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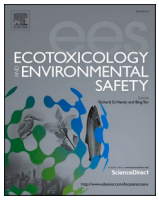

Association between endocrine-disrupting chemical mixtures and non-alcoholic fatty liver disease with metabolic syndrome as a mediator among adults: A population-based study in Korea

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ABSTRACT

Endocrine-disrupting chemicals (EDCs) may play a role in non-alcoholic fatty liver disease (NAFLD); however, studies on the combined effects of EDC mixtures on NAFLD development are limited. Here, we explored the association between exposure to EDC mixtures and NAFLD and investigated the potential mediating role of metabolic syndrome (MetS). We included participants from the Korean National Environmental Health Survey Cycle 4 (2018–2020) and quantified the urinary concentrations of various EDCs—eight phthalate metabolites, three phenols, one antibacterial compound, four parabens, four polycyclic aromatic hydrocarbons, and one pyrethroid pesticide metabolite—as well as serum concentrations of five perfluorinated compounds (PFCs). NAFLD was defined as a hepatic steatosis index (HSI) ≥36 or a fatty liver index (FLI) ≥60. Weighted quantile sum (WQS) regression was employed to evaluate the associations between EDC mixtures and the risk of MetS or NAFLD. Causal mediation analysis was conducted to explore the potential mediating effect of MetS on the association between mixtures of EDCs and NAFLD risk. All estimates were adjusted for age, sex, educational level, physical activity, smoking status, involuntary smoking, and drinking habits. A total of 2942 adults were included in the analysis. Moderate-to-high positive correlations were identified between phthalate metabolites and PFCs. Higher WQS scores were associated with an elevated risk of MetS and NAFLD. The sex-stratified WQS regression model showed that the interactions between the WQS index and sex were significant for MetS and NAFLD. According to the causal mediation analysis, both the direct and indirect effects of EDC mixtures on NAFLD, with MetS as a mediator, were significant in females. Collectively, these findings highlight the need for interventions that could address both EDC mixture exposure and metabolic status to effectively reduce the risks associated with NAFLD and its related complications.

Abbreviations: 3-PBA, 3-Phenoxybenzoic acid; BMI, body mass index; BP, butylparaben; BPA, Bisphenol A; BPF, Bisphenol F; BPS, Bisphenol S; CI, confidence interval; CMA, causal mediation analysis; DF, Detection frequency; EDCs, endocrine-disrupting chemicals; EP, ethyl paraben; FLI, fatty liver index; HCC, hepatocellular carcinoma; GM, Geometric mean; KoNEHS, Korean National Environmental Health Survey; LOD, Limit of detection; MBzP, Monobenzyl phthalate; MCPP, Mono 3-carboxypropyl phthalate; MDCs, metabolism-disrupting chemicals; MECPP, Mono 2-ethyl-5-carboxypentyl phthalate; MEHHP, Mono 2-ethyl-5-hydroxyhexyl phthalate; MEOHP, Mono 2-ethyl-5-oxohexyl phthalate; MEP, Mono-ethyl phthalate; MMP, Mono-methyl phthalate; MnBP, Mono-n-butyl phthalate; MP, methyl paraben; NAP, naphthol; NAFLD, Non-alcoholic fatty liver disease; OHFlu, hydroxyfluorene; OHPhe, hydroxyphenanthrene; OHP, hydroxypyrene; OR, Odds ratio; TCS, triclosan; PFOA, perfluorooctanoic acid; PFOS, perfluorooctane sulfonic acid; PFHxS, Perfluorohexane sulfonate; PFNA, perfluorononanoic acid; PFDeA, perfluorodecanoic acid; PP, propyl paraben; WQS, weighted quantile sum.

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1. Introduction

Non-alcoholic fatty liver disease (NAFLD) is a metabolic liver disorder characterized by the excessive but reversible buildup of lipids in hepatocytes in individuals without substantial alcohol consumption, with or without additional macrophage infiltration and inflammation. NAFLD has the potential to progress to irreversible fibrosis, cirrhosis, or hepatocellular carcinoma (HCC) (Chalasani et al., 2018; Hardy et al., 2016; Lim et al., 2010; Wesolowski et al., 2017; White et al., 2012; Wree et al., 2013). Currently, NAFLD is considered the most prevalent chronic liver condition worldwide (Loomba and Sanyal, 2013; Negi et al., 2022), affecting approximately 25 % of adults, with a pooled prevalence of approximately 27.4 % in Asian countries (Fan et al., 2017; Younossi et al., 2016).

The liver functions as the hub of metabolic homeostasis, regulating several processes such as the synthesis of blood-clotting enzymes, the biosynthesis and turnover of hormones, protein and bile synthesis, drug and lipid metabolism, glycogen storage and release, and gluconeogenesis (Erslev, 1994; Kuntz and Kuntz, 2008). Impairment of these functions, especially those related to lipid metabolism, is associated with various diseases. Consequently, NAFLD is identified as a risk factor for cardiovascular diseases, diabetes, and increased mortality risk (Lake et al., 2022; Mantovani et al., 2022). However, NAFLD is often disregarded during clinical examination until progression to advanced stages, where permanent liver damage occurs; consequently, several patients with NAFLD remain unaware of their condition. Furthermore, the therapeutic options available are limited to dietary modifications and lifestyle adjustments, given that no medications have obtained US Food and Drug Administration approval to date. Therefore, it is essential to clarify the factors that trigger NAFLD, leading to an increased demand for liver transplantation, as well as the occurrence of advanced complications, such as HCC.

NAFLD is a complex condition shaped by various factors that influence its development and progression, including genetics, overnutrition, a sedentary lifestyle, obesity, type 2 diabetes mellitus, and environmental factors (Fang et al., 2018; Geisler and Renquist, 2017; Lim et al., 2010; Negi et al., 2021). Endocrine-disrupting chemicals (EDCs), many of which undergo metabolism and storage in the liver, have been proposed as environmental factors that may exert detrimental effects on the liver, potentially contributing to NAFLD development (Al-Eryani et al., 2015; Jin et al., 2014; Lim et al., 2009).

EDCs comprise a diverse group of natural and synthetic substances that can adversely impact health by disrupting or altering endocrine and metabolic systems (Casals-Casas and Desvergne, 2011; Gore et al., 2015). Owing to their impact on adipogenesis, increased hepatic insulin resistance, elevated accumulation of hepatic triglycerides (TGs), mitochondrial dysfunction, inflammation, and oxidative stress, EDCs are classified as metabolism-disrupting chemicals (MDCs) (Armstrong and Guo, 2019; Heindel et al., 2017).

The presence of EDCs is ubiquitous; hence, daily exposure to them is inevitable. Moreover, numerous EDCs have been shown to exert distinct effects on health when acting in combination. Exposure to a mixture of EDCs has been associated with negative outcomes, including heightened oxidative stress, elevated activities of antioxidant enzymes, alterations in the estrous cycle, and reduced steroidogenesis. Furthermore, it has been observed that mixtures of EDCs adversely influence cognitive function in the elderly, and can lead to detrimental effects on liver and kidney function, the endocrine system, neurodevelopmental processes, and thyroid function (Hamid et al., 2021; Midya et al., 2022; Ribeiro et al., 2017; Yilmaz et al., 2020; Zuo et al., 2024). Therefore, an approach that only considers the effects of a particular EDC in isolation may fail to comprehensively capture the intricate nature of exposure to EDC mixtures. However, previous studies that have examined the impact of EDC exposure on NAFLD primarily concentrated on individual chemicals; research on the combined effects of EDC mixtures on the development of NAFLD is scarce.

Therefore, we investigated the effects of EDC mixtures on NAFLD and explored the potential role of metabolic disorders in determining the relationship between these two events. Examining these factors will provide novel insights into the complex interactions among EDCs, metabolic syndrome (MetS), and NAFLD.

2. Materials and methods

2.1. Data source and study population

We used publicly available data from the Korean National Environmental Health Survey (KoNEHS) cycle 4 (2018–2020), a cross-sectional survey conducted by the National Institute of Environmental Research (NIER-2018–01–01–001), to assess the prevalence of environmental chemical exposure within the general Korean population. KoNEHS participants were selected through a multistage stratified sampling approach, ensuring a study population representative of the entire nation. All procedures were performed in compliance with relevant laws and institutional guidelines. KoNEHS was approved by the Institutional Review Board of the NIER in Korea (approval number: NIER-2016-Br-003–01). All individuals provided written informed consent for study participation. Individuals aged 19 or older who participated in KoNEHS cycle 4 and provided complete data on all exposures, outcomes, and confounding variables were included in the final analysis.

2.2. Exposure analysis

Eight phthalate metabolites (mono-[2-ethyl-5-hydroxyhexyl] phthalate, mono-[2-ethyl-5-oxohexyl] phthalate, mono-n-butyl phthalate, monobenzyl phthalate, mono-[2-ethyl-5-carboxypentayl] phthalate, mono-[carboxyoctyl] phthalate, mono-[carboxynonyl] phthalate, and mono[3-carboxylpropyl] phthalate); three phenols (bisphenol A, bisphenol F [BPF], and bisphenol S [BPS]); one antimicrobial compound (triclosan [TCS]); four parabens (methyl paraben [MP], ethyl paraben [EP], propyl paraben [PP], butylparaben [BP]); four polycyclic aromatic hydrocarbons (PAHs) (hydroxypyrene [OHP], naphthol [NAP], hydroxyfluorene, and hydroxyphenanthrene [OHPhe]); one pesticide (3-Phenoxybenzoic acid [3-PBA]); and five perfluorinated compounds (PFC) (perfluorooctanoic acid [PFOA], perfluorooctane sulfonic acid [PFOS], perfluorohexane sulfonate [PFHxS], perfluorononanoic acid [PFNA], and perfluorodecanoic acid) were measured in KoNEHS cycle 4. Phthalate metabolites, phenols, TCS, and parabens from spot urine samples were quantified using high-performance liquid chromatography with tandem mass spectrometry (HPLC-MS/MS). PAHs and 3-PBA from spot urine samples were measured using gas chromatography-mass spectrometry. PFCs in the serum of participants were measured using HPLC-MS/MS.

The procedures for collecting biological samples, environmental chemical measurement, and quality control have been detailed previously (Jung et al., 2022). Values below the limit of detection (LOD) were replaced with the LOD value divided by the square root of two, and the final exposure concentrations were calculated by adjusting for urea creatinine concentration.

2.3. Outcome analysis

2.3.1. Metabolic syndrome

MetS was defined by the presence of three or more of the following criteria: 1) abdominal obesity: ≥90 cm in males or ≥85 cm in females (Korean-specific cutoffs defined by the Korean Society of Obesity; Lee et al., 2007); 2) hypertriglyceridemia: serum TG ≥150 mg/dL, or receiving treatment for lipid abnormality; (3) low high-density cholesterol (HDL-C): serum HDL-C <40 mg/dL in males and <50 mg/dL in females, or receiving treatment for dyslipidemia; (4) high blood pressure: systolic blood pressure (SBP) ≥130 mmHg or diastolic blood pressure (DBP) ≥85 mmHg, or receiving treatment for hypertension;

and (5) hyperglycemia: hemoglobin A1c (HbA1c) ≥5.7 % or receiving anti-diabetic medication. Given the absence of fasting glucose values in KoNEHS cycle 4 data, we utilized HbA1c values as a substitute for fasting glucose values in the definition of MetS (Cavero-Redondo et al., 2019; Park et al., 2012).

2.3.2. NAFLD

Although a liver biopsy is considered the gold standard for diagnosing NAFLD, the procedure is highly invasive. Therefore, non-invasive prediction scores for NAFLD, such as the fatty liver index (FLI) and hepatic steatosis index (HSI), have been developed (Bedogni et al., 2006; Calori et al., 2011; Kahl et al., 2014; Kozakova et al., 2012; Lee et al., 2010; Sviklāne et al., 2018). In the current study, NAFLD was defined as HSI ≥36 or FLI ≥60. HSI and FLI values were calculated as follows:

$$\text{HSI} = 8 \times \text{alanine aminotransferase/aspartate aminotransferase ratio} + \text{body mass index (BMI)} \ (+2 \text{ if diabetic; } +2 \text{ if female sex}).$$

$$\text{FLI} = (e^{0.953 \times \text{loge (TG)} + 0.139 \times \text{BMI} + 0.718 \times \text{loge (γ-glutamyl-transferase)} + 0.053 \times \text{waist circumference} - 15.745}/(1 + e^{0.953 \times \text{loge (TG)} + 0.139 \times \text{BMI} + 0.718 \times \text{loge (γ-glutamyl-transferase)} + 0.053 \times \text{waist circumference} - 15.745}) \times 100$$

2.3.3. Covariates

Based on previous studies, we included age, sex, education (≤ high school or ≥ university), physical activity (no physical activity, exercise without sweat, or exercise with sweat), smoking (never smoked, past smoker, or current smoker), involuntary smoking (never smoked, ≤ twice a week, or ≥ three times a week), and drinking habits (not drinking, ≤ twice a month, ≤ twice a week, or ≥ three times a week) as covariates.

2.4. Statistical analysis

For descriptive analysis, characteristics and biochemical measures are presented as mean (standard deviation) or median (interquartile range) for continuous variables, and numbers (percentages) for categorical variables. Spearman's correlations were calculated to evaluate pairwise correlations between chemical exposures.

A weighted quantile sum (WQS) regression analysis was employed for a mixture analysis to evaluate the combined effects of the 26 chemicals considered, as well as the individual contributions of each chemical on MetS and NAFLD. In instances characterized by a high degree of correlation among multidimensional mixtures, the WQS regression analysis was employed to derive a single score for the targeted outcome (Carrico et al., 2015). This approach consists of two steps: estimation of the weighted index of the concentrations of multiple exposures, considering the strength of association with the targeted outcome, and evaluation of the significance of the association between this weighted index and the specified outcome. Furthermore, this procedure allows for the assessment of weights assigned to individual chemicals, enabling the identification of key components in the mixture that significantly contribute to the association. We fitted the WQS based on the quartiles of the concentration of exposures and conducted a WQS regression analysis, assuming that all WQS indices were positively associated with MetS and NAFLD. The dataset was partitioned into two subsets: a training set (40 %) for estimating the weighted average results and a validation set (60 %) for estimating the association between the weighted index and outcome of interest across 100 bootstrap samples of observations, along with implementing 100 repeated holdout cross-validations (Tanner et al., 2019). The contribution of the estimated weights for each exposure to the overall mixture effect was evaluated using a threshold of 0.038 (1/n, where n represents the number of chemicals included in the exposure). Given the well-established sex differences in metabolic features, we performed all analyses separately for male and female participants. In addition, we conducted a WQS-stratified interaction model to assess potential variations in the contribution of the mixture based on sex. This involved estimating sex-stratified weights and testing for significant differences in slopes between sexes by incorporating an interaction term between the weighted index and sex in each model.

Finally, we performed causal mediation analysis (CMA) (VanderWeele, 2016) to evaluate the direct effect of WQS scores on MetS and NAFLD independently of the mediator, the indirect effect mediated by MetS, and the percentage mediated, using a counterfactual approach. All analyses were adjusted for covariates and performed using R software version 3.6.3 (R Foundation for Statistical Computing, Vienna, Austria). The WQS regression analysis was performed using the "gWQS" package, while CMA was performed using the "mediation" package. A P-value of <0.05 indicated statistical significance.

3. Results

Table 1 lists the characteristics and biochemical parameters of the study population discriminated by sex. Among the 2942 participating adults, 1272 were males and 1670 were females. Overall, 1371 (46.6 %) participants were considered to have MetS. Based on FLI values, a total of 669 (22.7 %) participants were considered to have NAFLD. Based on HSI values, a total of 884 (30.0 %) participants were considered to have NAFLD.

Table 2 summarizes the LOD, detection frequencies, and distribution of chemical exposure. Chemical exposure was detected in the range of 61–100 %. Among the chemicals analyzed, OHPhe exhibited the lowest detection rate (61.1 %), followed by PP (73.2 %) and OHP (74.0 %). The correlation coefficients of chemical exposure are shown in Fig. 1. The results indicate significant moderate-to-high positive correlations between phthalate metabolites and PFCs.

The multivariable WQS regression models showed a positive association between the EDC mixture and both MetS and NAFLD. The mixture of EDCs significantly increased the risk of MetS (Odds ratio [OR]: 1.28; 95 % confidence interval [CI]: 1.09–1.51), NAFLD based on FLI value (OR: 1.25; 95 % CI: 1.03–1.53), and NAFLD based on HSI value (OR: 1.30; 95 % CI: 1.11–1.52). EP (0.12), PFOS (0.11), and PFNA (0.10) were associated with the highest MetS weights. PFNA (0.13), EP (0.12), and PFOS (0.08) showed the highest FLI weights. PFNA (0.17), PFHxS (0.10), and PFOS (0.09) exhibited the highest HSI weights (Fig. 2).

Based on stratified weights, the mixture effect was greater in females (overall weight: 61 %, 63.8 %, and 67.6 % for MetS, NAFLD based on FLI, and NAFLD based on HSI, respectively) than in males (Fig. 3). The results of the sex-stratified WQS regression (with interaction term included) revealed a significant interaction between the weighted index and sex for MetS (OR: 3.52; 95 % CI: 1.39–8.91), NAFLD based on FLI (OR: 2.52; 95 % CI: 1.04–6.12), and NAFLD based on HSI (OR: 5.00; 95 % CI: 2.30–10.89).

We explored the mediating role of MetS in the association between WQS scores and NAFLD in both sexes. The direct and indirect effects of the WQS score on NAFLD, with MetS serving as a mediator, were significant in the total population and females but not in males (Table 3). In females, the mediated proportion was approximately 30–40 %, regardless of whether NAFLD risk was defined by FLI or by HSI, thereby suggesting that MetS is a significant mediator in the association between WQS scores and the risk of NAFLD.

4. Discussion

In the current study, we demonstrated a positive association between EDC mixtures and NAFLD, as well as a significant interaction effect on the relationship between EDC mixtures and NAFLD based on sex, suggesting sex-specific differences in susceptibility. Furthermore, our findings indicate that MetS plays a significant role in mediating the association between EDC mixtures and NAFLD, particularly in females. This highlights the importance of considering sex-specific factors to

Table 1
Basic characteristics of study participants from the KoNEHS cycle 4 (2018–2020).

Characteristics	Total (N = 2942)	Males (N = 1272)	Females (N = 1670)	*P*-value
Age[a]	54 (42–64)	55 (42–65)	54 (41.25–63)	0.129
Education[b]				<0.001
≤High school	1670 (56.8)	663 (52.1)	1007 (60.3)	
≥University	1272 (43.2)	609 (47.9)	663 (39.7)	
Physical activity[b]				0.533
No physical activity	1567 (53.3)	663 (52.1)	904 (54.1)	
Exercise without sweat	203 (6.9)	88 (6.9)	115 (6.9)	
Exercise with sweat	1172 (39.8)	521 (41.0)	651 (39.0)	
Smoking[b]				<0.001
Never smoker	1901 (64.6)	320 (25.2)	1581 (94.7)	
Past smoker	586 (19.9)	542 (42.6)	44 (2.6)	
Current smoker	455 (15.5)	410 (32.2)	45 (2.7)	
Involuntary smoking[b]				<0.001
Never smoker	2650 (90.1)	1086 (85.4)	1564 (93.7)	
≤ Twice a week	138 (4.7)	93 (7.3)	45 (2.7)	
≥ Three times a week	154 (5.2)	93 (7.3)	61 (3.7)	
Drinking[b]				<0.001
Not drinking	908 (30.9)	256 (20.1)	652 (39.0)	
≤ Twice a month	995 (33.8)	349 (27.4)	646 (38.7)	
≤ Twice a week	586 (19.9)	328 (25.8)	258 (15.4)	
≥ Three times a week	453 (15.4)	339 (26.7)	114 (6.8)	
BMI (kg/m^2)[a]	24.7 (22.6–27.1)	25.3 (23.3–27.4)	24.2 (22.1–26.7)	<0.001
Waist Circumference (cm)[a]	85.0 (78.0–92.0)	89.4 (84.0–95.1)	81 (75.0–88.0)	0.661
HDL (mg/dL)[a]	53 (44–63)	48 (41–57)	57 (48–67)	<0.001
SBP (mmHg)[a]	131 (120–144)	134 (124–145)	128 (117–143)	0.932
DBP (mmHg)[a]	79 (71–87)	82 (74–90)	77 (69–85)	0.951
HbA1c (%)[a]	5.5 (5.3–5.9)	5.6 (5.3–6.0)	5.5 (5.3–5.8)	<0.001
MetS[b]				<0.001
Yes	1371 (46.6)	657 (51.7)	714 (42.8)	
No	1571 (53.4)	615 (48.3)	956 (57.2)	
AST (U/L)[a]	24 (21–29)	26 (22–30)	23 (20–27)	<0.001
ALT (U/L)[a]	21 (16–29)	25 (19–34)	18 (15–25)	<0.001
GGT (U/L)[a]	20 (13–33)	29 (19–49)	15 (11–23)	<0.001
FLI[a]	31.3 (12.8–57.2)	47.2 (25.3–70.9)	20.8 (8.1–42.3)	<0.001
HSI[a]	33.3 (30.3–36.8)	33.8 (30.6–37.2)	33.0 (30.0–36.4)	<0.001
NAFLD based on FLI[b]				<0.001
FLI <60	2273 (77.3)	815 (64.1)	1458 (87.6)	
FLI ≥60	669 (22.7)	457 (35.9)	212 (12.7)	
NAFLD based on HSI[b]				<0.001
HSI <36	2058 (70.0)	850 (66.8)	1208 (72.3)	
HSI ≥36	884 (30.0)	442 (33.2)	462 (27.7)	

Abbreviations: ALT, alanine aminotransferase; AST, aspartate aminotransferase; BMI, body mass index; DBP, diastolic blood pressure; FLI, fatty liver index; HbA1c, hemoglobin A1c; HDL, high density lipoprotein; HSI, hepatic steatosis index; GGT, gamma-glutamyl transferase; KoNEHS, the Korean National Environmental Health Survey; MS, metabolic syndrome; NAFLD, non-alcoholic fatty liver disease; SBP, systolic blood pressure. Data are presented as N (%) or median (Q1–Q3).

[a] Student's *t*-test was used to compare normally distributed continuous variables between males and females groups.

[b] A Chi-square test was used to test the distributions of categorical between males and females groups.

understand the complex interplay between EDC exposure, MetS, and NAFLD. Targeted prevention strategies and tailored interventions that account for these sex-specific differences may afford substantial efficacy in reducing the incidence and severity of NAFLD. Overall, our study provides novel insights into the intricate relationships between EDC mixture exposure, MetS, and NAFLD.

Four primary pathways contribute to the development of NAFLD: (1) elevated lipid uptake in the liver, (2) decreased export of very-low-density lipoprotein (VLDL) particles, (3) reduced activity of fatty acid oxidation, and (4) enhanced *de novo* lipogenesis (Ipsen et al., 2018; Kawano and Cohen, 2013). Hepatic lipid homeostasis is regulated by the intake of free fatty acids (FFAs), their *de novo* synthesis in hepatocytes, and their elimination through oxidation and TG synthesis. TGs are subsequently exported from the hepatocytes as VLDL particles. Fatty liver or steatosis occurs when hepatic uptake or *de novo* synthesis of FFAs surpasses their oxidation and secretion as TGs.

The accumulation of TGs within hepatocytes results in steatosis, and this abnormal lipid buildup in the liver exerts distinct effects on various hepatic cell types (Jou et al., 2008). The aberrant processing of FFAs in hepatocytes through Toll-like receptor 4 triggers macrophage activation, setting off a pro-inflammatory pathway and leading to the development of NAFLD (De Taeye et al., 2007; Malaguarnera et al., 2005; Wree et al., 2013). In addition, excess hepatic lipids serve as an activation signal, initiating the fibrotic process observed in more advanced liver conditions, including NASH and HCC (Michelotti et al., 2013; Wree et al., 2013).

Although further research is necessary to validate these effects in humans, the existing evidence supports the findings of our study, demonstrating that exposure to EDCs is associated with NAFLD. EDCs induce a cascade of events by continuously activating molecular pathways, including heightened hepatic insulin resistance, increased hepatic TG buildup, and mitochondrial dysfunction. These processes ultimately increase oxidative stress and lipid peroxidation while diminishing antioxidation, which is pivotal in NAFLD etiology (Cano et al., 2021). This is facilitated through the interaction of EDCs with diverse receptors, encompassing nuclear receptors, peroxisome proliferator-activated receptor (PPAR)-α, and estrogen receptors (ER-α and -β) (Chen et al., 2022).

Regarding the effects of EDC exposure on NAFLD, most prior studies have focused on individual chemicals despite the more frequent simultaneous exposure to multiple chemicals. A few studies have evaluated the effects of EDC combinations on NAFLD. Nguyen and Kim (2022) reported an association between a mixture of chemicals, including heavy metals, volatile organic compound (VOC) metabolites, PAH metabolites, phthalate metabolites, phenols, parabens, TCS, 3-PBA, and cotinine, and biomarkers of liver function in Korean adults aged 19–86 years. Yang et al. (2022) demonstrated substantial positive associations between elevated concentrations of mixed PAH metabolites and MetS, along with its individual components. Li et al. (2022) explored the overall impact of a combination of metals, perchlorates, nitrates, thiocyanates, pesticides, phthalates, PAHs, pyrethroids, herbicides, and organophosphate pesticide metabolites on the risk of NAFLD. Furthermore, Choi et al. (2023) demonstrated a notable positive overall effect of VOCs and PAH metabolites on NAFLD prevalence in Korean adolescents. Hu et al. (2021) investigated the association between PAH mixture and NAFLD, revealing that HDL and TG played a mediating role in this association. Cheng et al. (2023) showed that mixed perfluoroalkyl substances are associated with hepatic fibrosis rather than with steatosis. Lei et al. (2023) demonstrated that EDCs metabolites exposure were significantly associated with metabolic dysfunction-associated fatty liver disease. In addition to uncovering a significant positive overall effect of the 26 chemicals on the risk of NAFLD, our study highlights specific substances that exert a particularly robust impact on this association. Among the investigated EDCs, EP, PFOS, PFNA, BPS, and NAP exerted the most substantial effects on MetS. PFNA, EP, PFOS, PFHxS, and BPF had the greatest influence on NAFLD as defined by the FLI, whereas PFNA,

Table 2
Distribution of chemical exposures among study participants from the KoNEHS cycle 4 (2018–2020).

Chemical exposure	LOD	DF (%)	GM	Mean	Percentile		
					25th	50th	75th
Phthalate metabolites (μg/L)							
MEHHP	0.069	99.9	11.30	18.35	6.28	12.42	21.76
MEOHP	0.052	99.0	5.96	10.45	3.29	6.81	12.50
MnBP	0.084	96.5	17.26	41.58	9.74	20.33	41.09
MECPP	0.073	99.9	15.23	23.27	8.52	16.19	28.32
MBzP	0.030	95.4	0.80	2.41	0.33	0.83	2.17
MCPP	0.072	91.9	0.33	0.62	0.17	0.34	0.63
MEP	0.108	91.8	4.23	77.62	1.22	3.54	13.46
MMP	0.069	97.8	2.86	4.78	1.61	3.41	13.46
Phenols (μg/L)							
BPA	0.029	95.6	0.85	2.54	0.42	1.08	2.30
BPF	0.036	91.0	0.17	0.70	0.06	0.15	0.38
BPS	0.022	85.9	0.11	0.38	0.05	0.11	0.26
Antibacterial (μg/L)							
TCS	0.041	82.9	0.18	1.06	0.08	0.18	0.36
Parabens (μg/L)							
MP	0.209	95.4	13.28	77.24	3.68	12.53	54.76
EP	0.124	99.5	44.42	174.31	12.40	52.28	193.62
PP	0.158	73.2	1.09	16.63	0.11	0.60	5.53
BP	0.109	99.4	1.19	1.87	0.79	1.10	1.55
Polycyclic aromatic hydrocarbons (μg/L)							
OHP	0.044	74.0	0.16	0.30	0.03	0.22	0.38
NAP	0.033	99.9	2.64	5.90	1.15	2.41	5.99
OHFlu	0.052	90.0	0.27	0.51	0.14	0.28	0.51
OHPhe	0.037	61.1	0.09	0.18	0.03	0.11	0.25
Pesticide (μg/L)							
3-PBA	0.020	99.4	0.88	1.80	0.41	0.87	1.97
Perfluorinated compounds (μg/L)							
PFOA	0.050	100	7.02	8.20	4.84	7.13	10.17
PFOS	0.056	100	16.67	20.02	11.08	17.04	25.49
PFHxS	0.071	99.8	4.47	6.28	2.73	4.33	6.94
PFNA	0.019	100	2.37	2.84	1.57	2.45	3.62
PFDeA	0.017	100	1.03	1.21	0.70	1.04	1.49

Abbreviations: 3-PBA, 3-Phenoxybenzoic acid; BPA, Bisphenol A; BP, butylparaben; BPF, Bisphenol F; BPS, Bisphenol S; DF, Detection frequency; EP, ethyl paraben; GM, Geometric mean; KoNEHS, Korean National Environmental Health Survey; LOD, Limit of detection; MBzP, Monobenzyl phthalate; MCPP, Mono (3-carboxypropyl) phthalate; MEHHP, Mono (2-ethyl-5-hydroxyhexyl) phthalate; MEOHP, Mono (2-ethyl-5-oxohexyl) phthalate; MEP, Mono-ethyl phthalate; MMP, Mono-methyl phthalate; MnBP, Mono-n-butyl phthalate; MP, methyl paraben; NAP, naphthol; OHFlu, hydroxyfluorene; OHP, hydroxypyrene; OHPhe, hydroxyphenanthrene; PFDeA, perfluorodecanoic acid; PFHxS, Perfluorohexane sulfonate; PFNA, perfluorononanoic acid; PFOA, perfluorooctanoic acid; PFOS, perfluorooctane sulfonic acid; PP, propyl paraben; TCS, triclosan.

PFHxS, PFOS, EP, and NAP had a pronounced impact on NAFLD as defined by the HSI. These findings underscore the necessity for comprehensive interventions targeting EDC exposure to mitigate the risk of NAFLD, with special attention paid to PFCs, EPs, and PAHs, including naphthol.

The interaction between sex and EDCs was analyzed by performing a WQS analysis stratified by sex. Our results showed that sex significantly interacted with EDC concentrations, with females exhibiting greater vulnerability to the effects of EDCs than males. We also found that although certain specific substances (MEP, BPS, TCS, EP, PP, OHP, and PFOA) had a greater effect in males, the overall chemical mixtures had a relatively greater effect on NAFLD in females. This suggests that females are more susceptible to the effects of EDC mixtures and may warrant additional interventions.

Understanding the pathways through which EDCs influence NAFLD will provide insights into potential interventions to prevent its development. Hence, we aimed to ascertain the degree to which EDCs affect NAFLD through metabolic abnormalities by conducting a CMA. Our results suggest that MetS acts as a mediator between EDCs and NAFLD, either in the total population or in females, accounting for approximately 30–40 % of the total effect. However, the total and natural direct and indirect effects were not statistically significant in males. He et al. (2023) reported increased NAFLD with higher di-(2-ethylhexyl) phthalate concentration, with BMI mediating approximately 46 % of the total effect and waist circumference mediating approximately 66 % of the total effect. Furthermore, Hu et al. (2021) explored the positive association between a PAH mixture and NAFLD, identifying HDL and TG as mediators and reporting that NAFLD triggered by EDC exposure could be mitigated to a certain extent by interventions for metabolic disorders. In other words, using MetS as a screening tool has the potential to enhance the identification of individuals at a higher risk, enabling targeted interventions to modify unhealthy lifestyle habits and reduce exposure to EDCs.

The findings of previous studies that have explored the impact of EDC exposure on metabolic disorders support the mediating role of MetS in the association between EDC exposure and NAFLD, as evidenced by our study. EDCs, also known as MDCs, play a crucial role in processes such as adipogenesis, lipid metabolism, and energy balance (Heindel et al., 2017) and are pivotal contributors to the onset of obesity, type 2 diabetes, dyslipidemia, and MetS. Several mechanisms have been proposed to elucidate the association between EDC exposure and metabolic abnormalities. EDCs bind to estrogen receptors (ER-α and ER-β), influencing the synthesis of adiponectin (Rochester and Bolden, 2015). In addition, EDCs and associated metabolites may enhance the differentiation of preadipocytes into adipocytes and increase lipid accumulation via the PPAR-γ signaling pathway (Boucher et al., 2016; Hao et al., 2012; Janesick and Blumberg, 2011). EDCs can also affect the metabolism of low-density lipoprotein-C, HDL-C, and TG, leading to increased serum lipid levels through PPAR-α activation (Kim et al., 2005). Moreover, EDCs can induce oxidative stress, characterized by the overproduction of reactive oxygen species and lipid peroxidation. Elevated oxidative stress and inflammation can potentially activate the nuclear factor-κB pathway, intensifying systematic inflammation associated with MetS (Li et al., 2023; Yang et al., 2022). Furthermore, EDCs disrupt the thyroid

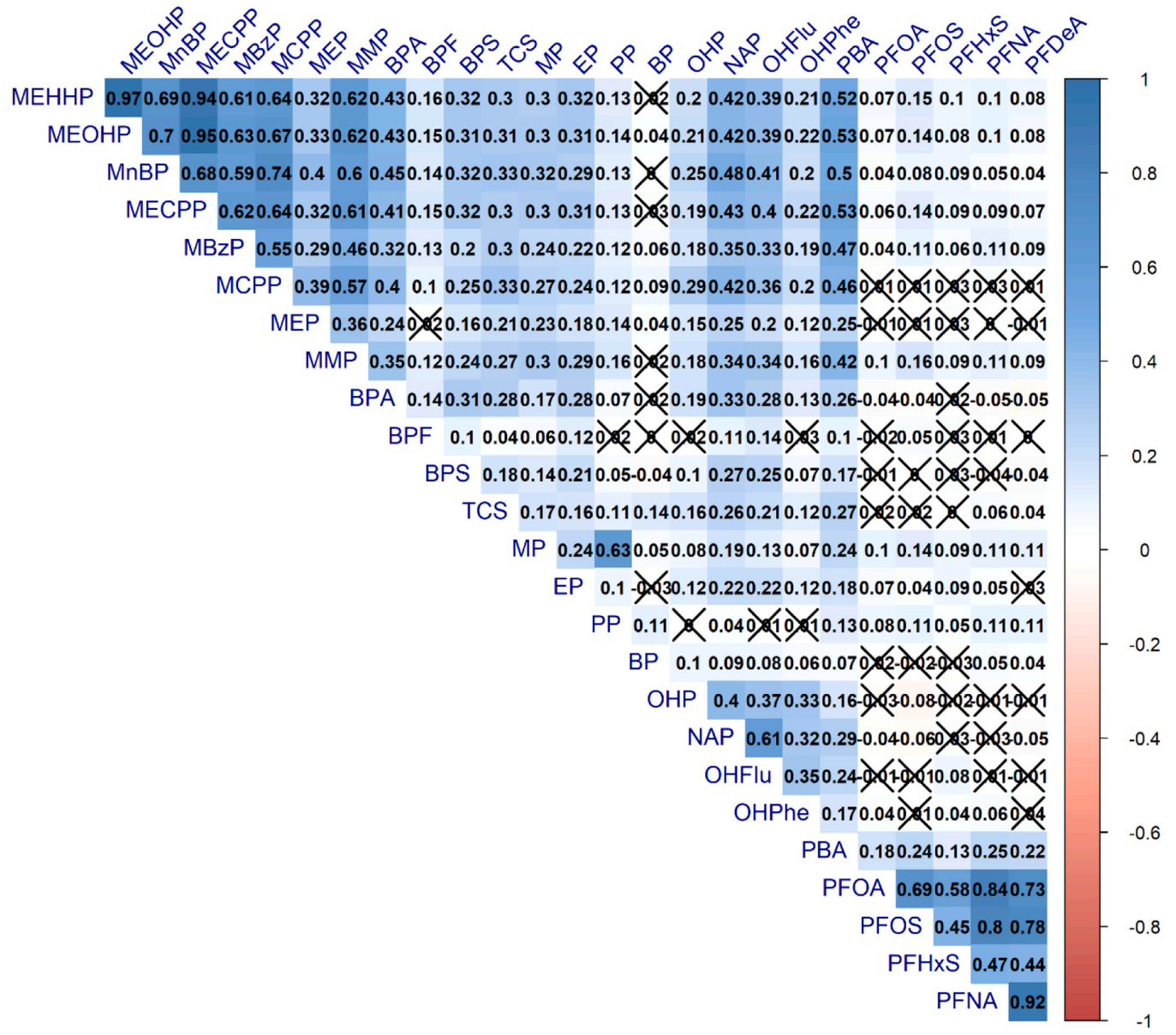

Fig. 1. Spearman correlations among the concentrations of chemical exposure. Abbreviations: 3-PBA, 3-Phenoxybenzoic acid; BP, butylparaben; BPA, Bisphenol A; BPF, Bisphenol F; BPS, Bisphenol S; EDC, endocrine-disrupting chemical; EP, ethyl paraben; FLI, fatty liver index; HSI, hepatic steatosis index; MBzP, Monobenzyl phthalate; MCPP, Mono (3-carboxypropyl) phthalate; MEP, Mono-ethyl phthalate; MECPP, Mono (2-ethyl-5-carboxypentyl) phthalate; MEHHP, Mono (2-ethyl-5-hydroxyhexyl) phthalate; MEOHP, Mono (2-ethyl-5-oxohexyl) phthalate; MetS, metabolic syndrome; MMP, Mono-methyl phthalate; MnBP, Mono-n-butyl phthalate; MP, methyl paraben; NAP, naphthol; OHFlu, hydroxyfluorene; OHPhe, hydroxyphenanthrene; OHP, hydroxypyrene; PFDeA, perfluorodecanoic acid; PFHxS, Perfluorohexane sulfonate; PFNA, perfluorononanoic acid; PFOA, perfluorooctanoic acid; PFOS, perfluorooctane sulfonic acid; PP, propyl paraben; TCS, triclosan.

hormone system and interfere with basal metabolism.

This study has several strengths. First, to overcome the methodological challenges presented by multiple comparisons and multicollinearity, common in the analysis of high-dimensional data from exposures to numerous chemicals, we implemented the weighted quantile sum regression (WQS) method. This advanced statistical approach, which is gaining prominence in epidemiological studies, addresses the questions of identifying a mixture effect on health outcomes and identifying the individual components responsible for these effects. The method employs an empirical weighted index that captures the aggregate influence of these components, then assesses its relationship with health outcomes. By effectively tackling issues related to dimensionality and multicollinearity, WQS regression significantly improves the validity of our results. This methodological advancement ensures robust protection against the prevalent risks of false positive and false negative errors, which are frequently observed in analyses conducted using conventional statistical models. This study provides compelling evidence regarding the detrimental effects of EDC mixtures on NAFLD (Renzetti et al., 2023; Schmidt et al., 2021; Zhang et al., 2019). Second, to identify subgroups at higher risk, we assessed the interaction effect of sex, revealing that females are more susceptible than males. Further research is warranted to gain deeper insights into the potential mechanisms underlying this sex difference. Third, we performed CMA to elucidate the underlying biological pathways linking EDC mixtures and NAFLD, demonstrating that metabolic abnormalities likely mediate these effects.

However, this study has a few limitations. The main limitation originates from the adoption of a cross-sectional design, in which EDC exposure, metabolic status, and liver function were assessed at a single time point. Particularly, this study's reliance on a single spot

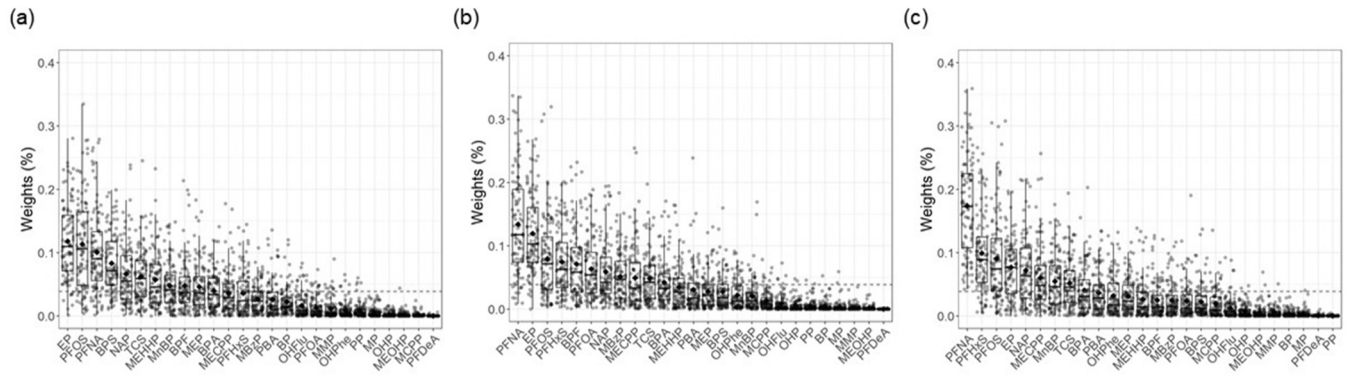

Fig. 2. Weights of each chemical contributing to the positive association between EDC mixtures and (a) MetS, (b) NAFLD based on FLI and (c) NAFLD based on HSI. Models were adjusted for age, sex, education, physical activity, smoking, involuntary smoking, and drinking habits. Abbreviations: 3-PBA, 3-Phenoxybenzoic acid; BP, butylparaben; BPA, Bisphenol A; BPF, Bisphenol F; BPS, Bisphenol S; EDC, endocrine-disrupting chemical; EP, ethyl paraben; FLI, fatty liver index; HSI, hepatic steatosis index; MBzP, Monobenzyl phthalate; MCPP, Mono (3-carboxypropyl) phthalate; MEP, Mono-ethyl phthalate; MECPP, Mono (2-ethyl-5-carboxypentyl) phthalate; MEHHP, Mono (2-ethyl-5-hydroxyhexyl) phthalate; MEOHP, Mono (2-ethyl-5-oxohexyl) phthalate; MetS, metabolic syndrome; MMP, Mono-methyl phthalate; MnBP, Mono-n-butyl phthalate; MP, methyl paraben; NAFLD, non-alcoholic fatty liver disease; NAP, naphthol; OHFlu, hydroxyfluorene; OHPhe, hydroxyphenanthrene; OHP, hydroxypyrene; PFDeA, perfluorodecanoic acid; PFHxS, Perfluorohexane sulfonate; PFNA, perfluorononanoic acid; PFOA, perfluorooctanoic acid; PFOS, perfluorooctane sulfonic acid; PP, propyl paraben; TCS, triclosan.

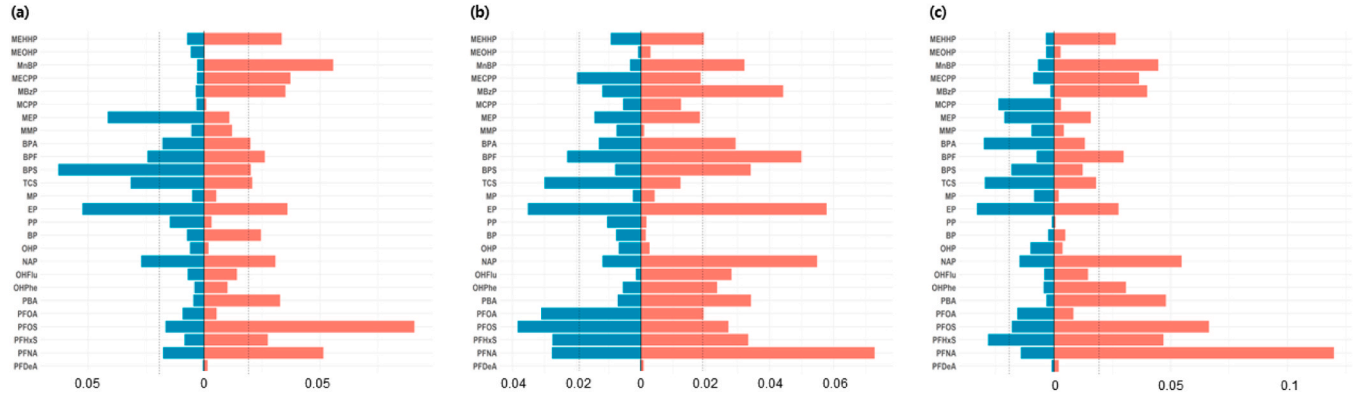

Fig. 3. Divergent plots comparing the mean estimated sex-specific weights for (a) MetS, (b) NAFLD based on FLI, and (c) NAFLD based on HSI. The dotted lines represent the threshold guideline derived from the equi-weighted index (1/n, where n represents the number of exposure chemicals), while the green and orange bars indicate weights for males and females, respectively. Abbreviations: 3-PBA, 3-Phenoxybenzoic acid; BP, butylparaben; BPA, Bisphenol A; BPF, Bisphenol F; BPS, Bisphenol S; EDC, endocrine-disrupting chemical; EP, ethyl paraben; FLI, fatty liver index; HSI, hepatic steatosis index; MBzP, Monobenzyl phthalate; MCPP, Mono (3-carboxypropyl) phthalate; MEP, Mono-ethyl phthalate; MECPP, Mono (2-ethyl-5-carboxypentyl) phthalate; MEHHP, Mono (2-ethyl-5-hydroxyhexyl) phthalate; MEOHP, Mono (2-ethyl-5-oxohexyl) phthalate; MetS, metabolic syndrome; MMP, Mono-methyl phthalate; MnBP, Mono-n-butyl phthalate; MP, methyl paraben; NAP, naphthol; OHFlu, hydroxyfluorene; OHPhe, hydroxyphenanthrene; OHP, hydroxypyrene; PFDeA, perfluorodecanoic acid; PFHxS, Perfluorohexane sulfonate; PFNA, perfluorononanoic acid; PFOA, perfluorooctanoic acid; PFOS, perfluorooctane sulfonic acid; PP, propyl paraben; TCS, triclosan.

measurement of EDCs concentration within a cross-sectional framework does not capture the dynamics of EDCs concentration over time. Consequently, this approach does not account for the clearance of each chemical. Longitudinal studies are thus warranted to validate these findings. Additionally, we utilized FLI and HSI values instead of biopsy results to determine NAFLD status, which might have introduced some measurement bias.

5. Conclusions

We found that exposure to a combination of EDCs was associated with an increased risk of NAFLD and that this was at least partially mediated by the MetS status. Overall, our study emphasizes the need for comprehensive approaches that encompass both EDC exposure reduction and improvements in metabolic health to effectively treat NAFLD and its associated complications. Addressing these factors will contribute to alleviating the burden of this condition and enhancing overall health outcomes for individuals at risk, as well as for those diagnosed with NAFLD.

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CRediT authorship contribution statement

Bomi Park: Writing – review & editing, Supervision, Funding acquisition, Conceptualization. **Bohyun Park:** Writing – original draft, Validation. **Byungmi Kim:** Writing – original draft, Validation. **Chung Ho Kim:** Formal analysis, Data curation. **Hyun Jin Oh:** Visualization, Validation, Methodology.

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.

Table 3
Marginal, direct, and indirect effects of WQS scores on NAFLD risk with metabolic syndrome as the mediator.

Outcomes	TE (95 % CI)	NDE (95 % CI)	NIE (95 % CI)	PM (95 % CI)
Total				
NAFLD using values of FLI	1.56 (1.14–2.13)	1.29 (1.06–1.58)	1.21 (1.08–1.35)	43 % (23–78)
NAFLD using values of HSI	1.59 (1.20–2.64)	1.38 (1.12–1.69)	1.15 (1.06–1.25)	30 % (13–56)
Men				
NAFLD using values of FLI	1.41 (0.92–2.10)	1.32 (1.02–1.69)	1.06 (0.90–1.24)	18 % (-65–73)
NAFLD using values of HSI	1.35 (0.86–2.09)	1.29 (0.93–1.77)	1.04 (0.92–1.18)	14 % (-77–98)
Women				
NAFLD using values of FLI	1.90 (1.31–2.73)	1.56 (1.23–1.97)	1.22 (1.07–1.39)	30 % (13–51)
NAFLD using values of HSI	1.68 (1.12–2.37)	1.44 (1.07–1.83)	1.17 (1.05–1.30)	30 % (11–63)

CI, confidence interval; FLI, fatty liver index; HSI, hepatic steatosis index; NAFLD, non-alcoholic fatty liver disease; NDE, natural direct effect; NIE, natural indirect effect; PM, estimated proportion mediated; TE, total effect. Models were adjusted for age, sex, education level, smoking, involuntary smoking, drinking, and physical activity.

Data availability

The authors do not have permission to share data.

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