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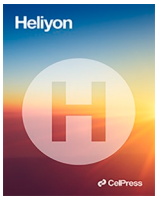

Research article

Association between heavy metal exposure and biomarkers for non-alcoholic fatty liver disease in Korean adolescents

Dong-Wook Lee [a], Jongmin Oh [b], Yu Min Lee [c], Hyun-Joo Bae [d], Youn-Hee Lim [e,*]

[a] Department of Occupational and Environmental Medicine, Inha University Hospital, Inha University, Incheon, Republic of Korea
[b] Institute of Ewha-SCL for Environmental Health (IESEH), Ewha Womans University College of Medicine, Seoul, Republic of Korea
[c] Department of Occupational and Environmental Medicine, Severance Hospital, College of Medicine, Yonsei University, Seoul, Republic of Korea
[d] Korea Environment Institute, Sejong, Republic of Korea
[e] Section of Environmental Health, Department of Public Health, University of Copenhagen, Copenhagen, Denmark

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ABSTRACT

Objectives: The global prevalence of non-alcoholic fatty liver disease (NAFLD) in adolescents has increased. In addition to childhood obesity, environmental risk factors, such as heavy metals that are known to be involved in hepatotoxicity, play role in NAFLD occurrence. However, their association with NAFLD remains unclear. This study aimed to investigate the association between heavy metal exposure and NAFLD biomarkers in adolescents.

Methods: In this cross-sectional study, we used the data of a total of 1505 adolescents aged 12–17 years who participated in the Korean National Environmental Health Survey III (2015–2017) and IV (2018–2020). The presence of blood lead (BPb), blood mercury (BHg), urinary mercury (UHg), and urinary cadmium (UCd) were measured. Liver enzymes including serum aspartate aminotransferase (AST), alanine aminotransferase (ALT), and gamma-glutamyl transferase (GGT) were evaluated. For NAFLD biomarkers, the hepatic steatosis index (HSI) was calculated. Multivariate linear regression models, weighted quantile sum (WQS) regression, and Bayesian kernel machine regression (BKMR) model were used to investigate the association between heavy metals and NAFLD biomarkers.

Results: Among heavy metals, mercury presence showed a significant association with NAFLD biomarkers. Two-fold increases in BHg and UHg were associated with 0.21 points (95 % confidence interval [CI]: 0.08–0.35) and 0.19 points (95 % CI: 0.09–0.30) higher HSI, respectively. In the WQS model, heavy metal mixture was significantly associated with increased HSI ($\beta = 0.06$, 95 % CI: 0.01–0.11). Similarly, in the BKMR model, heavy metal mixture was positively associated with NAFLD biomarkers, and BHg was the most important contributor in the association.

Conclusions: BHg and UHg were significantly associated with NAFLD biomarkers in adolescents, indicating that organic and inorganic mercury exposure could potentially be a risk factor for NAFLD. To mitigate and address the risk of NAFLD associated with heavy metal exposure, it is imperative to take measure to reduce avoidable mercury exposure is necessary.

* Corresponding author. Section of Environmental Health, Department of Public Health, University of Copenhagen, Øster Farimagsgade 5, Building 15, Copenhagen, 1014, Denmark.
E-mail address: younhee.lim@sund.ku.dk (Y.-H. Lim).

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1. Introduction

Non-alcoholic fatty liver disease (NAFLD) is an umbrella term encompassing wide spectrum of conditions that involve long-standing hepatic steatosis. The etiology of NAFLD involves neither genetic nor metabolic disorders, infections, side effects of medication, alcohol consumption, or malnutrition [1,2]. NAFLD often emerges during adolescence and poses a significant risk of serious health complications later in life, including cirrhosis and hepatocellular carcinoma [3]. Moreover, the life expectancy in children with NAFLD is shorter than that in the general population of the same age and sex, and some of them can develop cirrhosis and end-stage liver disease [4].

The global prevalence of NAFLD has escalated over the past three decades, making it a significant global health concern [5]. The estimates indicated that NAFLD affects 7.6 % of children, 34.2 % of obese children [6], and 25.2 % of adults worldwide [7]. In South Korea, the prevalence of NAFLD among children was 4.7 % in 2010 and increased to 5.9 % in 2015 [8]. Because obese adolescents have a risk to develop NAFLD [9], the increasing trend of obesity is the most important factor in increasing NAFLD in adolescents [10]. Moreover, emerging evidence suggests that environmental factors, including heavy metal exposure, may also play a significant role in the development of NAFLD [11,12].

Heavy metals, such as mercury (Hg), lead (Pb), and cadmium (Cd), are ubiquitous environmental contaminants, that have toxic effects on body organs, including the liver [13]. Several epidemiological studies have investigated the associations between these heavy metals and NAFLD. Blood Hg was significantly associated with NAFLD, as defined by liver enzyme levels among US adolescents [14]. Blood Pb showed a positive relationship with NAFLD, as determined by abdominal ultrasonography in Chinese adults [15]. Cd exposure in young adulthood assessed by toenail was associated with NAFLD in the US [16]. These heavy metals can induce hepatotoxicity by generating reactive oxygen species, altering the antioxidant system, and reducing the expression of genes related to oxidative stress [17]. For example, Pb enhances the peroxidation of membrane lipids, resulting in hepatocellular damage [18]. Chronic Cd exposure could result in a hepatic injury via the disruption in gene transcription and expression [19].

Given the well-documented hepatotoxicity of heavy metals and epidemiological evidence linking chronic exposure to heavy metals and liver diseases [20,21], it is imperative to identify modifiable risk factors during childhood is essential to prevent NAFLD. However, despite the significance of investigating potential risk factors, including heavy metal exposure, and the existing evidence in the adult population [19], no research has yet delved into the association between heavy metal exposure and NAFLD in adolescents. This study was undertaken to unveil the association between heavy metal exposure and the development of NAFLD in adolescents, offering valuable insights into the potential environmental risk factors contributing to NAFLD.

2. Material and methods

2.1. Study population

We used data from the Korean National Environmental Health Survey (KoNEHS). A survey was conducted to obtain national human biomonitoring data from the National Institute of Environmental Research (NIER). The third and fourth KoNEHS were conducted from 2015 to 2017 (III) and 2018–2020 (IV), respectively. The KoNEHS was designed to select participants to represent the national population using cluster sampling with stratified and randomly selected schools [22]. A total of 183 and 181 schools, with 922 and 828 students were included in the KoNEHS III and IV, respectively.

2.2. Sample collection

Blood and urine samples of study participants were collected from the clinics or hospitals close to each educational institution. Sample collection was performed by health professionals in the clinics or hospitals, according to the guideline provided by NIER. After cleaning the area with a 70 % alcohol swab, blood sample was collected from the superficial veins of the cubital fossa using BD Vacutainer® system. Blood samples to measure heavy metal concentration in Ethylenediaminetetraacetic Acid (EDTA) tubes (trace element EDTA tube, Royal Blue cap, BD #368381) were rolled five times and placed in a roll mixer for 30 min at room temperature. Blood samples to measure liver enzymes in serum separating tube (SST) tubes were centrifuged for 10 min at 3000 rpm to separate the serum at room temperature. EDTA tubes and SST tubes were stored at refrigerating temperature (2–6 °C) before sending to the sample management and storage sites. Urine samples were collected in polypropylene urine container specimen cup and stored at refrigerating temperature (2–6 °C) in a cooler with ice water. Trained investigators visited each medical institute, collected the samples, and transferred them to the sample management and storage sites within 24 h. The samples were aliquoted and stored at −70 °C, and were sent for analysis every 2 weeks.

2.3. Heavy metal concentration measurements

Total Hg and Pb were detected in blood (hereinafter referred to as 'BHg' and 'BPb,' respectively). In addition, Cd and total Hg were measured in urine (referred to as 'UCd' and 'UHg,' respectively). BPb was measured using a graphite furnace-atomic absorption spectrometer (GF-AAS, Analyst 800, PerkinElmer, MA, USA) [23]. For BPb measurement, whole blood samples and standard solutions (0.6, 1.2, 2.4, 4.8, 9.6 μg Pb/dL) were diluted (1:5) with a distilled water, and aqueous matrix modifier solution (2 % Triton X-100 and 0.2 % $(NH_4)_2HPO_4$). The measurement was repeated three times to create a calibration curve. UCd was measured using a GF-aas (240Z, Agilent, Santa Clara, CA, USA) [23]. For UCd measurement, urine specimen and standard solutions (0.5, 1, 2, 4, 6, 8 μg Cd/L) were

diluted (1:5) with a distilled water, and aqueous matrix modifier solution (2 % Triton X-100 and 0.2 % $(NH_4)_2HPO_4$). As BHg indicates exposure to seafood containing methylmercury and UHg indicates long-term exposure to inorganic mercury [24,25], this study used both BHg and UHg as individual mercury exposure markers. A gold amalgamation direct mercury analyzer (DMA-80, Milestone, CT, USA) was used to measure the total Hg in blood or urine. BHg and UHg were analyzed at a wavelength of 254.65 nm and 253.7 nm, respectively. Limit of detections (LODs) for BPb, BHg, UHg, and UCd were determined to be 0.17 μg/L, 0.1 μg/dL, 0.04 μg/L, and 0.04 μg/L, respectively. BPb, BHg, UHg, and UCd below LODs were substituted to LODs divided by $\sqrt{2}$. UHg and UCd were divided by urine creatinine to produce creatinine-corrected levels. Log-2-transformed heavy metal exposure levels were used as independent variables, and the association's effect size was presented as a 2-fold increase in exposure levels. External quality control program was performed for analytical laboratories, including G-EQUAS in Germany, and proficiency testing by NIER. Periodic quality assurance and quality control measurements were also performed [23,26].

2.4. Liver enzymes

Serum alanine aminotransferase (ALT) and aspartate aminotransferase (AST) levels and gamma-glutamyl transferase (GGT) in participants' serum were analyzed. The modified International Federation of Clinical Chemistry and Laboratory Medicine Reference Method was used to measure serum ALT, AST, and GGT levels using colorimetry. These spectrophotometric measurements were based on the rate reaction methods, which utilize changes in absorbance to determine reaction values. The ADVIA 1800 Chemistry system (Siemens, Erlangen-Germany), an automated clinical chemistry analyzer, was used to measure AST, ALT, and GGT using compatible biochemistry kits. All analyses followed the manual published by NIER [26]. AST, ALT, and GGT in serum were analyzed at the wavelengths of 340 nm, 340 nm, and 410 nm, respectively, using a colorimetry. The LODs for ALT, AST, and GGT were 3.4 IU/L, 4.2 IU/L, and 4.0 IU/L, respectively.

2.5. Biomarkers for NAFLD

The hepatic steatosis index (HSI) is a widely used screening tool for identifying NAFLD, particularly in the selection of individuals for further diagnostic assessment and the recommendation of lifestyle modifications. HSI was developed based on data from 2680 NAFLD cases diagnosed by medical professionals with ultrasonography, alongside 2680 age- and sex-matched individuals without NAFLD. The development process involved employing stepwise multiple logistic regression analysis to elucidate the presence of NAFLD considering a set of independent predictors, including body mass index (BMI), the presence of diabetes mellitus, waist circumference, systolic blood pressure, diastolic blood pressure, serum fasting glucose, Hemoglobin A1C, cholesterol levels, AST, ALT, GGT, high sensitive C-reactive protein, and uric acid, and ALT/AST ratio. HSI was calculated using the following formula: HSI = 8 × (ALT/AST ratio) + BMI + 2 (if diabetes mellitus) + 2 (if female). Higher HSI score significantly predicts the presence and severity of NAFLD status, as shown in the prediction performance in the independent validation dataset. The positive association with fatty liver grade according to the ultrasonography imaging had an area under receiver-operating characteristic curve (AUC) of 0.812 [27]. The performance in predicting predict NAFLD in 225 Korean adolescent was excellent, with an AUC of 0.929 [28]. HSI also showed moderate diagnostic accuracy in the study with 119 Dutch participants compared to magnetic resonance imaging [29]. ALT and AST levels, the indicators of hepatocellular injury or inflammation [30], and GGT, serve as biomarkers of liver disease, particularly bile duct obstruction, alcohol abuse, and non-alcoholic fatty liver disease [31].

2.6. Covariates

We considered several individual characteristics in the model. Maternal education level was classified into three categories: below high school graduate, college graduate, and above college (master's and PhD degrees). BMI in adolescents was calculated based on self-reported weight and height, where weight (kg) was divided by the height in meters squared (m^2). BMI was classified as normal (<23 kg/m^2), overweight (23 ≤ BMI <25 kg/m^2), or obese (≥25 kg/m^2). Smoking status was categorized into the following groups: current smoking, former smoking, or never smoking. Alcohol consumption status was defined based on lifetime drinking experience (yes: ≥ once; no: none). Similarly, physical activity was assessed and categorized into two groups based on the frequency of moderate exercise to make the body sweat per week (yes: ≥ once; no: none). After considering the covariates adjusted in previous studies [32,33], we utilized DAGitty, a tool for creating directed acyclic graphs, to select the covariates for the models (Supplementary Fig. S1). The selected covariates were age, sex, body mass index, maternal education level, smoking status, alcohol consumption, and physical activity.

2.7. Statistical analysis

In a cross-sectional study design, we investigated the association between individual heavy metal exposures and biomarkers for NAFLD. To visualize the exposure-response relationships of metals (BPb, BHg, UHg, and UCd) with HSI and liver function markers (ALT, AST, and GGT), we constructed a generalized additive model after adjusting for covariates. Natural cubic splines with degrees of freedom were selected based on the lowest Generalized Cross-Validation score. To estimate the association between heavy metal exposure and NAFLD biomarkers (HSI, ALT, AST, and GGT), we used multivariate linear regression models after adjusting for age, sex, BMI, maternal education level, alcohol consumption, and smoking status. To estimate the association of mixture exposures to heavy

metals with NAFLD biomarkers, we used two mixture models: weighted quantile sum (WQS) regression and Bayesian kernel machine regression (BKMR) models. WQS regression is a statistical method commonly used in environmental health studies to identify and estimate the effects of mixtures with a high number of components. This approach allows for the simultaneous consideration of multiple correlated exposures and identifies the important components of a mixture that are associated with a specific outcome [34]. Coefficients in WQS represents the effect size of the association of NAFLD biomarkers per one quantile increase in mixture exposures. BKMR is a semi-parametric statistical method that enables the simultaneous consideration of multiple exposures. Unlike WQS, BKMR could yield potential non-linear relationships between exposure and outcome variables, and individual exposure–response functions accounting for the other exposures. BKMR uses a hierarchical variable selection method to identify key exposures linked to the outcome [35]. Posterior inclusion probabilities (PIP) were calculated to determine their importance using a threshold of 0.5. The BKMR Models were run for up to 5000 iterations in each analysis. In BKMR, cumulative effect of the exposure to four heavy metals is presented by the estimated change in NAFLD biomarkers in quantiles compared to when all four heavy metals are present at 50th percentile to represents the effect size of the association of NAFLD biomarkers per one quantile increase in mixture exposures. Pearson's correlation coefficients among BPb, BHg, UHg, and UCd were calculated, and the results visualized. All statistical analyses were performed using R version 4.2.3. GAM, WQS, and BKMR were implemented with the R package *mgcv*, *gWQS*, and *bkmr*, respectively. SAS version 9.3 (SAS Institute, Cary, NC, USA). *P*-value <0.05 was considered statistically significant.

3. Results

A total of 1750 adolescents were recruited in the KoNEHS III and IV. We excluded participants with missing information on liver function tests (AST, ALT, or GGT, N = 8), blood and urine heavy metal concentration (N = 28), medication treatment for any disorder (N = 182), and covariates (N = 27). Finally, 1505 adolescents were included in the analysis (Fig. 1).

Table 1 shows the characteristics of the study participants. Among 1505 participants, 47 % were males, and 47 % were middle school students (12–14 years), whereas the rest were high school students (15–17 years). More than half of the mothers had higher education than high school (54.4 %). Nineteen percent of the participants were overweight or obese (BMI ≥25) and 5.2 % of adolescents were ever smokers (2.3 % were former smokers and 2.9 % were current smokers). Two-thirds of the participants reported that they had moderate physical activity.

Overall, the mean HSI score was 29.2 (standard deviation [SD] ± 5.3), with 9.2 % of participants demonstrating an elevated HSI score (Table 1). Mean AST, ALT, and GGT were 21.9 U/L (SD ± 7.7), 17.3 U/L (SD ± 15.9), and 14.6 U/L (SD ± 8.9), respectively. HSI was moderately associated with AST, ALT, and GGT (Pearson's correlation coefficient: 0.30–0.59) (Supplementary Fig. S2). HSI score, the percentage of elevated HSI score, AST, ALT, and GGT were higher among boys than girls. Obese adolescents showed significantly higher HSI score (37.2, SD ± 5.1) than normal-weight adolescents (31.2, SD ± 2.1) and overweight adolescents (26.4, SD ± 2.6). Similarly, AST, ALT, and GGT levels were significantly higher among obese adolescents than among normal or overweight adolescents (Table 1).

Table 2 presents the mean concentrations of heavy metals in blood and urine samples across various demographic and lifestyle

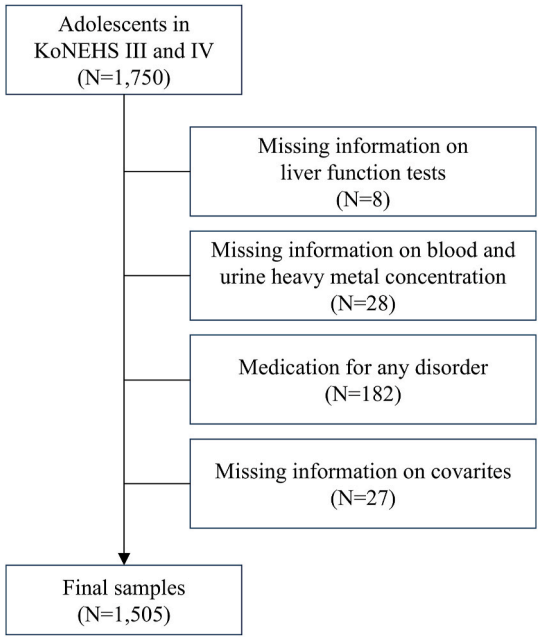

Fig. 1. A flowchart of the study participants.
KoNEHS, Korean National Environmental Health Survey.

Table 1
Characteristics of the study participants and descriptive statistics of biomarkers for NAFLD by individual characteristics.

Characteristics	N (%)	HSI		AST	ALT	GGT
		Score	HSI >36	U/L	U/L	U/L
		mean (±SD)	n (%[a])	mean (±SD)	mean (±SD)	mean (±SD)
Total	1505 (100)	29.2 (±5.3)	139 (9.2)	21.9 (±7.7)	17.3 (±15.9)	14.6 (±8.9)
Gender						
Girls	797 (53.0)	29.1 (±4.5)	51 (6.4)[c]	20.1 (±5.9)[c]	13.9 (±10.2)[c]	12.0 (±6.8)[c]
Boys	708 (47.0)	29.3 (±6.0)	88 (12.4)	23.9 (±9.0)	21.1 (±19.9)	17.5 (±10.1)
Age (years)						
Middle school (12–14)	707 (47.0)	28.2 (±5.0)[c]	51 (7.2)[b]	22.6 (±8.4)[b]	16.9 (±17.4)	13.6 (±8.7)[c]
High school (15–17)	798 (53.0)	30.1 (±5.3)	88 (11.0)	21.3 (±7.1)	17.6 (±14.5)	15.4 (±9.0)
Maternal education						
≤ High school	686 (45.6)	29.3 (±5.4)	68 (9.9)	21.6 (±6.8)[b]	17.1 (±14.7)	14.7 (±9.3)
College	738 (49.0)	29.0 (±5.1)	61 (8.3)	21.8 (±7.1)	17.0 (±14.1)	14.4 (±7.4)
Above college	81 (5.4)	29.8 (±6.0)	10 (12.3)	24.7 (±16.0)	22.4 (±32.2)	15.2 (±16.2)
BMI (kg/m^2)						
Normal (<23)	1000 (66.4)	26.4 (±2.6)[c]	2 (0.2)[c]	21.1 (±4.8)[c]	14.0 (±6.1)[c]	12.8 (±5.2)[c]
Overweight (23–25)	219 (14.6)	31.2 (±2.1)	5 (2.3)	21.2 (±4.8)	17.2 (±8.8)	15.0 (±6.4)
Obese (≥25)	286 (19.0)	37.2 (±5.1)	132 (46.2)	25.0 (±14.3)	29.1 (±31.2)	20.5 (±15.7)
Smoking						
Never smoker	1427 (94.8)	29.1 (±5.2)	129 (9.0)	21.8 (±7.7)	17.1 (±15.7)	14.4 (±8.4)[b]
Former smoker	35 (2.3)	28.8 (±4.2)	2 (5.7)	22.4 (±5.7)	17.5 (±11.0)	15.4 (±5.8)
Current smoker	43 (2.9)	30.9 (±6.5)	8 (18.6)	22.9 (±9.4)	22.5 (±25.3)	19.0 (±20.6)
Alcohol consumption						
No	1142 (75.9)	29.5 (±5.3)	102 (8.9)	21.8 (±7.6)	17.0 (±16.0)	14.2 (±8.1)[c]
Yes	363 (24.1)	29.4 (±5.5)	37 (10.2)	22.2 (±8.3)	18.1 (±16.3)	15.8 (±11.1)
Physical activity						
Yes	921 (61.2)	29.4 (±5.5)[b]	92 (10.0)	21.3 (±7.7)[c]	17.0 (±15.8)	14.0 (±8.6)[b]
No	584 (38.8)	28.8 (±4.8)	47 (8.0)	22.8 (±7.8)	17.8 (±16.1)	15.4 (±9.3)

NAFLD, non-alcoholic fatty liver disease; HSI, hepatic steatosis index; AST, aspartate aminotransferase; ALT, alanine aminotransferase; GGT, gamma-glutamyl transferase; BMI, body mass index; SD, standard deviation.
Student's T-test or analysis of variance (ANOVA) was performed to test the differences in heavy metal exposure markers according to demographic variables.
[a] Percentage of participants with HSI >36 in a row.
[b] $P < 0.05$.
[c] $P < 0.001$.

factors. The geometric mean (GM) of BPb, BHg, UHg, and UCd were 0.81 μg/dL, 1.36 μg/L, 0.22 μg/gCr, and 0.13 μg/gCr, respectively. The numbers of samples below LODs of BPb, BHg, UHg, and UCd were 32 (2.1 %), 1 (0.7 %), 52 (3.5 %), and 189 (12.6 %), respectively. Among BPb, BHg, UHg, and UCd, we observed weak correlations (Pearson's correlation coefficient: 0.08–0.15) (Supplementary Fig. S2). We observed that boys and those with unfavorable lifestyle (smoking, alcohol consumption, and lack of physical activity) showed higher mean concentration levels of heavy metals in blood (BPb, BHg) than the corresponding groups. In addition, obese adolescents showed slightly higher BHg than overweight and normal adolescents.

Fig. 2 depicts the exposure–response associations. Significant positive linear associations of BHg with HSI, ALT, and GGT were observed, whereas the association of BHg with AST showed a steep increase above 1.2 in log-2 transformed levels (or 1.4 μg/L in geometric level). Negative associations were observed between BPb and HSI and GGT, but they were not statistically significant. Unrinay exposure levels showed null associations with NAFLD biomarkers (see Fig. 3).

Table 3 shows the changes in biomarkers for NAFLD (HSI, AST, ALT, and GGT) per 2-fold increase in heavy metal exposure. Each 2-fold increase in BHg was strongly associated with increases in HSI (β = 0.21, 95 % confidence interval [CI]: 0.08–0.35), AST (β = 0.65, 95 % CI: 0.13–1.17), ALT (β = 1.70, 95 % CI: 0.70–2.71), and GGT (β = 0.76, 95 % CI: 0.21–1.32). Similarly, a 2-fold increase in UHg levels was associated with increases in his (β = 0.19, $P < 0.001$), ALT (β = 0.97, $P = 0.017$), and GGT (β = 0.80, $P < 0.001$), whereas the association of UHg with AST was not significant. However, exposure to BPb and UCd were not associated with biomarkers for NAFLD.

Mixture models showed significant associations of heavy metals and NAFLD biomarkers (Supplementary Table S1). WQS results showed HSI was significantly associated with the heavy metal mixture (β = 0.06, 95 % CI: 0.01–0.11) per one quartile increase in the metal mixture). In the WQS model, BHg was found to be the most important variable for the mixture association, as the final contributing weights of bHg, UCd, BPb, and UHg were 0.84, 0.08, 0.07, and <0.01, respectively (Supplementary Fig. S3). Similarly, we observed strong positive associations of the heavy metal mixture with ALT (β = 0.57; 95 % CI: 0.12–1.02 per one quartile increase in the metal mixture) and GGT (β = 0.33; 95 % CI: 0.10–0.55 per one quartile increase in the metal mixture)). Similarly, ALT and GGT showed positive associations with metal mixtures and BHg was most contributing variable followed by UHg. However, we did not observe any significant mixture association with AST.

The association of the overall mixture with the HSI was not significant in the BKMR model (Fig. 2). As the metal mixture increased, there was an increasing trend in HSI score, but this was not statistically significant. In this model, BPb, BHg, UHg, and UCd showed PIPs higher than 0.5 (Supplementary Table S2), and there were positive associations of BHg, and UHg with HIS when other heavy metal

Table 2
Heavy metal concentration in blood and urine among the participants.

Characteristics	N (%)	Geometric mean ± geometric standard deviation			
		BPb (μg/dL)	BHg (μg/L)	UHg (μg/gCr)	UCd (μg/gCr)
Total		0.81 ± 1.53	1.36 ± 1.65	0.22 ± 1.90	0.13 ± 2.61
below LOD, N (%)	1505 (100)	32 (2.1 %)	1 (0.7 %)	52 (3.5 %)	189 (12.6 %)
Gender					
Girls	797 (53.0)	0.70 ± 1.54[b]	1.28 ± 1.65[b]	0.25 ± 1.93	0.14 ± 2.59
Boys	708 (47.0)	0.94 ± 1.43	1.46 ± 1.63	0.19 ± 1.82	0.12 ± 2.60
Age					
Middle school (12–14)	707 (47.0)	0.81 ± 1.56	1.37 ± 1.66	0.23 ± 1.85	0.13 ± 2.68
High school (15–17)	798 (53.0)	0.81 ± 1.5	1.36 ± 1.64	0.21 ± 1.94	0.13 ± 2.55
Maternal Education					
≤ High school	686 (45.6)	0.82 ± 1.55	1.35 ± 1.59[a]	0.23 ± 1.98	0.14 ± 2.57[a]
College	738 (49.0)	0.80 ± 1.52	1.37 ± 1.67	0.22 ± 1.82	0.12 ± 2.64
Above college	81 (5.4)	0.82 ± 1.43	1.46 ± 1.81	0.19 ± 1.90	0.12 ± 2.50
Body mass index					
<23 kg/m^2	1000 (66.4)	0.80 ± 1.55	1.32 ± 1.64[b]	0.22 ± 1.91	0.14 ± 2.58
23–25 kg/m^2	219 (14.6)	0.84 ± 1.49	1.33 ± 1.63	0.21 ± 1.81	0.13 ± 2.59
≥25 kg/m^2	286 (19.0)	0.82 ± 1.50	1.55 ± 1.64	0.21 ± 1.92	0.12 ± 2.71
Smoking					
Never smoker	1427 (94.8)	0.80 ± 1.53[a]	1.36 ± 1.64[a]	0.22 ± 1.89	0.13 ± 2.62
Former smoker	35 (2.3)	0.98 ± 1.39	1.51 ± 1.89	0.20 ± 1.77	0.10 ± 2.56
Current smoker	43 (2.9)	0.93 ± 1.51	1.53 ± 1.71	0.18 ± 2.07	0.14 ± 2.40
Alcohol consumption					
No	1142 (75.9)	0.79 ± 1.54[b]	1.34 ± 1.65	0.22 ± 1.89	0.13 ± 2.61
Yes	363 (24.1)	0.88 ± 1.49	1.43 ± 1.64	0.21 ± 1.92	0.13 ± 2.61
Exercise					
Yes	921 (61.2)	0.77 ± 1.54[a]	1.34 ± 1.68	0.23 ± 1.89	0.14 ± 2.52
No	584 (38.8)	0.87 ± 1.51	1.40 ± 1.59	0.21 ± 1.91	0.12 ± 2.72

BPb, blood lead; BHg, blood mercury; UHg, urinary mercury; UCd, urinary cadmium; LOD, limit of detection.
Student's t-test or analysis of variance (ANOVA) was performed to test the differences in heavy metal exposure markers according to demographic variables.
[a] $P < 0.05$.
[b] $P < 0.001$.

exposure are median values, despite of the insignificance (Supplementary Fig. S4). ALT and GGT were significantly and positively associated with an increase of heavy metal mixture. In the association between AST and heavy metal exposures, BHg and UHg showed PIPs higher than 0.5. In the association between GGT and heavy metal exposures, BHg showed PIPs higher than 0.5, and increased BHg was significantly associated with increased GGT in case of other exposure variables are fixed.

4. Discussion

Recently, NAFLD has emerged as a substantial public health concern among adolescents, with an alarming increase in its incidence and prevalence. According to Schwimmer et al. (2019), the prevalence of NAFLD among adolescents is approximately 10 % in the U.S. and 5.9 % in South Korea, with a significantly higher rate among obese individuals [3,8]. This disease, characterized by excessive fat accumulation in the liver, has severe implications for future health outcomes, including the development of cirrhosis, hepatocellular carcinoma, and cardiovascular disease [2]. The etiology of NAFLD in adolescents is multifactorial. Notably, obesity, particularly central obesity, is a well-established risk factor for NAFLD, and this association has been suggested to be mediated by insulin resistance [36]. Dietary habits, such as a high intake of saturated fats, fructose, and sugar-sweetened beverages, have also been linked to NAFLD among adolescents [37].

In a cross-sectional study with 1505 adolescents in South Korea, we found significant associations of mercury levels with NAFLD with 0.21 points higher HSI, 0.50 U/L higher AST, 1.70 U/L higher ALT, and 0.76 U/L higher GGT by a 2-fold increase in blood mercury level. The results of mixture models support that the mercury level in blood was the most important contributor to the NAFLD among heavy metals. However, other metals (Pb and Cd) were not significantly associated with NAFLD. Some of our findings are in line with previous studies. A panel study of 508 Korean adults reported that a 2-fold increase in BHg was significantly associated with GGT levels increased by +11.0 % in women and +8.1 % in men [38]. Another panel study of 560 Korean elderly adults reported that, compared to the adults with lowest quartile of BHg, those with highest quartile of BHg showed 0.07, 0.12, and 0.12 U/L higher levels of AST, ALT, and GGT, respectively [39]. A recent study in the US using National Health and Nutrition Examination Survey (NHANES) data from 1999 to 2014 reported significant associations in subgroup analyses, as the highest quartile of mercury level group (vs. the lowest quartile) showed significantly elevated ALT levels (>22 U/L in girls and >25 U/L in boys) with the ORs of 1.76 among non-Hispanic white adolescents and 1.41 among normal or underweight adolescents [14]. Yang et al. analyzed 1143 adolescents aged 12–19 years using NHANES data (2011–2016) and reported that an increase of 1 μg/L in BHg was associated with 1.57 U/L elevated ALT levels in girls [40]. In addition to Hg exposure in childhood, in-utero exposure to Hg has been linked to elevated ALT levels at 8

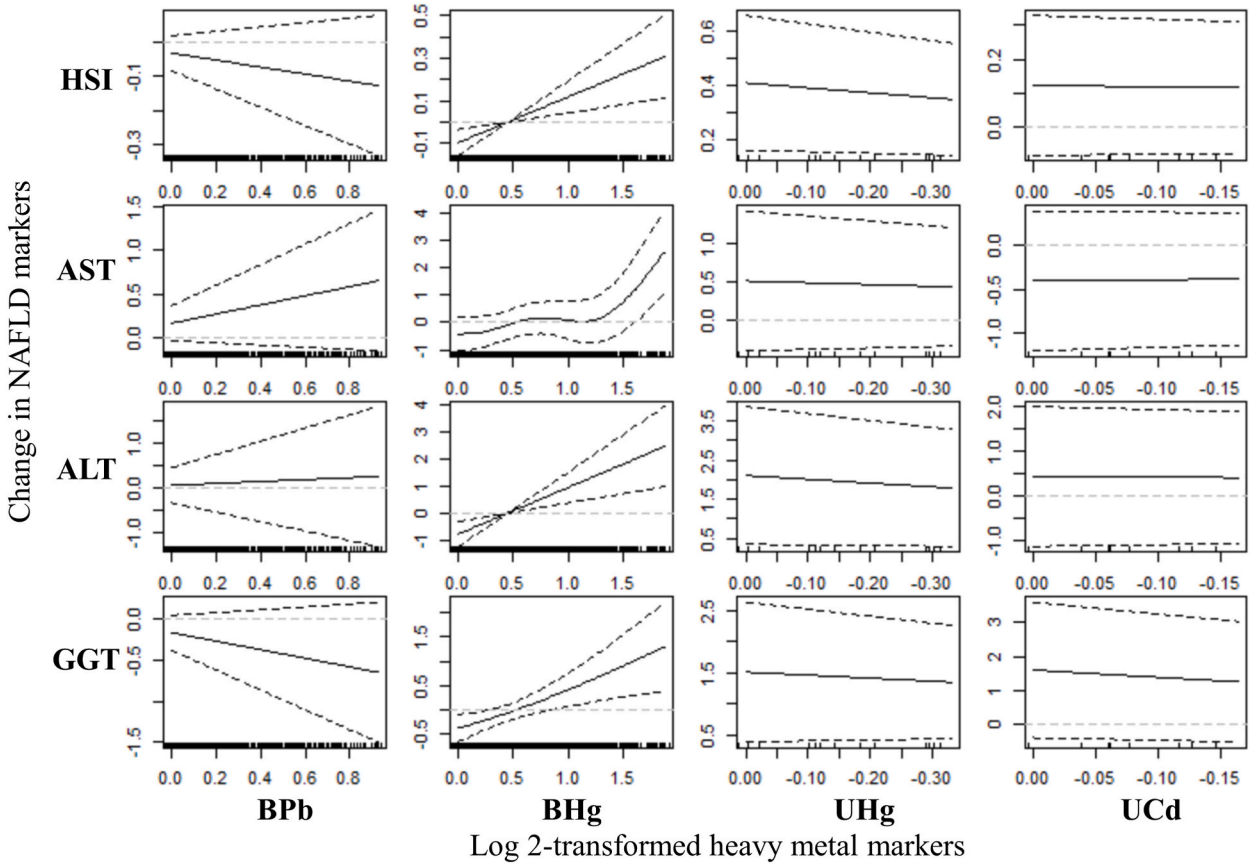

Fig. 2. Associations between the overall mixture metals and NAFLD biomarkers in BKMR BKMR, Bayesian kernel machine regression; HSI, hepatic steatosis index; AST, aspartate aminotransferase; ALT, alanine aminotransferase; GGT, gamma-glutamyl transferase
The cumulative effect of a mixture of four heavy metal exposure markers (mercury in blood, cadmium in urine, mercury in urine, and lead in blood) on HSI, AST, ALT, and GGT was shown. The models were adjusted for age, sex, maternal educational level, body mass index, smoking, alcohol consumption, and exercise.

years of age [41]. Despite these studies, there remains a dearth of comprehensive studies, particularly those focusing on adolescents, that investigate the potential influence of Hg exposure on NAFLD, underscoring the urgent need for further research in this field to fully elucidate the mechanisms and public health implications.

Adolescents are more sensitive to exposure to environmental heavy metals, because they experience rapid cell division, organ formation, and rapid growth [42]. NAFLD is closely associated with obesity. Therefore, heavy metals potential effects on obesity could explain the observed association with NAFLD. A recent systematic review and meta-analysis study showed a significant association between mercury and obesity [43]. Chronic exposure to low-level mercury disrupts metabolic activity and hormonal balance of adipose tissue, resulting in obesity [44]. In Korea, a study using nationally representative data showed an association between obesity and blood mercury level in adolescent [45]. Hg could also affect the liver directly, which plays a central role in detoxification and excretion of Hg from the body. Once Hg enters the body, it is transported to the liver, where it is biotransformed for excretion. Glutathione S-transferase, a liver enzyme, facilitates the conjugation of toxic Hg ions to glutathione (GSH), a tripeptide molecule with strong antioxidant properties. This reaction forms a less toxic and more soluble complex, which is easily excreted from the body. After conjugation, the Hg-GSH complex is transported out of the liver cells into the bile [46]. Excess absorption of Hg can lead to depletion of GSH [47], which can impede detoxification. Hg exposure also induces oxidative stress, apoptosis [48], mitochondrial dysfunction, and lipid peroxidation in the liver [49]. In the animal studies, mercury exposure induces the elevated liver enzymes in blood, and degenerative and necrotic changes in liver tissue [50,51]. Hg-induced toxicity can also results in the alterations in the immune system, related to the pathogenesis of NAFLD [52]. These processes are associated with NAFLD development and progression [53].

In the present study, the mean concentration (±SD) of BHg among Korean adolescents was 1.55 (±0.96) μg/L. BHg concentrations can vary significantly depending on dietary habits, Hg exposure, and other environmental and lifestyle factors. Adolescents in countries with high seafood consumption (e.g., Japan) may have relatively high BHg concentrations because fish and shellfish are the main sources of methylmercury exposure in humans. Although the data were not nationally representative, a study published in 2014 found that the GM of BHg concentration in Japanese adolescents was around 4.55 μg/L [54]. However, in countries such as the United States, where seafood consumption is relatively lower than Japan, BHg levels are expected to be lower. For example, a study using

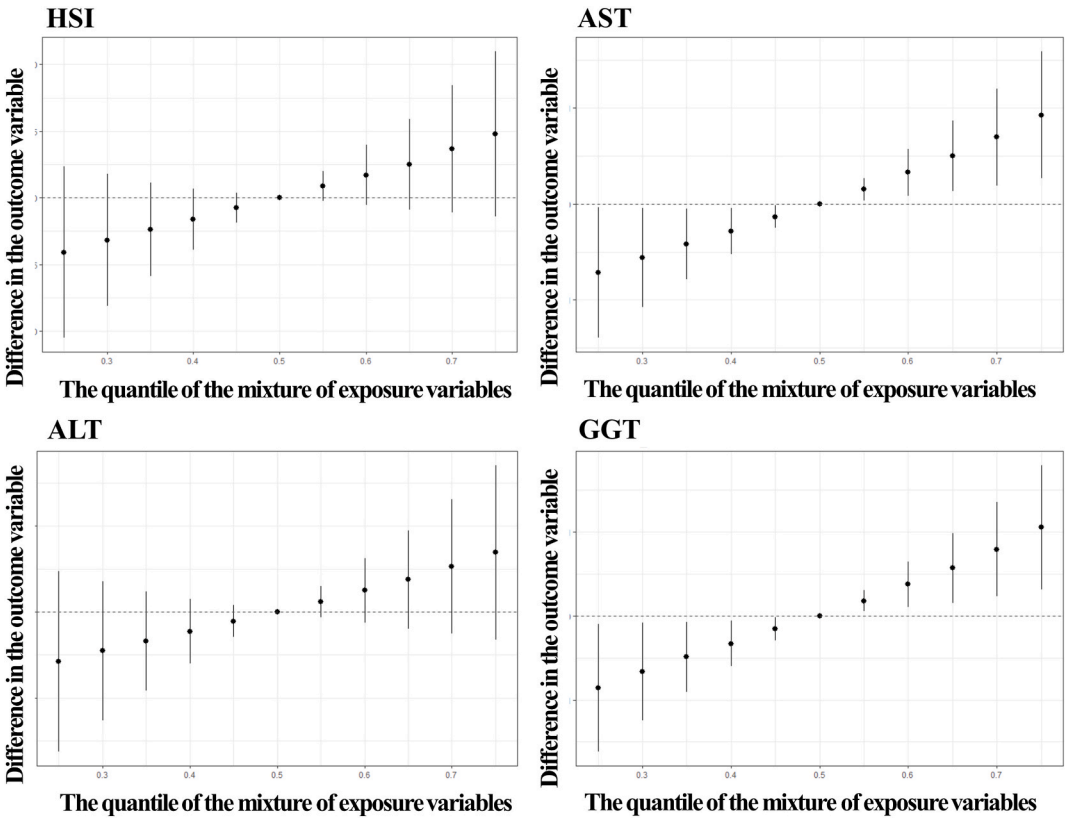

Fig. 3. Exposure-response associations between heavy metal exposure and non-alcoholic fatty liver disease biomarkers. HSI, hepatic steatosis index; AST, aspartate aminotransferase; ALT, alanine aminotransferase; GGT, gamma-glutamyl transferase; BPb, blood lead (ug/dL); BHg, blood mercury (ug/L); UHg, urinary mercury (ug/gCr); UCd, urinary cadmium (ug/gCr)
Models were adjusted for age, sex, maternal educational level, body mass index, smoking, alcohol consumption, and exercise.

Table 3
Multivariate linear regression models comparing between heavy metal exposure and non-alcoholic fatty liver disease biomarkers.

Biomarkers	Changes in biomarkers for NAFLD per 2-fold increase in heavy metals (95 % CI)			
	BPb	BHg	UHg	UCd
HSI	−0.10 (−0.26–0.06)	0.21 (0.08–0.35)[a]	0.19 (0.09–0.30)[b]	0.04 (−0.03–0.11)
AST	0.50 (−0.14–1.14)	0.65 (0.13–1.17)[a]	0.23 (−0.18–0.64)	−0.13 (−0.40–0.13)
ALT	0.20 (−1.05–1.44)	1.70 (0.70–2.71)[b]	0.97 (0.18–1.76)[a]	0.15 (−0.38–0.67)
GGT	−0.56 (−1.24–0.13)	0.76 (0.21–1.32)[a]	0.80 (0.37–1.23)[b]	0.11 (−0.18–0.40)

HSI, hepatic steatosis index; AST, aspartate aminotransferase; ALT, alanine aminotransferase; GGT, gamma-glutamyl transferase; CI, confidence interval; BPb, blood lead; BHg, blood mercury; UHg, urinary mercury; UCd, urinary cadmium.
Models were adjusted for age, sex, maternal educational level, body mass index, smoking, alcohol consumption, and exercise.
[a] $P < 0.05$.
[b] $P < 0.001$.

NHANES 2011–2012 data reported that the mean (±SD) BHg among U.S. adolescents was 0.67 (±0.85) μg/L. The German Human Biomonitoring Commission has presented guidance values based on epidemiological studies, indicating that BHg <5 μg/L has no adverse health effects in children and adults [55]. However, recent studies and our study suggest that the presence of BHg lower than 5 μg/L could have detrimental effects. Therefore, it is crucial to minimize Hg exposure as much as possible.

Dietary methylmercury is one of the major sources of Hg exposure, but it is not typically found in urine. Therefore, UHg could provide background exposure to inorganic and metal mercury, other than dietary methylmercury, while BHg could reflect methylmercury exposure [56] Inorganic and methyl mercury derived from industrial areas and commercial products, methyl mercury, is closely associated with fish and seafood consumption. As the half-life of each biological monitoring index is also different, BHg has been used to monitor the biological burden in acute exposure, and UHg has been widely used to monitor occupational exposure [57]. In our study, BHg and UHg were significantly associated with NAFLD. This result implies that all type of Hg across organic, inorganic, and metal Hg could have detrimental effects on human liver.

Previous studies have reported an association between the exposure to multiple heavy metals and NAFLD. Meiduo et al. studied the urine concentrations of chromium, Cd, Pb, and manganese and serum ALT and AST levels in 1171 participants and found a significant negative association between AST and ALT levels using BKMR models [58]. Chang et al. measured serum chromium, cobalt, Cd, and Pb levels and liver function in 785 adults with a mean age of 57 years and found a significant association between concurrent exposure to a heavy metal mixture and elevated AST, but the exposure was also associated with low ALT [59]. Furthermore, a significant association between heavy metal mixtures (Cd, Hg, and Pb) and NAFLD indices has been reported [60]. However, multiple exposures to Hg, Cd, or Pb were not significantly associated with NAFLD in our study. Although the results of the WQS regression for the association between heavy metal exposure and HSI and GGT levels were significant, Hg exposure comprised almost all weighted scores. Because adolescents have relatively lower levels of heavy metal exposure than adults, interactive effects among heavy metals on liver injury could be observed only at higher levels than the current levels among our participants. Studies on the effects of heavy metals on NAFLD among adolescents are scarce; therefore, more research is needed on this topic, especially among adolescents who are hexposed to high levels of heavy metals.

We used nationally representative samples including a relatively large number of adolescents and measured biological indices using accurate and validated methods. The association between Hg exposure and NAFLD as measured using the HSI is a novel finding. However, this study has several limitations. First, due to the cross-sectional nature of the KoNEHS, it is impossible to conclude causality between exposure to heavy metals and alterations in hepatic biomarkers. Second, we merged two survey cycles, and appropriate sampling weights were not assigned to the study population; therefore, the results could not represent the general population of Korean adolescents. Third, blood heavy metals, which are indicative of relatively recent exposure, unlike urine heavy metals, fall short of elucidating the body burden of long-term exposure. Hence, the results must be interpreted with caution. Fourth, although we excluded individuals with any disease status on medication, it is possible that the study included participants with mild liver dysfunction due to other factors, such as over-the-counter drugs. Fifth, although our study focused on Pb, Cd, and Hg exposure, the possibility of simultaneous exposure to other metals capable of inducing oxidative stress and impairing liver function could not be ruled out. Sixth, genetic predisposition to NAFLD may differ by race and ethnicity; therefore, our findings have limited generalizability to other populations. Finally, we were unable to obtain information on dietary intake and did not consider it in the model, although dietary pattern could influence heavy metal exposure levels [61]. However, as dietary pattern is related to BMI in *in vivo* studies, we used BMI as a proxy of dietary intake and adjusted for it in the model. Nevertheless, we cannot rule out residual confounding of dietary intake when estimating the association between heavy metal exposure and NAFLD.

5. Conclusion

We found a noteworthy association between mercury exposure and NAFLD in adolescents, taking into account exposure to lead and cadmium. This finding underscores the potential role of mercury exposure in increasing the incidence of NAFLD in adolescents. Because the primary approach to managing NAFLD in adolescents involves risk factor management, it is crucial to emphasize the necessity of efforts to reduce avoidable mercury exposure for the prevention and management of NAFLD in adolescents [62]. Further research is required to elucidate the exact physiological mechanisms that link Hg exposure to the development of NAFLD, and broad-spectrum studies are needed to understand the potential effects of the exposure to multiple heavy metals on NAFLD.

Ethical approval

The Institutional Review Board (IRB) of the National Institute of Environmental Research (NIER), Korea, approved the KoNEHS (IRB No. NIER-2016-BR-003-01, NIER-2016-BR-003-03, NIER-2018-BR-003-02).

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Data Availability

The database used in the present study are available upon request to the National Institute of Environmental Research in South Korea.

CRediT authorship contribution statement

Dong-Wook Lee: Writing – original draft, Investigation, Conceptualization. **Jongmin Oh:** Writing – review & editing, Methodology, Formal analysis. **Yu Min Lee:** Writing – review & editing. **Hyun-Joo Bae:** Writing – review & editing, Project administration. **Youn-Hee Lim:** Writing – review & editing, Supervision, Conceptualization.

Declaration of Generative AI and AI-assisted technologies in the writing process

Nothing to disclose.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.heliyon.2024.e37840.

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