusted for age, gender, race, education level, family PIR, BMI, hypertension, diabetes mellitus, smoking status, triglyceride, total cholesterol, LDL-cholesterol, direct HDL-cholesterol, urinary creatinine, except for the stratifying variable. BMI: Body Mass Index.

especially its impact on NAFLD in male individuals.

At present, there are few systematic studies on the effects of barium exposure on the liver. Mohammed et al. reported that oral administration of barium to rats elevated the levels of both aspartate transaminase and alanine transaminase, as well as infiltration of inflammatory cells and activation of hepatic stellate cells in the liver (22), illustrating that barium has potential toxicity to liver. However, the exact pathophysiology of barium-related hepatotoxicity and its correlation with the risk of NAFLD has not been completely elucidated in the literature. Barium is involved in the regulation of inflammation and oxidative stress, which can be potentially involved in the pathogenesis of NAFLD. A prior animal study unveiled that barium exposure could enhance the levels of reactive oxygen species (ROS), including hydroxyl, hydrogen peroxide, and superoxide, while markedly decreasing the activity of liver antioxidants, such as catalase, superoxide dismutase, glutathione reductase, and glutathione peroxidase (34). The disturbance of the balance between

ROS and antioxidants could expose the hepatocytes to oxidative stress and cause an overexpression of various proinflammatory cytokines such as TNF- α, IL-6 and IL-1β in the liver (34). These cytokines, according to existing evidence, are primarily responsible for aggravating the hepatic inflammation and mediate both the onset and progression of NAFLD (35-37). Moreover, excessive ROS production could further induce a rapid decline in hepatocyte mitochondrial respiration (38). The normal mitochondrial metabolic function is important for the maintenance of hepatic lipid homeostasis. Conversely, mitochondrial dysfunction can reduce the efficiency of the tricarboxylic acid (TCA) cycle and ATP production, which eventually results in the uptake of metabolites by the liver for lipogenesis (39).

Insulin resistance is another possible explanation for the effects of barium exposure on NAFLD, which is a key factor in both metabolic syndrome and NAFLD. A prior study proposed a close correlation between insulin resistance and barium exposure and that increased UBLs were closely correlated with impaired glucose tolerance and insulin resistance (26). At the molecular level, barium could substantially exacerbate hepatic gluconeogenesis and glucose production by competitively blocking the pump-mediated K^+ transport in hepatic sodium-potassium ATPase, thereby rendering hepatocytes more vulnerable to insulin resistance (40). In addition, the pro-inflammatory cytokines (such as TNF-α) directly induced by barium can interfere with insulin signaling via the repression of insulin receptor substrate phosphorylation, which in turn can lead to the state of insulin resistance (41). Therefore, oxidative stress, mitochondrial dysfunction and barium-induced insulin resistance may account for the fundamental mechanisms of barium exposure and the risk of NAFLD. Despite these promising results, questions remain, and further research should be conducted.

Subsequently, subgroup and interaction analyses were carried out in the current study. An interaction was noted between the effect of barium on NAFLD and gender, which illustrated a markedly higher risk of NAFLD in men than in women subsequent to exposure to barium. However, the reasons behind the variable correlation between barium exposure and the risk of NAFLD among males and females remain unclear. A possible cause may be related to the different effects of barium exposure on sex hormones in different genders. As reported, sex-hormone binding globulin (SHBG) is one of the main hormones in the body and low serum SHBG levels are thought to play a crucial role in the pathogenesis of NAFLD (42,43). Interestingly, Galloway et al. analyzed the relationship between barium and sex hormone levels in the U.S. population, which unravelled no significant association between urinary barium concentration and serum SHBG levels in females but a considerable association between barium exposure and SHBG down-regulation in males (44). On this basis, we speculated that barium-induced gender-specific reduction of SHGB levels might explain the interaction effect observed in our study. However, future research is merited to validate this speculation and to examine the various possible relationships among barium exposure, SHBG levels, and the risk of NAFLD.

Our study has several strengths worthy of note. (1) To the best of our knowledge, no prior research examining the correlation between urinary barium levels and NAFLD has been conducted previously. The present study is the first report to investigate this association. (2) The data of our study is primarily based on a large nationally representative survey in the US, which has strengthened its statistical credibility and reliability. (3) The stratification and interaction effect analyses in this study was able to make better use of data and could identify potential interactions with other variables.

However, certain limitations of this study should be recognized. Firstly, NHANES currently contains no data on liver biopsies and rare data on liver B-ultrasound. Hence, NAFLD was diagnosed with the USFLI, rather than ultrasonography or liver biopsy. However, it was previously validated that the USFLI had an area under the receiver-operating characteristic curve value of 0.80 (95%CI: 0.77-0.83) for the detection of NAFLD in participants with USFLI scores ≥ 30 (45). Therefore, the use of the USFLI alone might cause the misdiagnosis of NAFLD. Secondly, patients in the current study were selected based on a negative diagnosis of NAFLD, which might introduce a potential bias of selection, because patients with chronic liver diseases, such as viral hepatitis and autoimmune hepatitis, were not excluded. Under such conditions, the prevalence of NAFLD might be overestimated, resulting in falsely higher calculated values. To avoid the miscalculation of NAFLD incidences, it is necessary to conduct further studies with the exclusion of patients with chronic liver diseases. Thirdly, barium exposure was quantified with urinary samples from individuals, yet the single measurement may not reflect long-term exposure or account for intra-individual variability. Fourth, consistent with other observational studies, residual confounders may bias our analyses. In addition, our analyses were performed on the U.S. population. Thus, the findings may not be generalizable to populations in other regions.

To summarize, our findings established a positive correlation between urinary barium levels and NAFLD prevalence. In particular, this association was modified by gender and was noticeably more pronounced in males. Further research should be conducted to investigate the potential fundamental mechanisms through which barium exposure can increase the risk of NAFLD.

Conflicting Interest

There are no conflicts of interest

Ethics approval and informed consent

The National Centre for Health Statistics Research Ethics Review Board reviewed and approved NHANES,

and all the participants signed written consent prior to the survey every year. This research analyzed de-identified information downloaded from the NHANES public database, which is exempt from future Institutional Review Board approval

References

1. COTTER T., RINELLA M. Nonalcoholic Fatty Liver Disease 2020: The State of the Disease. *Gastroenterology.*, 2020, **158**: 1851-1864.
2. YOUNOSSI Z., KOENIG A., ABDELATIF D., FAZEL Y., HENRY L., WYMER M. Global epidemiology of nonalcoholic fatty liver disease-Meta-analytic assessment of prevalence, incidence, and outcomes. *Hepatology.*, 2016, **64**: 73-84.
3. ESTES C., RAZAVI H., LOOMBA R., YOUNOSSI Z., SANYAL A. Modeling the epidemic of nonalcoholic fatty liver disease demonstrates an exponential increase in burden of disease. *Hepatology.*, 2018, **67**: 123-133.
4. ALVAREZ C., GRAUBARD B., THISTLE J., PETRICK J., MCGLYNN K. Attributable Fractions of Nonalcoholic Fatty Liver Disease for Mortality in the United States: Results From the Third National Health and Nutrition Examination Survey With 27 Years of Follow-up. *Hepatology.*, 2020, **72**: 430-440.
5. Lin X., Shi K., You J., Liu W., Luo Y., Wu F., *et al*. Increased risk of colorectal malignant neoplasm in patients with nonalcoholic fatty liver disease: a large study. *Mol Biol Rep.*, 2014, **41**: 2989-2997.
6. TARGHER G., BYRNE C., LONARDO A., ZOPPINI G., BARBUI C. Non-alcoholic fatty liver disease and risk of incident cardiovascular disease: A meta-analysis. *J Hepatol.*, 2016, **65**: 589-600.
7. MANTOVANI A., ZAZA G., BYRNE C., LONARDO A., ZOPPINI G., BONORA E., *et al*. Nonalcoholic fatty liver disease increases risk of incident chronic kidney disease: A systematic review and meta-analysis. *Metabolism.*, 2018, **79**: 64-76.
8. MAKRI E., GOULAS A., POLYZOS S. Epidemiology, Pathogenesis, Diagnosis and Emerging Treatment of Nonalcoholic Fatty Liver Disease. *Arch Med Res.*, 2021, **52**: 25-37.
9. CHEN J., WU L., YANG G., ZHANG C., LIU X., SUN X., *et al*. The influence of PM2.5 exposure on non-alcoholic fatty liver disease. *Life Sci.*, 2021, **270**: 119135.
10. CAI S., FAN J., YE J., RAO X., LI Y. Phthalates exposure is associated with non-alcoholic fatty liver disease among US adults. *Ecotoxicol Environ Saf.*, 2021, **224**: 112665.
11. PRAGST F., STIEGLITZ K., RUNGE H., RUNOW K., QUIG D., OSBORNE R., *et al*. High concentrations of lead and barium in hair of the rural population caused by water pollution in the Thar Jath oilfields in South Sudan. *Forensic Sci Int.*, 2017, **274**: 99-106.
12. PEANA M., MEDIC S., DADAR M., ZORODDU M., PELUCELLI A., CHASAPIS C., *et al*. Environmental barium: potential exposure and health-hazards. *Arch Toxicol.*, 2021, **95**: 2605-2612.
13. KRAVCHENKO J., DARRAH T., MILLER R., LYERLY H., VENGOSH A. A review of the health impacts of barium from natural and anthropogenic exposure. Environ *Geochem Health.*, 2014, **36**:797-814.
14. BRENNIMAN G., NAMEKATA T., KOJOLA W., CARNOW B., LEVY P. Cardiovascular disease death rates in communities with elevated levels of barium in drinking water. *Environ Res.*, 1979, **20**: 318-324.
15. KRISHNA S., JAISWAL A., SUJATA G., SHARMA D., ALI Z. Barium poisoning with analytical aspects and its management. *Int J Adv Res Med Chem.*, 2020, **2**: 20-27
16. RHYEE S., HEARD K. Acute barium toxicity from ingestion of "snake" fireworks. *J Med Toxicol.*, 2009, **5**: 209-213.
17. JOSHI N., SHARMA C., SAI., SHARMA J. Acute barium intoxication following ingestion of soap water solution. *Indian J Crit Care Med.*, 2012, **16**: 238-240.
18. BHOELAN B., STEVERING C., VAN DER BOOG A., VAN DER HEYDEN M. Barium toxicity and the role of the potassium inward rectifier current. *Clin Toxicol (Phila).*, 2014, **52**: 584-593.
19. DALLAS C., WILLIAMS P. Barium: rationale for a new oral reference dose. *J Toxicol Environ Health B Crit Rev.*, 2001, **4**: 395-429.
20. WANG C., PI X., CHEN Y., WANG D., YIN S., JIN L., *et al*. Prenatal exposure to barium and the occurrence of neural tube defects in offspring. *Sci Total Environ.*, 2021, **764**: 144245.
21. PI X., JIN L., LI Z., LIU J., ZHANG Y., WANG L., *et al*. Association between concentrations of barium and aluminum in placental tissues and risk for orofacial clefts. *Sci Total Environ.*, 2019, **652**: 406-412.
22. MOHAMMED A., ISMAIL H. Hematological, biochemical, and histo-pathological impacts of barium chloride and barium carbonate accumulation in soft tissues of male Sprague-Dawley rats. *Environ Sci Pollut Res Int.*, 2017, **24**: 26634-26645.
23. ANANDA, S., SHAOHUA, Z., LIANG, L. Fatal barium chloride poisoning: four cases report and literature review. *Am J Forensic Med Pathol.*, 2013, **34**: 115-118.
24. LIU B., FENG W., WANG J., LI Y., HAN X., Hu H., *et al*. Association of urinary metals levels with type 2 diabetes risk in coke oven workers. *Environ Pollut.*, 2016, **210**: 1-8.
25. WANG X., MUKHERJEE B., PARK S. Associations of cumulative exposure to heavy metal mixtures with obesity and its comorbidities among U.S. adults in NHANES 2003-2014. *Environ Int.*, 2018, **121**: 683-694.
26. MENKE A., GUALLAR E., COWIE C. Metals in Urine and Diabetes in U.S. Adults. *Diabetes.*, 2016, **65**:164-171.
27. HONG SH., CHOI K. Sarcopenic Obesity, Insulin Resistance, and Their Implications in Cardiovascular and Metabolic Consequences. *Int J Mol Sci.*, 2020, **21**: 494.
28. CESBRON A., SAUSSEREAU E., MAHIEU L., COULAND I., GUERBET M., GOULLÉ, J. Metallic profile of whole blood and plasma in a series of 106 healthy volunteers. *J Anal Toxicol*, 2012, **37**: 401-405.
29. PODDALGODA D., MACEY K., ASSAD H., KRISHNAN K. Development of biomonitoring equivalents for barium in urine and plasma for interpreting human biomonitoring data. *Regul Toxicol Pharmacol*., 2017, **86**: 303-311.
30. MEFFERT P., BAUMEISTER S., LERCH M., MAYERLE J., KRATZER W., VöLZKE H. Development, external validation, and comparative assessment of a new diagnostic score for hepatic steatosis. *Am J Gastroenterol.*, 2014, **109**: 1404-1414.
31. RUHL C., EVERHART J. Fatty liver indices in the multiethnic United States National Health and Nutrition Examination Survey. *Aliment Pharmacol Ther.*, 2015, **41**: 65-76.
32. CHEN C., ZHOU Q., YANG R., WU Z., Yuan H., ZHANG N., *et al*. Copper exposure association with prevalence of non-alcoholic fatty liver disease and insulin resistance among US adults (NHANES 2011-2014). *Ecotoxicol Environ Saf.*, 2021, **218**: 112295.
33. BARR D., WILDER L., CAUDILL S., GONZALEZ A., NEEDHAM L., PIRKLE J. Urinary creatinine concentrations in the U.S. population: implications for urinary biologic monitoring measurements. *Environ Health Perspect.*, 2005, **113**: 192-200.
34. ELWEJ A., GROJJA Y., GHORBEL I., BOUDAWARA O., JARRAYA R., BOUNDAWARA T., *et al*. Barium chloride induces redox status unbalance, upregulates cytokine genes expression and confers hepatotoxicity in rats-alleviation by pomegranate peel. *Environ Sci Pollut Res Int.*, 2016, **23**: 7559-7571.
35. LU S., WANG Y., LIU J. Tumor necrosis factor-α signaling in nonalcoholic steatohepatitis and targeted therapies. *J Genet Genomics.*, 2022, **49**: 269-278.
36. ANDERSON N., BORLAK J. Molecular mechanisms and therapeutic targets in steatosis and steatohepatitis. *Pharmacol Rev.*, 2008, **60**: 311-357.
37. GAUL S., LESZCZYNSKA A., ALEGRE F., KAUFMANN B., JOHNSON C., ADAMS L., *et al*. Hepatocyte pyroptosis and release of inflammasome particles induce stellate cell activation and liver fibrosis. *J Hepatol.*, 2021, **74**:156-167.
38. KOWALTOWSKI A., VERCESI A. Mitochondrial damage induced by conditions of oxidative stress. *Free Radic Biol Med*., 1999, **26**: 463-471.
39. MAILLOUX R., LEMIRE J., APPANNA V., Aluminum-induced mitochondrial dysfunction leads to lipid accumulation in human hepatocytes: a link to obesity. *Cell Physiol Biochem.*, 2007, **20**: 627-638.
40. SUN H., CAO L., ZHU M., WU Z., SHEN C., NIE X., *et al*. DR-region of Na+/K+-ATPase is a target to ameliorate hepatic insulin resistance in obese diabetic mice. *Theranostics.*, 2020, **10**: 6149-6166.
41. WEICKERT M., PFEIFFER A. Signalling mechanisms linking hepatic glucose and lipid metabolism. *Diabetologia.*, 2006, 49:1732-1741.
42. LEE S., LEE Y., YANG H., LEE H., LEE G., AN B., *et al*. Sex hormone-binding globulin suppresses NAFLD-triggered hepatocarcinogenesis after menopause. *Carcinogenesis.*, 2019, **40**: 1031-1041.
43. SAEZ-LOPEZ C., BARBOSA-DESONGLES A., HERNANDEZ C., DYER R., INNIS S., SIMó R., *et al*. Sex Hormone-Binding Globulin Reduction in Metabolic Disorders May Play a Role in NAFLD Development. *Endocrinology.*, 2017, **158**: 545-559.
44. TAO C., LI Z., FAN Y., LI X., QIAN H., YU H., *et al*. Independent and combined associations of urinary heavy metals exposure and serum sex hormones among adults in NHANES 2013-2016. *Environ Pollut.*, 2021, **281**: 117097.
45. Ruhl C., Everhart J. Fatty liver indices in the multiethnic United States National Health and Nutrition Examination Survey. *Aliment Pharmacol Ther.*, 2015, **41**:65-76.