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Dietary exposures to environmental pollutants: Integrated multimedia perspectives

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Dietary exposures to environmental pollutants: Integrated multimedia perspectives

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Editorial: Dietary exposures to environmental pollutants: integrated multimedia perspectives

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Editorial on the Research Topic
Dietary exposures to environmental pollutants: integrated multimedia perspectives

The global challenge of understanding the intricate links between dietary exposures and environmental pollutants is crucial for public health and necessitates a comprehensive and multifaceted approach. Environmental pollutants, broadly encompassing chemicals and particles present in our air, water, soil, and food, have long been a cause of concern for their potential adverse effects on human health. Among the multiple pathways of exposure to these pollutants, diet is particularly significant as it is a primary route through which humans are exposed to various environmental contaminants. This is because pollutants can accumulate in the food chain, leading to higher concentrations in the foods consumed by humans. Moreover, the interaction between diet and environmental pollutants is complex and multifactorial, involving various biological, environmental, and lifestyle factors. Therefore, a thorough understanding of the relationships between dietary exposures and environmental pollutants is essential for developing effective strategies to minimize the risk and impact of these pollutants on human health. This Research Topic, entitled "*Dietary exposures to environmental pollutants: integrated multimedia perspectives*," includes 15 articles that delve into various aspects of dietary exposures and their associations with environmental pollutants, providing integrated perspectives on this critical subject.

Several articles in this issue address the broader environmental context. Azanaw et al. conducted a multilevel analysis of improved drinking water sources and sanitation facilities in Ethiopia, which is fundamental to public health. The study revealed that while access to improved water sources is moderate, access to improved sanitation was significantly lower, underlining the need for significant improvements in providing access to improved water sources and sanitation facilities in Ethiopia. Additionally, He et al. investigated the spatial and temporal patterns, exposure risks, and drivers of surface ozone (O_3) pollution in China, providing a broader perspective on air pollution and its effects. The study revealed a significant increase in O_3 concentrations and associated premature deaths from respiratory

diseases, emphasizing the need for region-specific O_3 control policies. These two articles highlighted the broader environmental context of dietary exposures and environmental pollutants and called for a comprehensive and targeted approach to address the fundamental public health issues related to environmental pollutants.

Other studies explore the impacts of specific pollutants. Jhuang et al. investigated the latency period between aristolochic acid (AA) exposure and the development of upper urinary tract urothelial carcinoma (UTUC) in a Taiwanese cohort. The study highlighted a decreased risk of UTUC after the ban on AA in Taiwan, particularly among middle-aged women with moderate to high AA exposure and men with moderate AA exposure. The findings underscored that the latency period of UTUC varies with age, AA exposure dose, and gender. de Morais Valentim et al. conducted a multiscale analysis of pesticide residues in food in Brazil, estimating dietary cancer risks and associated mechanisms. The study provided a critical analysis of the pesticide scenario in Brazil from geographical, political, and public health perspectives, highlighting human rights violations due to food and water contamination. Additionally, Wang G.-X. et al. examined the association between urinary nickel and obesity status in adults using data from the National Health and Nutrition Examination Surveys. The study found a correlation between urinary nickel levels and body mass index and waist circumference in adult males, suggesting the need for obese men to reduce nickel exposure. These articles explored the impacts of specific pollutants, including aristolochic acid, pesticide residues, and urinary nickel, on various health outcomes. The findings highlighted the need for targeted interventions, such as the ban on aristolochic acid in Taiwan, and a comprehensive analysis of pesticide residues in food in Brazil to address human rights violations due to food and water contamination.

Several articles explore the relationship between environmental pollutants and specific health outcomes. Wei et al. conducted an age-period-cohort analysis to explore trends in cardiovascular disease (CVD) mortality attributable to non-optimal temperatures in China. Despite reductions in CVD mortality attributable to non-optimal temperatures, the study revealed a steady increase in ischemic heart disease mortality and the burden from high temperatures, calling for more strategies to protect human health from climate change impacts. Xiao et al. conducted a systematic review and meta-analysis to explore the association between per- and polyfluoroalkyl substances (PFAS) and the risk of hypertension. The study found that higher levels of certain PFAS, namely perfluorooctane sulfonate, perfluorooctanoic acid, and perfluorohexane sulfonate, were correlated with an increased risk of hypertension. Moreover, Zhang Y. et al. performed a bibliometric analysis of the association between drinking water pollution and bladder cancer. The study provided an overall and intuitive understanding of this topic in recent years, highlighting key research hotspots and revealing trends for further in-depth study in the future. These articles discussed the relationship between environmental pollutants and specific health outcomes, including cardiovascular disease mortality, hypertension, and bladder cancer. The findings suggested the need for strategies to protect human health from the impacts of climate change, a deeper understanding of the association between PFAS and hypertension, and a comprehensive analysis of the association between drinking water pollution and bladder cancer.

A group of articles delves into more specific dietary exposures. Jiao et al. investigated the association between egg intake and cardiometabolic factors (CMFs) in Chinese adults. They observed a U-shaped association between egg intake and CMFs, indicating that moderate egg consumption might lower the risk of central obesity, elevated triglycerides, decreased HDL-C, and elevated plasma glucose, whereas higher egg intake did not show a significant association with CMFs. Zhang S. et al. evaluated the relationship between body fat percentage (BF%) and the risk of type 2 diabetes (T2D) in Chinese adults. Analyzing data from 5,595 adults aged 18-65, they found that the incident risk of T2D significantly increased over specific levels of total and trunk BF% in both Chinese males and females. Their findings suggest that optimal BF% cut-off values may be valuable for clinical applications in the prevention and treatment of T2D in China. Additionally, Wang G. et al. explored the association between intestinal microbiota markers, dietary patterns, and T2D in Chinese patients. The study revealed significant associations between dietary intake patterns and gut flora, highlighting the crucial role of gut microbiota in linking dietary intake and the etiology of T2D. Another study by Wang N. et al. examined the role of rare earth elements (REEs) and dietary intake in the development of tongue cancer in southeast China. The findings suggest that some REEs interact with food intake to influence tongue cancer risk, while others act as mediators. These articles delved into specific dietary exposures and their association with health outcomes, including cardiometabolic factors, T2D, and tongue cancer. The findings suggest that moderate egg consumption may lower the risk of central obesity and other cardiometabolic factors, while optimal body fat percentage cut-off values may be valuable for the prevention and treatment of T2D. Additionally, the association between dietary intake patterns, gut flora, and T2D, and the interaction between REEs and food intake in the development of tongue cancer, highlight the crucial role of diet in disease development.

The final set of articles focuses on emerging contaminants and their health effects. Chen X. et al. discussed the adverse health effects of emerging contaminants on inflammatory bowel disease (IBD). The authors highlighted that emerging contaminants, such as microplastics, endocrine-disrupting chemicals, chemical herbicides, heavy metals, and persisting organic pollutants, may lead to many chronic diseases, including IBD. The article underscores the need to understand the impact of these new emerging contaminants on IBD and minimize their exposures to lower the future incidence of IBD. Liu et al. presented a nested case-control study examining the relationship between urinary selenium levels and the risk of gestational diabetes mellitus (GDM). The study found a significant negative association between urinary selenium and the risk of GDM, with this association varying depending on the fetal sex. The findings suggest that lower urinary selenium levels are associated with a higher risk of GDM, particularly among pregnant women with female fetuses. In another study, Chen Y. et al. investigated the role of endocrine-disrupting chemicals (EDCs) as a promoter of non-alcoholic fatty liver disease (NAFLD). The authors summarized the major EDCs contributing to the growing burden of NAFLD and aimed to raise

public awareness regarding the hazards posed by EDCs to reduce the incidence of NAFLD. Overall, these articles focused on the adverse health effects of emerging contaminants on specific diseases like IBD, GDM, and NAFLD. The findings underscored the need to understand the impact of emerging contaminants on chronic diseases and to minimize exposures to lower the incidence of them.

Collectively, the articles in this Research Topic offer an integrative framework for dissecting the nuanced interplay between dietary exposures, environmental pollutants, and a range of health outcomes. The research presented not only deepens our understanding of these complex relationships but also serves as a crucial foundation for developing evidence-based public health policies and interventions. Specifically, these findings underscore the pressing need for targeted strategies that can mitigate the harmful impacts of environmental pollutants through informed dietary choices and lifestyle modifications. As our understanding evolves, it remains crucial to perpetually scrutinize these interconnections and their mechanistic underpinnings, intending to formulate more refined and effective preventative and management approaches for the myriad health risks associated with environmental exposures. In light of the comprehensive insights offered, the editors are optimistic that this Research Topic will engross the readership of Frontiers in Public Health and are confident that this topic will act as a catalyst for future research endeavors, encouraging multidisciplinary approaches to explore the multifaceted dimensions of dietary exposures to environmental pollutants.

Author contributions

PG: Conceptualization, Writing – original draft, Writing – review & editing. YM: Writing – review & editing. GD: Writing – review & editing. QX: Writing – review & editing.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Association of egg intake with risks of cardiometabolic factors among adults in China

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Objective: To explore the association between egg intake and cardiometabolic factors (CMFs) in Chinese adults.

Method: The subjects were 6,182 adults aged 18–64 who had complete survey data and had no CMFs at baseline. Egg intake was assessed with 3 days−24 h dietary recalls in all waves of the China Health and Nutrition Survey (CHNS). Multivariate Cox proportional risk regression model and restricted cubic spline (RCS) model were used to analyze the association and dose-response relationship between egg intake and CMFs.

Results: Of the 6,182 participants who did not have metabolic syndrome (MetS) at baseline, 1,921 developed this disease during an average follow-up of 5.71 years, with an incidence of 31.07%. Central obesity, elevated TG, decreased HDL-C, elevated blood pressure and elevated plasma glucose were 38.65, 26.74, 30.21, 40.64, and 30.64%, respectively. After adjusting for demographic characteristics, lifestyle, energy and BMI, using the lowest quintile (Q1) as a reference, the risk of central obesity, elevated TG, decreased HDL-C, and elevated plasma glucose in the highest quintile (Q5) were reduced by 15% (HR = 0.85, 95% CI = 0.73–0.98, $P = 0.16$), 33% (HR = 0.67, 95% CI = 0.57–0.78), 25% (HR = 0.75, 95% CI = 0.63 0.90, $p = 0.05$), and 28% (HR = 0.72, 95% CI = 0.63–0.83, $p < 0.05$), respectively. The risk of elevated blood pressure was reduced by 26% in the fourth quintile (HR = 0.74, 95% CI = 0.64–0.85, $P = 0.85$). RCS analysis show that the overall correlation and nonlinear relationship between egg intake and CMFs were statistically significant ($P < 0.05$). When the intake was lower than 20 g/days, the risk of MetS, central obesity, elevated blood pressure and elevated plasma glucose were negatively correlated with egg intake, while elevated TG was negatively correlated with eggs when the intake was lower than 60 g/days. There was no statistically significant association between egg intake and CMFs at higher egg intake.

Conclusion: There was a *U*-shaped association between egg intake and CMFs in Chinese adults.

KEYWORDS

adults, egg intake, cardiometabolic risk factors, dose-response relationship, China

Introduction

Cardiovascular disease (CVD) is the main cause of death and disability all over the world (1), ranking first among the causes of death of urban and rural residents in China and 46. 66% in rural and 43.81% in urban. Two out of every five deaths are due to CVD (2). Among many risk factors, cardiometabolic factors (CMFs), including obesity, elevated blood pressure, elevated plasma glucose and dyslipidemia, are the main risk factors of CVD, which are also the important components of the "health factors" in the "Life's Essential 8" newly proposed by the American Heart Association (3). The prevalence of each CMF increased by 20–50% from 2009 to 2018 in China (4). Studies have found that more than half of people had at least two CMFs in China (5), and the prevalence of metabolic syndrome (MetS) is increasing at home and abroad, and the data from China Health and Nutrition Survey (CHNS) in 2015 showed that the prevalence reached 18.1% (6). It places a heavy burden on individuals and societies.

Eggs are the main source of dietary cholesterol, containing about 71% of the recommended daily intake of cholesterol (7). Because of its affordable market price, it is widely consumed worldwide. Evidence for the relationship between egg consumption and human health is controversial, with some studies finding a reduction in the risk of hemorrhagic stroke and levels of inflammatory factors, but no association in others (e.g., cancer, CVD, etc.) (8, 9). Eggs were found to be negatively associated with MetS in a prospective study in Korea and a cross-sectional study in China (7, 10), while a positive association was found in an Iranian cohort (11), and no significant association was found in an Australian and another Korean cohort (12, 13). At present, there are limited studies on eggs and CMFs at home and abroad, and most of them are cross-sectional studies. Therefore, this study uses the follow-up data from CHNS in 2009, 2015, and 2018 to analyze the association between egg intake and CMFs, and provide scientific basis for effective prevention and control of related diseases.

Materials and methods

Study population

We used data from CHNS, a long-term longitudinal follow-up project jointly conducted by the Institute of Nutrition and Health, Chinese Center for Disease Control and Prevention and the University of North Carolina at Chapel Hill. The project was launched in 1989 and has completed 11 waves of follow-up. And it was conducted in 15 provinces, the specific provinces or cities are shown in Supplementary Table 1. A stratified multistage random cluster sampling method was used, with county neighborhood committee, urban neighborhood committee, village and suburban village as the basic survey points. Twenty households were randomly selected from each survey point, and all household members were investigated. The same households and household members were tracked as far as possible in each round of survey. The survey content included questionnaire survey (community, household and personal information), medical physical examination (blood biochemical test was added in 2009, 2015, and 2018) and dietary survey. For specific sampling methods, survey scheme and content, please refer to the literature (14–16).

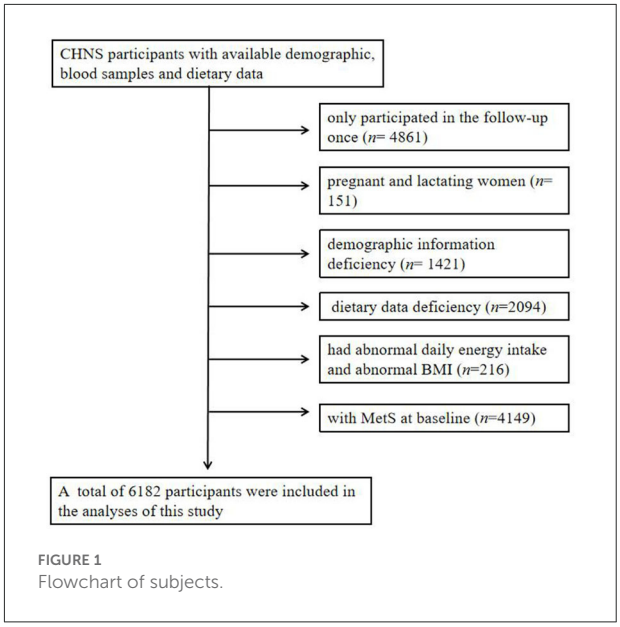

FIGURE 1
Flowchart of subjects.

In this study, we selected adults aged 18–64 as subjects, who participated in at least two follow-up surveys in 2009, 2015, and 2018. We excluded pregnant and lactating women (n = 151), those having demographic information deficiency (n = 1,421), having dietary data deficiency (n = 2,094), having abnormal daily energy intake (man: >6,000 or <800 kcal; women: 4,000 or <600 kcal) (17) and abnormal body mass index (BMI) (<14.0 or > 45.0 kg/m^2) (n = 216) (5) and those having MetS at baseline (n = 4,149). Finally, 6,182 subjects were included in this study (Figure 1). In addition, we identified specific study population subgroups for each single CMFs. The project was reviewed by the Ethics Review Committee of Institute of Nutrition and Health of Chinese Center for Disease Control and Prevention (No. 201524), and all the participants signed the informed consent.

Dietary assessment

In each wave of surveys, the consumption of food data was collected by the consecutive 3 days−24 h dietary recalls, cooking oil and condiments was collected by household weighing method and distributes them to individuals according to the individual energy consumption ratio in the household. Then, Food Composition Table was used to convert the collected

consumption of various foods, edible oils and condiments into the intake of energy and nutrients. Egg and energy intake were included in this study. In prospective analyses, considering that potential changes in diet after the development of the disease may confound the relationship between egg intake and CMFs, updating of dietary information was stopped upon diagnosis of the disease. If the subjects entered the cohort in 2009 and developed the disease in 2015, the egg consumption in 2009 was used; if the subjects developed the disease in 2018, the average egg consumption in 2009 and 2015 was used. If the subjects entered the cohort in 2015 and developed disease in 2018, the egg consumption in 2015 was used.

Diagnostic criteria of CMFs

We defined CMFs according to the joint statement of the International Diabetes Federation (IDF) in 2009 (18), Central obesity: waist circumference ≥85 cm in men and ≥80 cm in women; Elevated triglyceride (TG): ≥1.7 mmol/L or under treatment; Decreased HDL-C: <1.0 mmol/L in men and <1.3 mmol/L in women or under treatment; Elevated blood pressure: systolic blood pressure ≥130 mmHg, diastolic blood pressure ≥85 mmHg or being treated for essential hypertension; Elevated plasma glucose: fasting plasma glucose (FPG) ≥5.6 mmol/L or previously diagnosed with diabetes. The presence of any 3 of 5 risk factors constitutes a diagnosis of MetS. Detailed description of measurement methods was provided in this literature (19).

Covariates

The demographic data, lifestyle, dietary information were obtained through face-to-face surveys with special questionnaires by uniformly trained and qualified investigators. Such as age (18–49 and 50–64 years), gender, annual household income, education (low: primary school and below; medium: middle or high school; high: college and above), residential area (urban or rural), smoking (yes or no), drinking habit (yes or no), physical activity, energy and BMI (<18.5, 18.5∼23.9, and ≥24.0 kg/m^2). Annual household income and energy were categorized into three groups according to the tertiles (low, medium, and high). Physical activity was assessed by the metabolic equivalent (MET) and duration of each activity (hours/week) (20, 21) and was divided into three groups according to the tertiles. Energy was adjusted according to the tertiles. BMI is calculated by height and weight measurements.

Statistical analysis

Continuous and categorical variables were described by mean ± standard deviation and percentage (%), respectively. Chi-square test and ANOVA were used to analyze the baseline characteristics of the subjects according to the quintile of egg intake. The association between egg intake and CMFs was analyzed by multivariate Cox proportional risk model across quintile groups of egg intake, and we calculated p-values for linear trend by using the median value for each group of egg intake. We calculated hazard ratios (HRs; 95% CIs) and constructed three sequential models including demographic characteristics, lifestyle, energy and BMI. Finally, RCS model with 5 knots was used to analyze the dose-response relationship between egg intake and CMFs. All data were analyzed by SAS (version 9.4, SAS Institute, Inc., Cary, NC, USA) and R software version 4.1.0 (The R Foundation for Statistical Computing), and we defined statistical significance as $p < 0.05$.

Results

Baseline characteristics

As presented in Table 1, using the lowest quintile (Q1) as a reference, people with higher egg intake were more likely to be male, with higher income and education, and had lower physical activity, higher energy intake, higher BMI and had a drink habit. Baseline waist circumference, systolic blood pressure, diastolic blood pressure, and fasting plasma glucose were different between groups ($p < 0.05$), other variables including age, BMI, smoking, and baseline TG and HDL-C levels were not significantly different between egg intake levels ($p > 0.05$).

Association of egg intake with CMFs

Among the 6,182 participants who did not have MetS at baseline, 1,921 developed during an average follow-up of 5.71 years, with an incidence of 31.07%. After adjusting for demographic characteristics, lifestyle, energy, and BMI, the risk of MetS was reduced by 15% (HR = 0.85, 95% CI = 0.74–0.97) in the highest quintile group (Q5) using the lowest quintile group (Q1) as a reference. The trend test had no statistical significance ($p = 0.12$; Table 2).

Analysis of CMFs found that the incidence of central obesity, elevated TG, decreased HDL-C, elevated blood pressure and elevated plasma glucose were 38.65, 26.74, 30.21, 40.64, and 30.64%, respectively. After adjusting for all the covariables, taking Q1 as the reference group, the risk of central obesity, elevated TG, decreased HDL-C and elevated plasma glucose were reduced by 15 (HR = 0.85, 95% CI = 0.73–0.98), 33 (HR = 0.67, 95% CI = 0.57–0.78), 25 (HR = 0.75, 95% CI = 0.63–0.90) and 28% (HR = 0.72, 95% CI = 0.63–0.83) in the highest intake (Q5), respectively. Elevated blood pressure was associated with a 26% lower risk (HR = 0.74, 95% CI = 0.64–0.85) in the Q4 group, and there was no statistically significant association

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TABLE 1 Characteristics of participants according to quintile of egg intake.

	Egg intake (g/day)					*p*-value
	Q1 (0.00)	Q2 (0.00∼14.67)	Q3 (14.67∼29.63)	Q4 (29.63∼50.00)	Q5 (50.00∼)	
Age, %						0.275
18∼49	59.48	57.21	61.34	61.72	59.27	
50∼64	40.52	42.79	38.66	38.28	40.73	
Male,%	45.40	42.21	43.07	44.59	50.00	0.002
Household income per capita,%						<0.001
Low	39.30	30.29	30.51	32.95	29.47	
Median	33.65	32.94	33.61	33.28	32.91	
High	27.05	36.76	35.89	33.77	37.62	
Education,%						<0.001
Primary and below	38.86	31.18	28.87	29.67	27.00	
Middle and high	47.01	48.82	51.55	49.10	50.48	
College and above	14.14	20.00	19.58	21.23	22.52	
Urban,%	31.49	34.56	34.50	36.23	34.19	0.091
Never smoked,%	68.74	71.32	72.19	71.89	71.09	0.223
Never drunk alcohol,%	68.51	70.15	69.25	67.38	63.90	0.017
Physical activity,%						<0.001
Low	29.21	35.88	34.09	32.62	37.78	
Median	31.93	33.24	32.30	36.07	33.79	
High	38.86	30.88	33.61	31.31	28.43	
BMI (kg/m^2),%						<0.001
<18.5	7.10	7.94	6.69	5.57	3.67	
18.5∼23.9	61.09	65.74	60.03	58.93	58.31	
≥24.0	31.82	26.32	33.28	35.49	38.02	
Energy (kcal/d)						<0.001
Low	35.48	39.85	36.30	30.33	26.68	
Median	30.60	31.76	34.75	37.05	33.15	
High	33.92	28.38	28.96	32.62	40.18	
WC (cm)	79.32 ± 9.85	78.88 ± 9.21	79.61 ± 0.49	80.21 ± 0.29	81.62 ± 10.48	<0.001
TG (mmol/l)	1.25 ± 0.88	1.22 ± 0.96	1.22 ± 0.78	1.21 ± 0.80	1.19 ± 0.72	0.935
HDL-C (mmol/l)	1.45 ± 0.35	1.45 ± 0.34	1.46 ± 0.35	1.50 ± 0.53	1.48 ± 0.40	0.457
SBP (mmHg)	118.84 ± 14.95	118.70 ± 15.59	119.01 ± 15.07	119.29 ± 14.56	120.20 ± 15.01	0.009
DBP (mmHg)	77.35 ± 9.64	76.95 ± 9.57	77.66 ± 9.83	77.78 ± 9.94	78.71 ± 9.75	<0.001
FPG (mmol/l)	5.05 ± 0.97	5.00 ± 0.94	4.98 ± 0.73	5.06 ± 0.97	5.11 ± 0.85	<0.001

WC, waist circumference; TG, triglyceride; HDL-C, high density lipoprotein cholesterol; SBP, systolic blood pressure; DBP, diastolic blood pressure; FPG, fasting plasma glucose.

in the Q5 group. The trend test was statistically significant only for elevated TG and elevated plasma glucose ($p < 0.05$; Table 2).

Dose-response relationship between egg intake with CMFs

RCS analysis showed a *U*-shaped association between egg intake and CMFs. Taking no egg intake as reference, when the egg intake was <20 g/days, the risk of CMFs except TG decreased significantly with the increase of egg intake. When the intake was >20 g/days, the risk increased, but egg intake still had a protective effect (HR < 1). When the intake was >100 g/days, egg intake was not statistically associated with MetS and central obesity, and there was no statistical association with decreased HDL-C, elevated blood pressure and elevated plasma glucose when the intake was >150, 75, and 150 g/days, respectively. In addition, Egg intake was negatively correlated with elevated TG when the intake was <60 g/days, and no longer had protective effect after 150 g/days (Figure 2).

TABLE 2 Hazard ratio (HR) and 95% confidence interval (CI) for association of egg intake with CMFs.

	Egg intake (g/day)					*P* trend
	Q1	Q2	Q3	Q4	Q5	
MetS[a]						
Median	0.00	10.00	20.00	37.05	66.66	
Model1	1.00	0.62 (0.52, 0.74)*	0.81 (0.71, 0.93)*	0.78 (0.68, 0.89)*	0.92 (0.81, 1.04)	0.953
Model2	1.00	0.61 (0.51, 0.73)*	0.82 (0.71, 0.94)*	0.78 (0.68, 0.89)*	0.90 (0.79, 1.02)	0.796
Model3	1.00	0.64 (0.53, 0.78)*	0.76 (0.66.0.88)*	0.71 (0.62, 0.83)*	0.85 (0.74, 0.97)*	0.119
Central obesity[b]						
Median	0.00	8.00	20.00	35.14	65.88	
Model1	1.00	0.56 (0.46, 0.68)*	0.82 (0.71, 0.95)*	0.73 (0.63, 0.84)*	0.87 (0.76, 1.00)	0.394
Model2	1.00	0.56 (0.45, 0.68)*	0.82 (0.71, 0.95)*	0.71 (0.61, 0.83)*	0.86 (0.74, 0.99)*	0.255
Model3	1.00	0.59 (0.47, 0.73)*	0.80 (0.69, 0.93)*	0.70 (0.60, 0.82)*	0.85 (0.73, 0.98)*	0.158
Elevated TG[c]						
Median	0.00	10.00	20.00	36.67	65.60	
Model1	1.00	0.77 (0.64, 0.91)*	0.75 (0.65, 0.88)*	0.65 (0.56, 0.76)*	0.68 (0.59, 0.79)*	<0.001
Model2	1.00	0.76 (0.64, 0.90)*	0.76 (0.65, 0.88)*	0.65 (0.55, 0.75)*	0.67 (0.58, 0.78)*	<0.001
Model3	1.00	0.75 (0.63, 0.91)*	0.75 (0.64, 0.88)*	0.63 (0.53, 0.74)*	0.67 (0.57, 0.78)*	<0.001
Decreased HDL-C[d]						
Median	0.00	10.00	21.82	40.00	70.00	
Model1	1.00	0.53 (0.42, 0.66)*	0.74 (0.63, 0.88)*	0.72 (0.60, 0.85)*	0.76 (0.64, 0.90)*	0.044
Model2	1.00	0.51 (0.40, 0.64)*	0.73 (0.61, 0.86)*	0.69 (0.58, 0.83)*	0.75 (0.63, 0.89)*	0.040
Model3	1.00	0.50 (0.39, 0.63)*	0.70 (0.59, 0.84)*	0.67 (0.56, 0.81)*	0.75 (0.63, 0.90)*	0.055
Elevated BP[e]						
Median	0.00	10.00	20.00	36.67	66.08	
Model1	1.00	0.59 (0.49, 0.71)*	0.84 (0.74, 0.96)*	0.79 (0.70, 0.91)*	0.92 (0.82, 1.05)	0.877
Model2	1.00	0.58 (0.48, 0.70)*	0.84 (0.74, 0.97)*	0.79 (0.69, 0.90)*	0.92 (0.81, 1.05)	0.852
Model3	1.00	0.55 (0.46, 0.67)*	0.80 (0.69, 0.91)*	0.74 (0.64, 0.85)*	0.92 (0.80, 1.05)	0.850
Elevated FPG[f]						
Median	0.00	10.00	20.00	36.27	65.38	
Model1	1.00	0.52 (0.43, 0.62)*	0.73 (0.64, 0.84)*	0.68 (0.59, 0.78)*	0.74 (0.64, 0.84)*	0.003
Model2	1.00	0.51 (0.42, 0.61)*	0.75 (0.65, 0.86)*	0.69 (0.59, 0.79)*	0.74 (0.64, 0.84)*	0.004
Model3	1.00	0.50 (0.41, 0.61)*	0.72 (0.62, 0.83)*	0.66 (0.57, 0.76)*	0.72 (0.63, 0.83)*	0.002

[a]N = 6,182. [b]N = 4,075. [c]N = 5,520. [d]N = 3,774. [e]N = 4,718. [f]N = 5,611. Model 1 adjusted age, sex, education, urban and rural areas, and income; In model 2, smoking, drinking and physical activity levels were further adjusted based on Model 1. In Model 3, energy intake and BMI were further adjusted based on Model 2.
*$P < 0.05$.

Discussion

The study analyzed the association and dose-response relationship between egg intake and CMFs in adults aged 18–64 years in 15 provinces of China and showed a *U*-shaped association between them. Consistent results were found in a cross-sectional study of 23,993 Korean adults aged 19 and above from the Korea National Health and Nutrition Examination Survey (KNHANES) 2007–2011, but dietary survey was conducted using a food frequency method in this study (22).

Association between eggs and MetS and its mechanism

A prospective study of 1,633 Koreans showed that compared with those who consumed no eggs a week, those who consumed >3 eggs per week had a 54% (RR = 0.46, 0.26–0.82) and 46% (RR = 0.54, 0.31–0.93) lower risk of MetS in men and women, respectively (7). In a study of 3,616 iranians, the risk of egg consumption was 2.7 times than that of non-consumption (11). In the two cohorts of 5,251 Koreans and 5,324 Australians, compared with the lowest quartile group, no

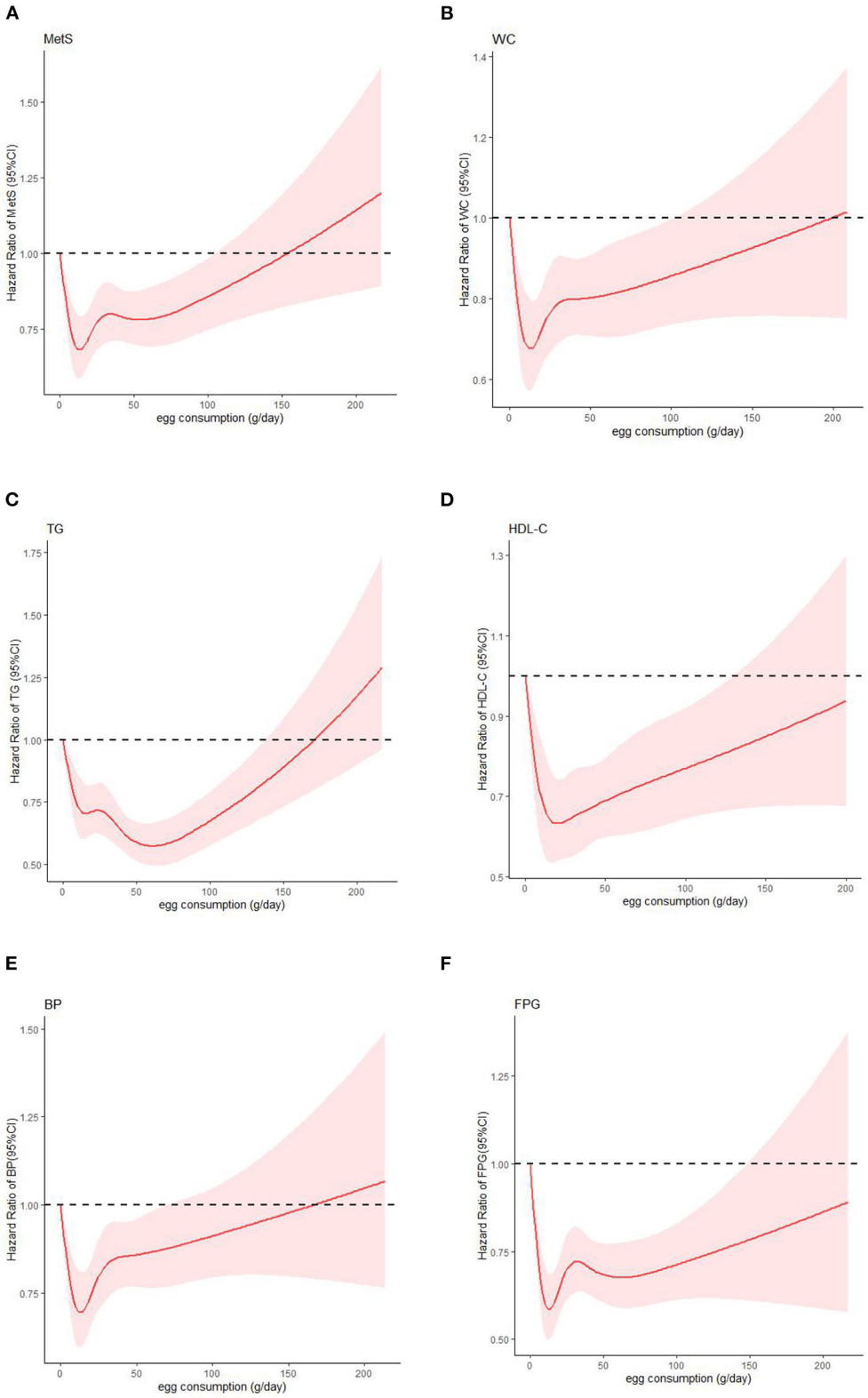

FIGURE 2
Dose-response relationship of egg intake with CMFs: **(A)** MetS: metabolic syndrome; **(B)** WC: waist circumference; **(C)** TG: triglyceride; **(D)** HDL-C: high density lipoprotein cholesterol; **(E)** BP: blood pressure; **(F)** FPG: fasting plasma glucose.

significant association was found between egg intake and MetS in the highest quartile group (12, 13). In a cross-sectional study of 11,529 people in China, it was found that the risk of MetS in people who consumed >7 eggs per week was 18% lower than that those who consumed <3 eggs (OR = 0.82, 95% CI = 0.74–0.91) (10). In another cross-sectional study of 8,241 people in China, it was found that the risk of MetS of consuming >1 egg per day was 1.18 times that of consuming <1/2 egg per day (40–50 g per egg) (23).

The results are controversial between egg consumption and MetS, which may have the following reasons. 1) There may be an effect of racial differences; 2) some studies have found that the association may be primarily driven by egg consumption patterns (7, 24). For example, In the United States, egg consumption reflects adherence to Western dietary patterns, as eggs are often eaten with red or processed meat, refined grains, and sugary beverages. Even careful adjustment of foods commonly consumed with eggs will not completely eliminate residual confounding associated with egg consumption habits; 3) In addition, the length of follow-up in the cohort study may also be related to the inability to draw conclusions about the long-term effects of egg intake; 4) Differences in the covariates adjusted for in the study may have influenced the results.

It has been found that the inverse association between egg intake and MetS may be attributed to other components of eggs rather than cholesterol, such as lutein and zeaxanthin, which may influence the development of MetS by improving lipoprotein metabolism and plasma carotenoid status (25). The explanation for the positive correlation was mainly attributed to cholesterol (23). In addition, eggs are rich in choline, and the plasma trimethylamine-N-oxide produced by intestinal microbiota metabolism may contribute to the positive correlation between egg intake and MetS (26). Studies have shown that the nutritional benefits of an egg far outweigh the adverse effects of the cholesterol it contains. The Dietary Guidelines for Americans (2020–2025) recommend eggs as a part of a healthy diet and remove the daily dietary cholesterol limit of 300 mg. The dietary Guidelines for Chinese Residents in 2022 put forward the recommended intake of eggs of 280∼350 g per week, but the population within the recommended range only accounts for 13.9% (27). Therefore, it is suggested to strengthen the propaganda and education on the nutritional value and health effects of eggs, improve the health awareness of residents, and increase the egg intake of Chinese residents in an appropriate amount.

Association between eggs and five CMFs and its mechanism

Our study found that egg intake reduced the risk of CMFs within a range. Similar results were found in a cross-sectional study of 23,993 subjects in Korea (22). In South Korea's Yangpyeong Cohort ($n = 3,616$), the study found that compared with no consumption of eggs, consuming >3 eggs per week was negatively associated with elevated TG and elevated plasma glucose only in men (7). Another large-scale genomic community-based study conducted in Korea ($n = 130,420$) found that compared with <1 egg per week, higher egg intake (≥7 eggs per week) was associated with lower risk of five CMFs in women and lower risk of decreased HDL-C in men (28). This indicates that gender may also affect the association between egg intake and disease in addition to racial differences. In our study, gender interaction was conducted on the sample population in advance and no statistical difference was found ($p = 0.14$), so there was no stratified analysis of gender.

Eggs serve as a source of high-quality protein, some studies have reported that egg intake can increase satiety and reduce calorie intake (29, 30), and it has also been shown to promote weight loss in limited human studies (31, 32), which may be related to the reduced incidence of central obesity. Another intervention study found that eggs decreased small LDL particles (33), which were highly correlated with decreased HDL-C and elevated TG (34). The negative correlation between eggs and elevated plasma glucose can be explained by the egg-induced decrease in inflammation (35), which may be due to the increased insulin sensitivity from monounsaturated fatty acids, polyunsaturated fatty acids, and antioxidants (lutein, zeaxanthin, and folic acid) in eggs (7). However, a positive association was found between eggs and diabetes, mainly in Americans, which was believed to be driven by egg consumption patterns. In addition, choline metabolites may be related to the relationship between egg intake and diabetes mellitus (24). Finally, omega-3 fatty acids high in eggs can compete with arachidonic acid in the cyclooxygenase pathway to decrease blood pressure (7).

Our study provided prospective evidence of non-linear association between egg intake and CM risk factors. In addition, for the classification of continuous variable, the number and boundary value of the classification are often subjective, which may lead to bias of research results (36). Therefore, the dose-response relationship between egg intake and CMFs was further analyzed by RCS model, and the results were consistent. However, there are still some limitations: 1) The 3 days−24 h dietary recalls has the recall bias, and it usually cannot evaluate daily dietary intake. To investigate long-term dietary behavior, it is better to use the food frequency questionnaire (FFQ) to collect dietary information, but in calculating nutrients, the 3 days−24 h dietary recalls is more accurate than FFQ; 2) We cannot rule out the possible influence of healthy dietary patterns associated with egg consumption; 3) Menopausal status, hormone use and oral contraceptives were not examined in this study, which may have influenced the results; 4) The different cooking methods of eggs may have influenced the results; 5) The influence of unknown confounding factors may exist; 6) We found that most dietary surveys abroad use FFQ method, while China mostly uses 3 days−24 h dietary recalls,

which should be cautious when comparing and extrapolate the results.

Conclusion

In summary, our results suggest an overall *U*-shaped association between egg intake and CMFs. More prospective studies are needed to verify the differences in association and the possible mechanisms among different CMFs in future.

Data availability statement

The original contributions presented in the study are included in the article/Supplementary material, further inquiries can be directed to the corresponding author/s.

Ethics statement

The studies involving human participants were reviewed and approved by the Ethics Review Committee of Institute of Nutrition and Health of Chinese Center for Disease Control and Prevention (No. 201524). The patients/participants provided their written informed consent to participate in this study.

Author contributions

YJ data collation, statistical analysis, and paper writing. WL, HJ, LW, SW, LH, and XJ data collection, paper revision, and guidance. HW and BZ research guidance, paper review, and administrative support. ZW and GD research design, funding support, paper revision, and review. All authors have read and agreed to the published version of the manuscript.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fpubh.2022.1010539/full#supplementary-material

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Association of intestinal microbiota markers and dietary pattern in Chinese patients with type 2 diabetes: The Henan rural cohort study

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Studies on intestinal microbiota in Chinese type 2 diabetes mellitus (T2DM) patients are scarce and correlation studies with dietary intake are lacking. The case-control study included 150 participants (74 T2DM patients and 76 controls) and microbiome analysis was performed using 16S rDNA sequencing. Principal component analysis was used to determine dietary patterns and correlation analysis was used to evaluate the associations between microbiota diversity, T2DM indicators and dietary variables. Compared to controls, the T2DM group had different gut flora characteristics, including lower alpha diversity, higher Firmicutes/Bacteroidetes ratios, statistically significant beta diversity and other specific bacterial species differences. Gut microbiota was associated with several diabetes-related metabolic markers including HOMA2-β, fasting plasma glucose, HbA1c and fasting insulin. Significant associations were also observed between dietary intake pattern and gut flora. The animal foods pattern scores were positively correlated with the relative abundance of the phylum Fusobacteria, and the vegetarian diet pattern scores were positively correlated with the relative abundance of the phylum Actinobacteria. Phylum Actinobacteria mediated the association of vegetarian diet pattern with fasting insulin and HOMA2-β (all $P < 0.05$). Composition of intestinal microbiota in Chinese T2DM patients differs from that of control population, and the intestinal flora is affected by dietary intake while being associated with several diabetes-related metabolic markers. The gut microbiota may play an important role in linking dietary intake and the etiology of T2DM.

KEYWORDS

T2DM, gut microbiota, 16S rDNA, diet, dietary pattern

Introduction

For Almost 500 million people worldwide have diabetes, and it is expected to exceed 600 million by 2030 and 800 million by 2045 with the majority of them being T2DM, according to International Diabetes Federation (1). T2DM is a complex chronic metabolic disease, and its occurrence is associated with genetic and various environmental factors. Previous studies have shown that intake of specific types of food was associated with the risk of T2DM (2, 3). Recent studies also suggest that the development of T2DM may also be associated with altered intestinal microbiota and that dietary intake might play a role in the process (4).

Several previous studies have attempted to explore the relationship between intestinal microbiota, dietary intake and T2DM. A number of experimental animal studies have shown that consumption of specific dietary components can alter the structure and function of the murine gut microbial community, and gut microbes are associated with the metabolism of substances which play an important role in the regulation of food and energy intake, glucose tolerance, and insulin sensitivity ((5–8)). Similar results have been observed in observational studies of population where intestinal bacteria affect the immune and metabolic activities of the host (9, 10). In addition, gut microbiota are associated with glucose regulation, insulin resistance, and glucose tolerance, and these results have been confirmed by various animal studies and population research from Europe (11–14). These findings suggest that changes in the composition and function of the intestinal microbiota are associated with T2DM and may be involved in the relationship of T2DM with dietary factors.

China has a large number of diabetic and pre-diabetic patients, and diabetes has become a critical health issue (15). Given the special dietary habits of Chinese people and the differences in intestinal microbiota indicators for T2DM between Chinese and European or American populations (11, 16–19), the results of studies in other countries may not be generally applicable to Chinese people. Thus, the study of the relationship between intestinal microbiota, T2DM and dietary factors is of great importance for the prevention and treatment of diabetes in China (15), and few studies have analyzed these three factors comprehensively. In this study, the case-control study method was used to explore the characteristics of intestinal microbiota in Chinese T2DM patients and to investigate the association between gut microbes, T2DM and dietary intake and the mediating effect of intestinal hygiene products. Our findings could provide basis for the further understanding of the pathogenesis and precise prevention of T2DM.

Materials and methods

Study participants

Participants in this community-based study were drawn from the Henan Rural Cohort, a large population-based study of chronic non-communicable diseases in five regions of Henan Province, China (20). In the present study, 74 T2DM patients and 76 matched controls (new-onset T2DM patients randomly selected from 1 site of the cohort; controls matched according to age, gender and other social-demographic features) were included. Of note, unlike other epidemiological or clinical studies, there is no precise formula for calculating the sample size of a survey for intestinal flora studies. Based on the literature of relevant similar studies and previous experience with 16S rDNA gene sequencing analysis, the minimum sample size for each group was 15 cases. The present study has an adequate sample size of 150 subjects, with 74 in the case group and 76 in the control group.

Inclusion criteria were as follows: (1) T2DM patients aged 35 to 79 years old; (2) no intention to change diet or physical activity or lose weight during the study; and (3) informed consent to the study by the participants themselves and their families. Exclusion criteria were as follows: (1) treatment with antibiotics or ingestion of other medications affecting intestinal microbiota within 2 months prior to stool sampling; (2) recent history of diarrhea, inflammatory bowel disease, or other conditions that may affect intestinal microbes; (3) missing information on dietary intake questionnaires or other covariates; and (4) normal energy intake.

Ethical approval was obtained from the Ethics Committee of Zhengzhou University ([2015] MEC (S128)). Written informed consent was obtained from all the participants before the study began.

Data collection and anthropometric measurements

Basic information about each participant was obtained through a questionnaire administered by trained staffers. Face-to-face interviews were conducted to collect information on demographic characteristics (age, gender, income, marital status and education level), lifestyle behaviors (smoking, alcohol consumption, physical activity and eating habits), personal and family history of disease and other aspects.

In this study, dietary intake was assessed according to the Dietary Guidelines for Chinese Residents and the dietary characteristics of residents in Henan Province using a food frequency questionnaire (FFQ) consisting of thirteen major food items (the reliability and validity of the FFQ have been verified)

(21). Food items included staple foods, red and white meat, fish, eggs, dairy, fruits, vegetables, beans, nuts, grains, pickles, and animal oils. Of the major food groups, it was emphasized that Chinses diet had a lower consumption of grains (wheat, rice, fine flour and their products) compared with staple food (Maize, oats, Korghum flour and red thistle), thought to be the primary sources of energy. According to the questionnaire, participants were asked to report the frequency (never, day, week, month, and year) and quantity (kg, g) of food consumed in the past year. For each major food item, dietary recall was assessed using visual photo mapping, allowing participants to assess the specific intake of each food compared to standard doses.

Anthropometric measurements were taken on the same morning as the questionnaire. For weight measurement, participants were dressed lightly without shoes and their weight was rounded to the nearest 0.1 kg; height was measured to the nearest centimeter using a standard ruler. Body mass index (BMI) was then calculated by dividing weight (kg) by height squared (m^2). After a rest period of at least 5 min, blood pressure was measured three times with an electronic sphygmomanometer (Omron HEM-7071A, Japan) on the right arm at 30-s intervals. The average of the three measurements was used for statistical analysis.

Blood and fecal samples collection and laboratory analysis

Venous blood samples were taken from overnight fasting participants for laboratory measurements. Fasting plasma glucose (FPG), total cholesterol (TC), triglycerides (TG), high-density lipoprotein cholesterol (HDL-C), and low-density lipoprotein cholesterol (LDL-C) were measured either directly or enzymatically using a ROCHE Cobas C501 automated biochemistry analyzer. Homeostatic model assessments of insulin resistance (HOMA2-IR) and beta-cell function (HOMA2-β) were calculated according to the designed equations based on fasting glucose and insulin levels (https://www.dtu.ox.ac.uk/homacalculator/) (22).

Participants were given a specimen collection kit and stool samples were obtained at their homes. Fecal samples were first stored in their home freezer and then transferred and stored at −80°C until further use. DNA quantity and quality were analyzed using the Nanodrop 2,000 spectrophotometer (Thermo Scientific) and molecular size agarose was estimated by gel electrophoresis.

The V3 and V4 region of the bacterial 16S rRNA gene was amplified from extracted fecal DNA through degenerate primers (338F: 5'-ACTCCTACGGGAGGCAGCA-3', 806R: 5'-GGACTACHVGGGTWTCTAAT-3'). The amplicons were purified and quantified according to manufacturer's protocols. The procedure of polymerase chain reaction (PCR) is as follows: denaturation (2 min at 98°C), followed by 30 cycles consisting of denaturation (30 s at 98°C), annealing (30 s at 50°C), extension (60 s at 72°C) and a final extension at 72°C for 5 min. PCR products were detected by 1.8% agarose gel electrophoresis. The magnetic bead system was used to purify the replicate PCR reactions. Purified PCR amplicons of each sample were mixed, according to the amplicon concentration of samples detected by Nanodrop. The amplicons were sequenced in a single pool in one run with the Hiseq 2,500 platform (250 PE, Illumina).

Covariate definition

Educational attainment was classified into four categories based on the questionnaire report: illiterate, primary school, junior high school, senior high school and above. Marital status was grouped into two levels, including married/cohabiting and other/widowed. The per capita monthly household income was calculated on a household basis and divided into three categories: <500 RMB/month, 500–1000 RMB/month and ≥1,000 RMB/month. Smoking and drinking status were both divided into two levels according to whether the subjects have a history of smoking or drinking. Family history of diabetes is defined as any immediate family member (e.g., parent, sibling or child) of the study participant having diabetes. The International Physical Activity Questionnaire (IPAQ) classifies physical activity levels into three levels: heavy physical activity, moderate physical activity and light physical activity.

Bioinformatics analysis

The raw sequences were processed to concatenate forward and reverse reads using FLASH (version 1.2.11), and resulting sequences were quality filtered by Trimmomatic (version 0.33) and chimeras removed with UCHIME (version 8.1) (23–25). Sequences were aligned through USEARCH (version 10.0) and clustered into OTUs by 97% similarity, and taxonomy was assigned using RDP Classifier (version 2.2) with Silva (Release 132) as a reference base (26–28). The phylogenetic tree was built with PyNAST (version 1.2.2) with the Neighbor-Joining method (29).

Alpha diversity (Chao1 index, Ace index, Shannon index and Simpson index) was calculated using Mothur (version v.1.30). Beta diversity (weighted and unweighted UniFrac distance metrics) were calculated and visualized using R by principal coordinates analysis (PCoA).

Linear discriminant effect size analysis (LEfSe) based on OTU level was performed to find differentially represented features between control and T2DM groups (30).

Statistical analysis

Given the differences between the two groups compared, normally distributed data were expressed as mean ± standard deviation (SD) and were analyzed using the independent *t*-test, while non-normally distributed data (continuous variables) were expressed as medians and interquartile ranges (IQR) and analyzed using the Wilcoxon rank sum test. The χ2 test was used to test for differences in categorical variables between cases and controls. The alpha diversity was tested with the Wilcoxon rank-sum test among groups. Permutational multivariate ANOVA (PERMANOVA) was performed to test beta diversity dissimilarity. Wilcoxon rank sum test was used to assess the association of relative abundance among each taxonomic level. We used a Spearman correlation analysis to examine the correlations between dietary intake with intestinal microbiota abundance, and dietary intake with diabetes-related metabolic markers. Mediation analysis was conducted using the relative abundance of intestinal microbiota as a mediator of the relationship between dietary intake and diabetes-related metabolic markers to investigate the relevance of significant findings related to type 2 diabetes, with potential covariates adjusted. False discovery rate (FDR) using the Benjamini-Hochberg method was applied to correct for the significant *P*-values. The PCA method was used to extract data and reduce the dimensionality of dietary intake information (in 11 food groups except for pickles and animal oils) and the factors were variationally orthogonal transformed. Identified factors were retained by scree plot, evaluated eigenvalues (>1) and interpretability. The sample adequacy was examined using the Kaiser-Mayer-Olkin (KMO) test for factor analysis (KMO = 0.69 > 0.6, Bartlett's test of sphericity $p < 0.01$). All results were deemed significant if the *p*-value was below 0.05 and data analysis was performed using R software (version 4.0.3; R Project for Statistical Computing, Vienna, Austria).

Results

Demographic characteristic

Basic information on participants is shown in Table 1. Among the 150 participants, the mean (SD) age was 59.39 (8.01) years, of which 61.3% were female individuals. The distribution of age, gender, demographic information and socioeconomic background, as well as other lifestyle habits and physical activity data were similar between T2DM and control individuals, for which the differences were not statistically significant. Compared to controls, T2DM participants had higher BMI, WC, FPG, fasting insulin, HbA1c, TC and TG (P <0.05 for all), but no significant differences in HDL-C and LDL-C (Table 1).

TABLE 1 Characteristics of T2DM cases and controls.

Characteristics	T2DM (*n*=74)	Controls (*n*=76)	*P*
Gender (%)			1
Male	29 (0.4)	29 (0.4)	
Female	45 (0.6)	47 (0.6)	
Age (year)	59.46 ± 7.88	59.32 ± 8.18	0.763
Education level			0.831
Illiterate	20 (0.3)	21 (0.3)	
Elementary school	29 (0.4)	26 (0.3)	
Junior high school	20 (0.3)	21 (0.3)	
High school or above	5 (0.1)	8 (0.1)	
Marriage			0.566
Married/cohabiting	64 (0.9)	69 (0.9)	
Other/solitary	10 (0.1)	7 (0.1)	
Income			0.533
<500 CNY	30 (0.4)	37 (0.5)	
500–1,000 CNY	19 (0.3)	19 (0.3)	
>1,000 CNY	25 (0.3)	20 (0.3)	
Alcohol			0.447
Y	13 (0.2)	9 (0.1)	
N	61 (0.8)	67 (0.9)	
Tobacco			0.888
Y	12 (0.2)	14 (0.2)	
N	62 (0.8)	62 (0.8)	
Family history of diabetes			0.114
Y	5 (0.1)	1 (0)	
N	69 (0.9)	75 (1)	
Physical activity			0.244
Low	16 (0.2)	19 (0.3)	
Moderate	38 (0.5)	45 (0.6)	
High	20 (0.3)	12 (0.2)	
BMI (kg/m^2)	25.5 ± 3.5	23.5 ± 3.0	<0.001
WC (cm)	86.8 ± 9.2	80.8 ± 8.9	<0.001
FPG (mmol/L)	9.3 ± 2.5	5.0 ± 0.4	<0.001
Fasting insulin (μU/L)	14.5 ± 6.6	11.7 ± 4.3	0.003
HbA1c (mg/dL)	8.3 ± 1.9	5.3 ± 0.3	<0.001
TC (mmol/L)	5.0 ± 1.0	4.7 ± 0.8	0.036
TG (mmol/L)	2.3 ± 1.6	1.7 ± 0.8	0.002
HDL-C (mmol/L)	1.3 ± 0.3	1.4 ± 0.3	0.331
LDL-C (mmol/L)	3.2 ± 0.93	3.0 ± 0.8	0.180

χ^2 tests for categorical variables, *t*-test or Wilcoxon rank sum test for continuous variables; Continuous variables are presented as mean±SD, and categorical variables are expressed as *n* (%); CNY, Chinese yuan; BMI, body mass index; WC, waist circumference; FPG, fasting plasma glucose; TC, total cholesterol; TG, Triglyceride; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol.

Intestinal microbiome profile

A total of 11,529,795 reads were sequenced from 150 samples, and 10,838,351 clean tags were generated after splicing

and filtering, averaging 72,256 clean tags per sample. 432 OTUs were obtained from all samples at the 97% similarity level.

Alpha diversity analysis results are shown in Figure 1. The T2DM group had lower microbial richness compared to the control group participants (ACE index: 259.02 (229.33–284.84) vs. 275.75 (251.29–297.80), $P = 0.035$; Chao index: 256.82 (228.06–282.33) vs. 283.00 (249.28–302.08), $P = 0.002$). There is no difference in microbial diversity indices between T2DM individuals and controls (Simpson index: 0.108 (0.074–0.154) vs. 0.093 (0.072–0.128), $P = 0.21$; Shannon index: 3.068 (2.793–3.364) vs. 3.198 (2.968–3.455), $P = 0.07$) (Figure 1).

Both Weighted UniFrac distance metrics and unweighted UniFrac distance metrics to some extent distinguished the intestinal microbiota of T2DM subjects from that of the control subjects. There were significant differences in β-diversity between the T2DM and control groups ($P < 0.001$) (Figure 2).

Microbial composition difference between T2DM and controls groups

At the phylum level, most of the sequencing reads were classified into four phylum groups (98.61% of total): Firmicutes (65.33%), Bacteroidetes (18.33%), Actinobacteria (8.03%) and Proteobacteria (6.94%). The relative Abundance of each gut microbiota at the phylum level was not significantly different between the T2DM group and the control group. The ratio of Firmicutes/Bacteroidetes was significantly higher in T2DM group [5.69 (2.30–19.14)] than that in the control group [2.89 (1.85–10.62)] ($P = 0.037$). At class level, the Bacteroidia and Melainabacteria showed lower abundances among T2DM cases compared to those of controls, whereas the relative abundances of Erysipelotrichia were higher in T2DM cases than in controls. At the order level, Pasteurellales, Bacteroidales and Gastranaerophilales were less abundant while Erysipelotrichales and Erysipelotrichales were more abundant in the T2DM group compared to non-T2DM controls. At family level, the results showed that the case group had higher abundance of Enterobacteriaceae and Enterococcaceae but lower abundance of Acidaminococcaceae, Clostridiaceae and an uncultured bacterium belonging to Bacteroidales (Supplementary Table 1). At the genus level, 21 genera with significantly different relative abundances were identified between the T2DM and control groups, of which 7 were more abundant in the case group and 14 were more abundant in the control group (All $P < 0.05$) (Table 2).

LEfSe analysis identified eight discriminatory features with significant differences in relative abundance between the T2DM and control groups (LDA score $\geq$4). From the phylogenetic dendrogram, it is clear that the differences mainly occurred in Enterobacteriales and Bacteroidales, which is consistent with the results of the previous analysis of bacterial composition (Figure 3).

Associations of gut microbiota and T2DM metabolic markers

To explore the relationship between intestinal microbiota, dietary intake and diabetes-related metabolic markers, Spearman's correlation analysis was performed and significant correlations between them were observed. For example, between intestinal microbiota and metabolic markers, alpha diversity (Chao1 index and Ace index) was negatively correlated with FPG and HbA1c, and significantly positively correlated with HOMA2-β; F/B ratio was positively correlated with BMI, FPG and HbA1c, and negatively correlated with HOMA2-β. Phylum Bacteroidetes and Tenericutes were negatively correlated with FPG and significantly positively correlated with HOMA2-β; Fusobacteria was positively correlated with FPG; Proteobacteria was positively correlated with HDL-C, LDL-C and TC (All P <0.05) (Figure 4). And the correlative heat map between metabolic markers and gut microbial genus was displayed in the Supplementary Figure 1.

Associations of dietary intake and gut microbiota

Correlation analysis of dietary intake and intestinal microbiota revealed that phylum Actinobacteria was positively correlated with dairy intake; Patescibacteria was negatively correlated with beans and grains; WPS-2 was negatively correlated with nuts; and white meat was positively correlated with Firmicutes (Supplementary Figure 2). The correlation analysis of intestinal microbiota at genus level with dietary intake was shown in the Supplementary Figure 3.

Three dietary patterns were obtained by principal component analysis: animal foods pattern, grain pattern and vegetarian diet pattern (patterns were named based on higher food group factor loading) (Supplementary Figure 4). The three factors explained 47% of the variance in the total food intake, with the animal foods pattern explaining 18% and the latter two patterns both accounting for 14% of the variance in food intake. The animal foods pattern was dominated by the intake of white meat, fish, red meat and egg. The grain pattern was characterized by higher intakes of grains (i.e., sweet potatoes/corn), nuts (i.e., peanuts, melon seeds) and legumes, whereas the vegetarian diet pattern was characterized by a predominant intake of staple foods, fruits, dairy products, and vegetables. Correlation analysis of dietary pattern scores with intestinal microbiota indicated that animal foods pattern scores were positively correlated with the relative abundance of the

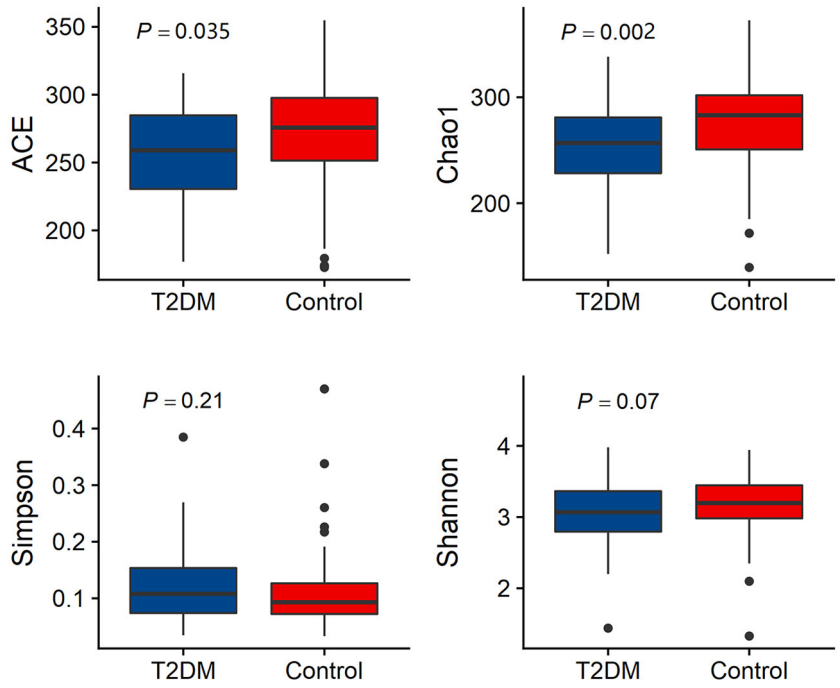

FIGURE 1
Comparison of alpha diversity (Ace index, Chao1 index, Simpson index and Shannon index) between the gut microbiota of T2DM and controls.

phylum Fusobacteria ($r = 0.17$; $P = 0.03$), and the vegetarian diet pattern scores were positively correlated with the relative abundance of the phylum Actinobacteria ($r = 0.21$; $P = 0.01$).

The mediating effects of intestinal microbiota

To explore the relationship among intestinal microbiota, dietary intake and diabetes-related metabolic markers, the mediation analysis was performed and significant association between them were observed. The mediation analysis showed that the association of the vegetarian diet pattern with fasting insulin (Indirect effect; β (95%CI): −0.269 (-0.684, −0.01); $P = 0.022$) and HOMA2-β (Indirect effect; β (95%CI): −2.365 (−4.762, −0.45); $P = 0.01$) was mediated by the phylum Actinobacteria (Figure 5).

Discussion

In this case-control study, we analyzed the intestinal microbiota characteristics of type 2 diabetic patients in a Chinese population, and explored the relationship between intestinal microbiota, type 2 diabetes and dietary intake pattern. To our knowledge, this is the first study to focus on the association between gut microbes, dietary component intake and T2DM in Chinese patients. We found that intestinal microbiota correlated with multiple diabetes-related metabolic markers. ACE index, Chao1 index, and phylum Bacteroidetes were negatively correlated with FPG and positively correlated with HOMA2-β, whereas the F/B ratio was the opposite. In addition, we also had several novel findings that dietary intake also had an impact on gut microbiota, for example, vegetarian dietary patterns were positively correlated with the phylum Actinobacteria and Actinobacteria also mediated the relationship of vegetarian dietary patterns with fasting insulin and HOMA2-β. These findings could help to explain the pathogenesis of T2DM and provide a basis for subsequent intervention, treatment and further research.

Although with different ethnicities, geographic locations, and habitual diets, our findings of intestinal microbiota characterization in Chinese T2DM patients agree well with findings from other populations (31, 32). T2DM patients had lower gut microbiota ACE index and Chao index than the control population; both the ACE index and the Chao index were composite indicators of richness and evenness. Correlation analysis also showed that alpha diversity was negatively correlated with FPG and HbA1c and positively correlated with HOMA2-β. We note that the F/B ratio was higher in diabetic patients and it was positively correlated with BMI, WC, FPG and HbA1c, which is similar to results of an animal experiment and a Ukrainian population study (33–35), suggesting that the F/B ratio is associated with obesity and glucose metabolism,

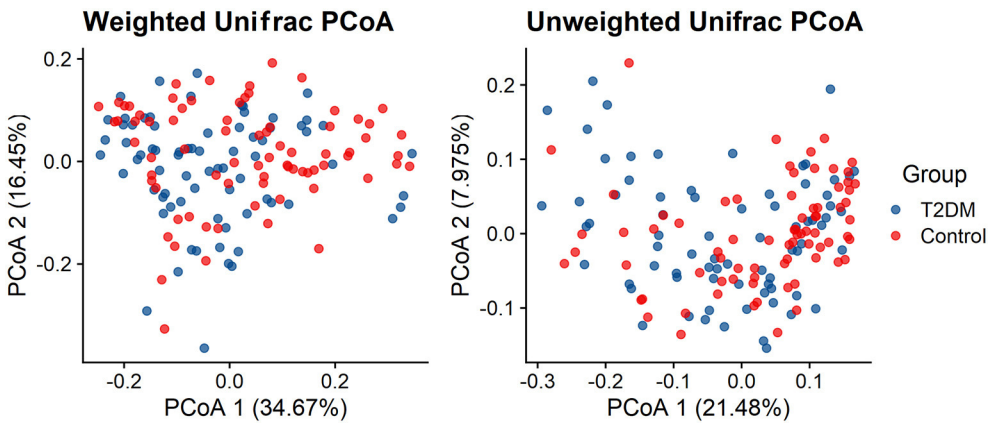

FIGURE 2
Principal coordinate analysis illustrating the grouping patterns of the T2DM and control individuals based on the unweighted UniFrac and weighted UniFrac distances (both $p < 0.001$).

TABLE 2 Comparison of genus abundance between T2DM cases and controls.

Genus	T2DM	Control	*P*	*P*-adjusted
Blautia	0.061	0.044	0.001	0.018
Citrobacter	0.002	0.000	0.003	0.032
Clostridium_sensu_stricto_1	0.019	0.026	0.000	0.012
Coprococcus_2	0.005	0.007	0.000	0.013
Enterobacter	0.003	0.000	0.006	0.048
Enterococcus	0.001	0.000	0.006	0.048
Fournierella	0.000	0.000	0.001	0.017
Haemophilus	0.001	0.002	0.008	0.047
Klebsiella	0.027	0.005	0.001	0.015
Lachnospira	0.001	0.002	0.000	0.002
Lachnospiraceae_NK4A136_group	0.005	0.011	0.007	0.048
Paraprevotella	0.000	0.001	0.001	0.017
Phascolarctobacterium	0.006	0.007	0.006	0.044
Roseburia	0.011	0.022	0.001	0.014
Ruminococcaceae_UCG-010	0.001	0.001	0.005	0.048
Ruminococcus_1	0.005	0.007	0.008	0.048
[Eubacterium]_eligens_group	0.009	0.007	0.006	0.046
[Eubacterium]_xylanophilum_group	0.001	0.002	0.006	0.043
Uncultured_bacterium_f_Peptostreptococcaceae	0.000	0.000	0.000	0.015
Uncultured_bacterium_o_Bacteroidales	0.004	0.003	0.001	0.018

and can be used as a marker for intestinal abnormalities in obese patients. In terms of specific microbial categories, we found that the relative abundance of the phylum Proteobacteria was positively correlated with blood lipid composition, whereas Tenericutes was negatively correlated with FPG and TG and positively correlated with islet secretory function. These findings are further supported by other observational and interventional studies from Chinese and European populations, revealing adverse impacts of Proteobacteria and the probiotic effects of Tenericutes (36–39). All these findings suggest that intestinal microbiota can be used as an entry point for further exploration of T2DM pathogenesis and even diagnosis and treatment.

Other significant findings of our study include the diet's impact on the regulation, structure and diversity of the intestinal microbiota. Diet is an important factor in the regulation of intestinal microbiota and has an important impact on the

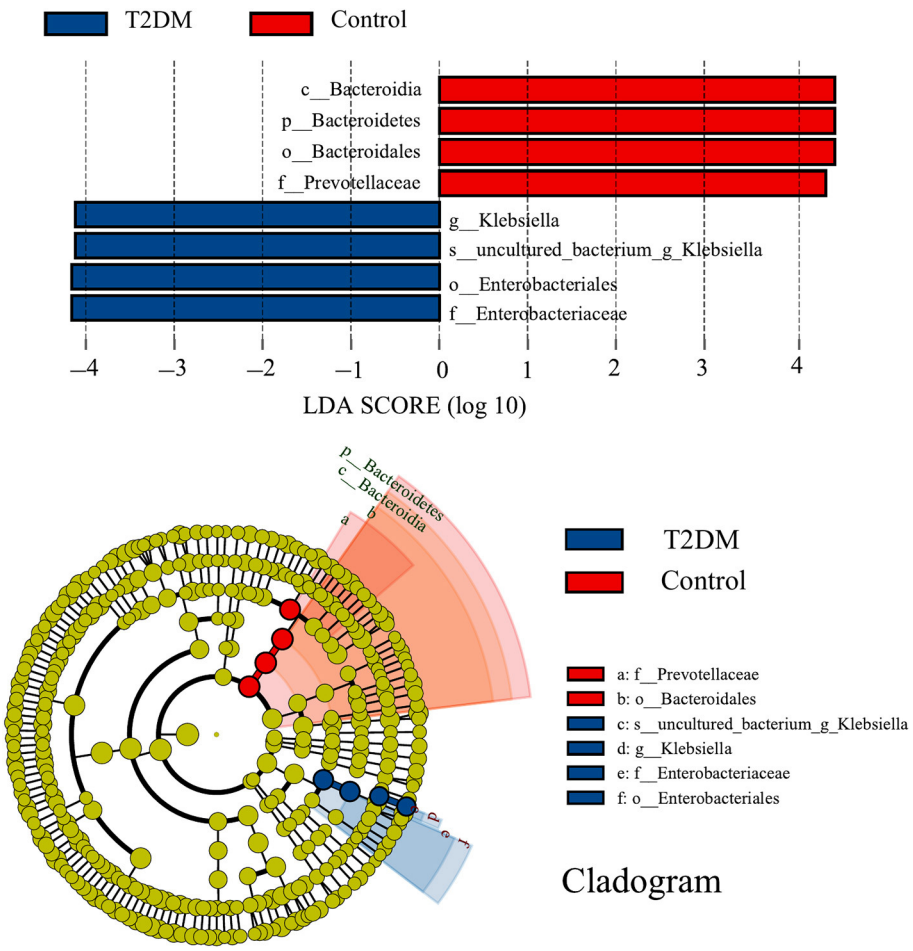

FIGURE 3
LEfSe analysis and corresponding phylogenetic dendrograms between T2DM and control groups (LDA score ≥ 4). The circles radiating from inside to outside of the phylogenetic dendrograms represent taxonomic levels from phylum to species; each small circle at a different taxonomic level represents a taxon at that level, and the diameter size of the small circles is proportional to the relative abundance size; species with no significant differences are colored uniformly in yellow.

structure and diversity of the microbiota (4). Several studies have shown that dietary intake plays an important role in the formation of intestinal microbiota and the maintenance of intestinal health (4, 9, 10, 40); for example, dietary fiber, vegetarian dietary patterns, and higher dietary quality have promotive effects on beneficial intestinal bacteria, while high-fat or high-energy diets have detrimental effects (7, 37, 41–44). In this study, we discovered that the dietary pattern of animal foods is positively correlated with the relative abundance of Fusobacteria. Fusobacteria is a common oral microbiota, and studies have shown that Fusobacteria found in the gut is associated with cancer, colitis and T2DM, possibly related to its influence on inflammation and immune responses (45–49). Another study also found similar relationships between animal food intake and Fusobacterium (47). In addition, it was also found that Fusobacteria was positively correlated with beans intake, and negatively correlated with fat intake, seems to be in conflict with the opinion above (50). That means how Fusobacterium can be regulated by dietary factors, even as an intervention or treatment method, is worthy of further study.

Another notable finding from our study is the effects of a vegetarian dietary pattern. Results suggest that the vegetarian diet pattern was positively correlated with Actinobacteria, while Actinobacteria was negatively correlated with FPG and HOMA2-β. Actinobacteria mediated the relationship of vegetarian diet pattern with fasting insulin and HOMA2-β. The vegetarian diet pattern includes ingredients such as vegetables and fruits rich in dietary fiber, which contribute to the growth of fiber-fermenting bacteria. Actinobacteria is the dominant intestinal micromicrobiota in most mammals, and those detected in the gut are usually beneficial, such as Streptomyces and Bifidobacterium, which can ferment fiber and secrete bioactive metabolites including butyrate, lactic acid and B vitamins (51, 52). Based on these properties, a number of

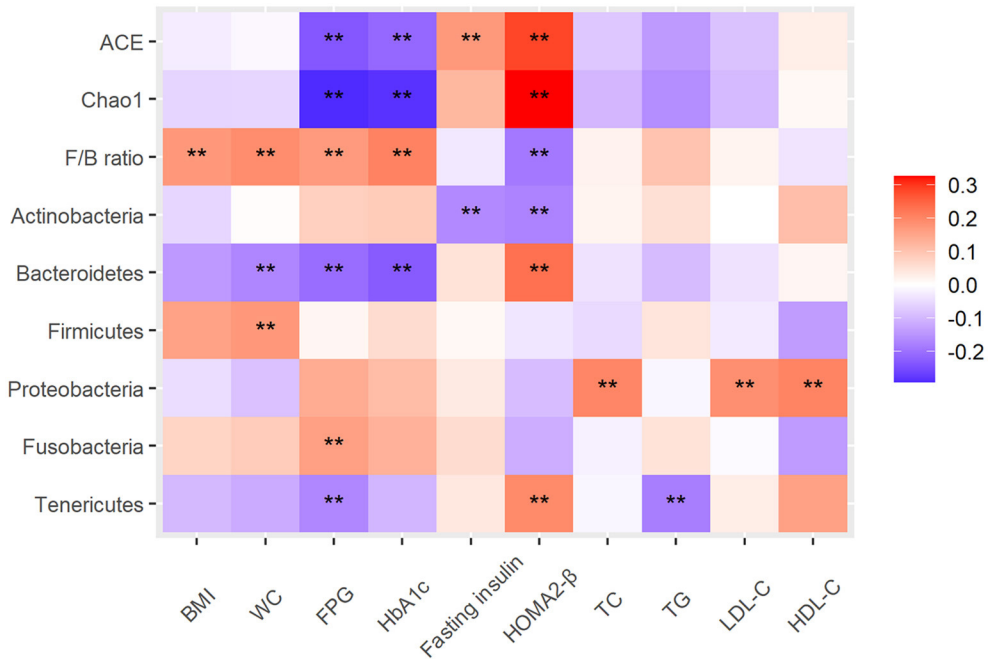

FIGURE 4
Heat map of the Spearman correlation between intestinal microbiota and type 2 diabetes–related traits. The intensity of the colors represents the degree of association as measured by the Spearman correlation. All significant correlations are marked with an asterisk (P <0.05). Alpha diversity index, ACE, Chao1; F/B ratio, ratio of Firmicutes/Bacteroidetes; BMI, body mass index; WC, FPG, fasting plasma glucose; HOMA2-β, Homeostatic model assessments2 of beta-cell function; TC, total cholesterol; TG, triglycerides; LDL-C, low-density lipoprotein cholesterol; HDL-C, high-density lipoprotein cholesterol.

Actinobacteria strains are considered to be a probiotic and are added to yogurt or other fermented foods to promote human health (53). Although no studies have reported this mediating role of intestinal microbiota modulating the relationship between vegetarian diet and insulin metabolism indicators, similar direct association results between vegetarian diet with intestinal microbiota and intestinal microbiota with insulin metabolism indicators have been reported in other population studies and interventional trials or animal experiments (36, 40, 41, 48). However, our study found only superficial associations, and further and more robust studies are needed to investigate deeper mechanisms.

Our study has several strengths. First, our study is the first observational study to explore comprehensively the association between dietary intake, intestinal microbiota and T2DM risk. Second, T2DM patients included in this study were all new-onset diabetes patients so as to avoid recall bias and changes in eating habits due to illness. Our study also has its potential limitations that should be taken into account when interpreting the results. This study is a case-control study that cannot yield an accurate causality, so caution is needed in the interpretation of the causality relationship. Second, this study only explored the effects of dietary factors and intestinal microbiota on T2DM patients, while other factors such as lifestyle and socioeconomic conditions were not addressed. Third, the dietary intake data were obtained by the respondents' review and assessment of their food intake in the past year, with some recall bias. Nevertheless, the reliability and validity of this food frequency questionnaire were also assessed by our team. Finally, this study used 16S rDNA sequencing of the intestinal flora, whereas the latest macro-genome sequencing technology can provide more accurate and richer analysis, and the combination with analysis of metabolic substances and functional pathways of the intestinal flora can provide more in-depth and convincing results. Thus, subsequent large metagenome cohort studies, with full consideration of lifestyle, dietary habits, socioeconomic and other factors that may influence gut microbiota are needed to elucidate the relationship between intestinal microbiota, dietary intake and T2DM.

Conclusion

In summary, in Chinese patients with T2DM, the intestinal microbiota was significantly different from normal individuals, with lower alpha diversity, higher F/B ratio and significantly different beta diversity. Several metabolic markers associated with diabetes, such as HOMA2-β, FPG, HbA1c and fasting

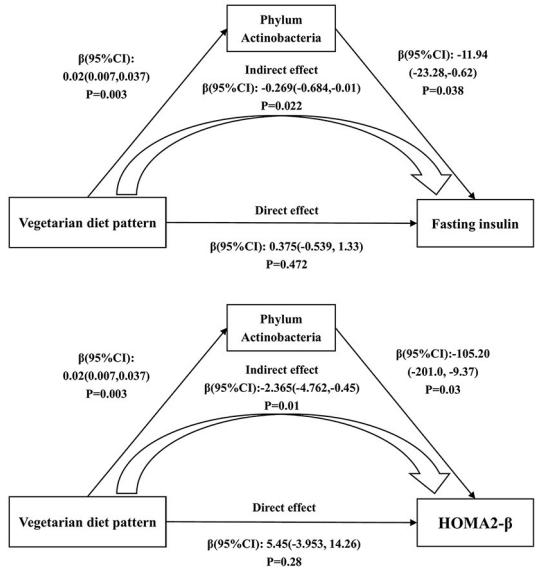

FIGURE 5
The mediating effects of phylum Actinobacteria on the associations of the vegetarian diet pattern score with fasting insulin and HOMA2-β. Adjusted for age, gender, education level, marriage, income, smoking, alcohol consumption and physical activity.

insulin, were significantly associated with intestinal microbiota. In addition, there were significant correlations between dietary intake and intestinal microbiota. Intestinal microbiota can, to some extent, explain the relationship between diet and diabetes. These findings could expand our insights into the mechanisms of diet-induced diabetes.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Ethics statement

The studies involving human participants were reviewed and approved by Zhengzhou University ethics committee. The patients/participants provided their written informed consent to participate in this study.

Author contributions

GW: writing—original draft preparation. QL, SC, and TY: data analysis and validation. TY and SC: writing—review and editing. KN and RG: investigation. YL, JL, WX, and GW: conceptualization. LL: project administration and supervision. All authors have read and agreed to the published version of the manuscript.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fpubh.2022.1046333/full#supplementary-material

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Longitudinal relationship between body fat percentage and risk of type 2 diabetes in Chinese adults: Evidence from the China Health and Nutrition Survey

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Objective: Body fat percentage (BF%) might be an alternative index of obesity which is the major risk factor for developing type 2 diabetes (T2D). We aim to longitudinally evaluated the relationship between BF% and risk of T2D.

Methods: A sample of 5,595 adults aged 18–65 who participated in two waves of China Health and Nutrition Survey (CHNS 2015 and 2018) was analyzed. Two level mixed-effects modified Poisson regression with robust estimation of variance stratified by sex was used to evaluate the risk ratios (RRs) for T2D according to quintiles of BF%, and the curves of receiver operating characteristic (ROC) were plotted to identify the optimal total and trunk BF% cut-off points for predicting an increased T2D risk.

Results: In males, compared with subjects in the first quintile of total BF%, those in the third (RR = 2.03, 95% CI 1.09–3.79), fourth (RR = 2.56, 95%CI 1.46–4.48), and fifth (RR = 2.16, 95%CI 1.22–3.82) quintile had higher risk of T2D after adjusting for all potential confounders (p-trend < 0.001). For females, the RR (95% CI) was 1.92 (1.14, 3.24) in the fifth quintile (p-trend $= 0.014$). Males and females with a trunk BF% >25.5 and 34.4% ($\geq$ quintile 4), respectively, were at significantly increased risk of T2D (p-trend = 0.001). Besides, the optimal cut-off values of total and trunk BF% were 21.9 and 25.2% for males, and 36.7 and 30.3% for females, respectively.

Conclusions: The incident risk of T2D significantly increased over specific level of total and trunk BF% in both Chinese males and females, and the optimal BF% cut-off values were valuable for clinical application of BF% based on sex difference, which may be a cost-effective implementation for prevention and treatment of T2D in China.

KEYWORDS

body fat percentage, cut-off points, type 2 diabetes, obesity, China

Introduction

Urbanization, energy-dense diets, and physical inactivity, with a consequent epidemic of obesity have resulted in the rapid escalation of type 2 diabetes (T2D) around the world (1), particularly in some developing countries (2). Substantial evidence has demonstrated the cause effect of obesity on risk of T2D and insulin resistance (3). Due to their simplicity and ease, body mass index (BMI) and waist circumference (WC) have been routinely employed to identify obese individuals in most epidemiologic studies (4–6). However, these anthropometric measures are unable to directly evaluate body fat and its distribution, and likely to underestimate the prevalence of obesity among people with normal weight but high body fat (7). Body fat percentage (BF%) determined by impedance is increasingly advocated as a favorable measurement of body composition at home and in medical check-ups owing to its safety, simplicity and affordability.

A growing body of studies have shown significant associations between BF% and cardiovascular disease risk factors, such as diabetes mellitus, hypertension, and dyslipidemia, irrespective of BMI and abdominal obesity (8–10). For example, subjects with normal or obese BMI but excess BF% had increased risks of developing T2D compared to those with normal BF% in White populations (8), as well as in Chinese populations (10). Besides, one study with cross-sectional design indicated that obesity measured by BF% could be a better predictor of T2D risk than BMI (11). Although several studies have explored the threshold values of BF% for obesity in different ethnic groups, such as the American Association of Clinical Endocrinology (males: 25%, females: 35%) (12) and Korean adults (males: 21%, females: 37%) (13), the cut-off points of BF% that reflect an increased risk of obesity-related disease remains unclear. Thus, it is crucial to determine the optimal BF% values that indicated the increased risk of T2D.

Given the cross-section design of previous studies (13–15) and ethnicity difference in body fat distribution (16), the current evidence seems insufficient to propose appropriate modification of body fat mass and its distribution based on risk of T2D for Chinese population. Therefore, the present study aimed to examine the longitudinal associations of total and trunk BF% with risk of T2D, using data from the China Health and Nutrition Survey (CHNS), and further identify the optimal cut-off values of total and trunk BF% to predict an increase in T2D risk. Such findings would be valuable for developing and implementing public health actions to provide guidance for reduction and intervention of body fat and improve T2D status in Chinese individuals.

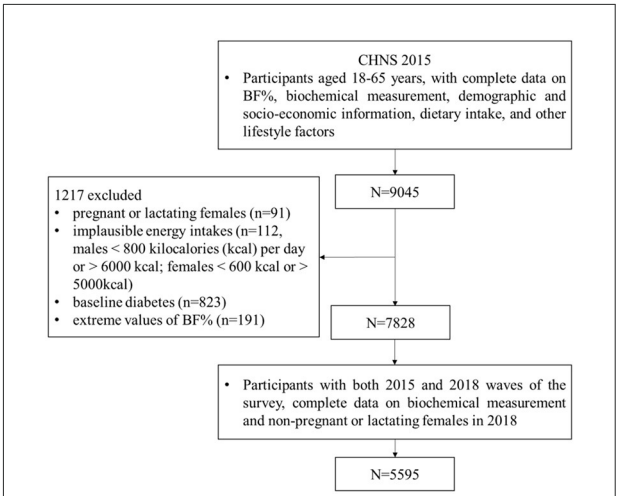

FIGURE 1
Flow chart of study population selection.

Methods

Study population

The current study utilized data from the CHNS, a population-based longitudinal survey with a focus on the relationships between sociological, economic and demographic changes and the effects on numerous nutritional and health status of social Chinese population. The CHNS was initiated in 1989 and carried out ten consecutive rounds of follow-up surveys during the period of 1991–2018, the details of the survey have been described elsewhere (17, 18).

This study was based on the data from the 2015 and 2018 waves of the CHNS. We chose the adults aged 18 to 65 who participated in both rounds of survey and were not diabetes at baseline with complete data on BF%, biochemical measurements, demographic information, socio-economic information, dietary intake, and other lifestyle factors, and then we excluded pregnant or lactating females, those having implausible energy intakes, and those with extreme values of BF%. Figure 1 presents the flow chart of participant selection. We investigated the association between BF% at baseline and risk of T2D in 2018 considering the prospective nature with clear temporal characteristic. The final analysis therefore consisted of 5,595 participants (2,471 males; 3,124 females) clustered in 338 communities. Incidence of T2D means number of diabetes in 2018 among 5,595 adults without diabetes at baseline.

The survey protocol was approved by the Institutional Review Committees of the University of North Carolina at Chapel Hill and the Chinese Center for Disease Control and

Prevention (No. 201524), and all subjects provided written informed consent.

Assessment of body fat percentage

Trained health workers measured BF% including total BF%, trunk BF%, and arm and leg BF%, using a body composition analyzer (TANITA BC601) with the participants in lightweight clothing and without shoes, in the fasting state and before working in the morning. Based on the bioelectrical impedance analysis (BIA), BF% was calculated to the nearest 0.1% according to a proprietary algorithm that required age, sex, height, and physical activity level inputs by investigators. The method has been validated previously and employed regularly in other studies (17, 19). The current study recorded the total BF% (ratio of total body fat mass and total body mass) and trunk BF% (ratio of trunk fat mass and trunk mass) separately, and performed quintiles of them for analysis.

Diagnosis of type 2 diabetes

Experienced nurses or phlebotomists collected overnight fasting blood samples *via* venipuncture. Blood samples were centrifuged within 3 h and preserved at −2∼8°C refrigerator for short-term storage in order to obtain reliable test results for later laboratory analysis. Fasting blood glucose (FPG) and glycated hemoglobin A1c (HbA_{1c}) were detected by GOD-PAP (Randox, UK) and HLC/HLC/HPLC (Tosoh, Japan/ Bio-Rad, USA/ Primus, USA) methods, respectively.

Based on the guideline for the prevention and treatment of type 2 diabetes mellitus in China (2020 edition) (20) and the World Health Organization criteria for diabetes mellitus (21), the diagnostic criterion for T2D was fasting glucose ≥7.0 mmol/L or HbA1c ≥6.5%, or a self-reported diagnosis of diabetes and treatment with antidiabetic pharmacotherapy.

Assessment of covariates

Information on socio-demographic and lifestyle variables were collected through standard questionnaires by trained interviewers, and only baseline covariates in round 2015 were assessed in the present study. The following variables were included: age (in years); per capita family income (tertiles: low, medium, high); individual educational level (primary school and below, completed middle school, high school and above); residence (rural and urban); smoking and alcohol drinking status (current vs. former or non-); community urbanization index (score) calculated based on 12 dimensions of the community level including physical, social, economic, cultural and sanitary environments (22); sleep duration (6–9 h vs. <6 h or 9 h) (23); physical activity (in MET-h/week) referred to the Compendium of Physical Activities (24). In addition, the intake of other dietary factors were also regarded as potential confounders, including total energy intake (TEI), percentage of total energy comes from fat, dietary fiber (25), calcium (26), magnesium (27), and Vitamin C, which were assessed by three consecutive days of 24-h recalls for each individual and the weighing of seasonings in the household inventory over the same period.

Statistical analysis

First, as shown in Supplementary Table 1, we performed statistical interaction tests between BF% and sex, and found significant interaction. We categorized the total and trunk BF% into five levels (quintiles of BF%) by sex, respectively. Baseline characteristics of participants were summarized and examined by Chi-square test for categorical variables, and Wilcoxon rank-sum and Kruskal-Wallis H test for non-normally distributed continuous variables.

To evaluate the association between the quintiles of total and trunk BF% and risk of T2D, a modified Poisson regression with robust (sandwich) estimation of variance was performed, which is an appropriate and reliable approach to estimate relative risk for the binary outcomes (28). Also, considering the hierarchical data structure of the CHNS, we used a two-level mixed-effects modified Poisson regression with robust (sandwich) estimation of variance to estimate the risk ratio (RRs) of T2D, taking communities as the second level and individual as the first level. We constructed three sequential models for analysis: Model 1 adjusted for no covariates; Model 2 adjusted for age, income level, education, residence, urbanicity index, physical activity, sleep duration, smoking and alcohol drinking; Model 3 further adjusted for TEI, percentage of total energy comes from fat, fiber, and other related dietary factors. In addition, linear trends across increasing categories of total and trunk BF% were assessed by assigning median values to levels of total and trunk BF%, and the variable modeled as a continuous term.

Furthermore, we plotted the curves of receiver operating characteristic (ROC) for the total and trunk BF% by sex to identify the cut-off points of BF% that predicted risk of T2D. Sensitivity, also known as true positive rate, reflects the ability of a screening test to detect patients; specificity, also known as true negative rate, reflects the ability of a screening test to identify non-patients. An ROC curve is produced by plotting a list of sensitivity on the y-axis against "1 – specificity" on the x-axis for different values of a continuous test measure. Two methods were used to determine the optimal cut-off points, which were the Youden's index reaching its maximum value (sensitivity + specificity – 1) and the shortest distance from the corner. The area under the curve (AUC) shows the authenticity of a test to classify the participants as likely to have disease or not, and the value of AUC is usually used to compare overall performances of different screening test, which is between 0 and 1, the closer

it is to 1, the higher its application value (29). Additionally, potential covariates including socio-demographic, lifestyle and dietary variables were also adjusted among the ROC curves.

All statistical analyses were conducted using SAS version 9.4 (SAS Institute Inc., Cary, NC, USA) and Stata version 15SE (Stata Corp., College Station, TX, USA). Two-tailed $p < 0.05$ was considered statistically significant.

Results

Baseline characteristics

The baseline characteristics of participants across total BF% levels by sex are summarized in Tables 1A,B, respectively. Females tended to have an obviously higher total BF% than males. The mean age of the participants were 46.3 (10.4) years. Both males and females with higher total BF% levels were older. It is notable that males whose total BF% were higher tended to have lower physical activity levels, higher income levels and educational levels, and live in urban area ($p < 0.05$). On the contrary, females with higher total BF% had lower socioeconomic status ($p < 0.05$).

Associations of total and trunk BF% with type 2 diabetes

A total of 282 participants were found to have T2D, and Table 2 presents the numbers of outcome events for each subtype stratified by sex. The associations between quintiles of total and

TABLE 1A Baseline characteristics of adult males according to the quintiles of the total BF%, CHNS ($n = 2,471$).

Baseline characteristics	Total	Q1	Q2	Q3	Q4	Q5	*p*-value
		<17.2%	17.2–21.1%	21.1–24.0%	24.0–27.3%	≥27.3%	
Age (years)	46.3 ± 10.4	44.2 ± 11.5	47.2 ± 10.4	46.5 ± 10.0	46.1 ± 10.0	47.3 ± 9.8	<0.001
Income level (%)							<0.001
Low	31.8	39.4	34.7	27.2	28.4	29.4	
Medium	33.5	32.7	31.5	34.4	34.9	34.0	
High	34.7	27.9	33.8	38.4	36.7	36.6	
Education level (%)							<0.001
Primary school and below	16.5	21.9	17.6	16.1	13.7	13.3	
Middle school	39.1	46.3	38.7	36.8	35.9	37.6	
High school and above	44.4	31.8	43.7	47.1	50.4	49.1	
Residence (%)							<0.001
Rural	66.4	79.7	71.5	63.4	60.1	57.3	
Urban	33.6	20.3	28.5	36.6	39.9	42.7	
Urbanicity index	71.4 ± 17.6	66.0 ± 16.9	70.0 ± 17.2	73.0 ± 17.6	74.0 ± 17.6	74.2 ± 17.5	<0.001
Smoking (%)							0.061
Former/non-smoker	42.9	38.7	40.7	44.7	43.2	47.3	
Current smoker	57.1	61.3	59.3	55.3	56.9	52.7	
Alcohol drinking (%)							0.632
Former/non-drinker	40.2	42.4	37.9	40.3	41.1	39.0	
Current drinker	59.8	57.6	62.1	59.7	58.9	61.0	
Sleep duration (%)							0.647
6~9 h	83.9	85.4	82.4	85.2	83.5	83.3	
<6/>9 h	16.1	14.6	17.6	14.8	16.5	16.7	
Physical activity (MET h/week)	184.4 ± 175.6	213.2 ± 203.8	188.8 ± 186.3	189.6 ± 179.5	166.3 ± 156.1	164.5 ± 141.6	<0.001
Dietary intake							
TEI (kcal/d)	2,189.1 ± 788.2	2,206.6 ± 833.3	2,161.8 ± 805.4	2,178.2 ± 801.5	2,166.7 ± 769.8	2,232.4 ± 728.6	0.146
Fat (% of total energy)	34.4 ± 12.0	34.5 ± 12.5	34.3 ± 12.3	34.5 ± 11.9	34.5 ± 12.0	34.1 ± 11.5	0.949
Fiber (g/d)	12.8 ± 8.8	13.0 ± 9.9	12.3 ± 8.1	12.7 ± 7.9	12.3 ± 7.1	13.5 ± 10.3	0.297
Calcium (mg/d)	372.1 ± 202.7	373.0 ± 202.3	356.6 ± 183.6	359.4 ± 190.2	380.0 ± 218.4	391.3 ± 215.5	0.043
Magnesium (mg/d)	288.3 ± 131.4	287.8 ± 125.5	285.2 ± 147.7	282.0 ± 115.3	284.7 ± 129.5	301.8 ± 135.9	0.039
Vitamin C (mg/d)	75.4 ± 111.1	72.3 ± 73.1	75.8 ± 123.1	74.8 ± 109.5	76.4 ± 102.1	77.8 ± 137.0	0.749

TABLE 1B Baseline characteristics of adult females according to the quintiles of the total BF%, CHNS (n = 3,124).

Baseline characteristics	Total	Q1	Q2	Q3	Q4	Q5	p-value
		<27.6%	27.6–31.6%	31.6–34.9%	34.9–38.6%	≥38.6%	
Age (years)	46.3 ± 10.2	41.9 ± 11.4	45.2 ± 10.2	47.0 ± 9.6	47.7 ± 9.3	49.6 ± 8.4	<0.001
Income level (%)							0.005
Low	34.6	33.0	32.1	35.3	35.0	37.3	
Medium	33.2	30.9	32.9	32.3	32.5	37.6	
High	32.2	36.1	35.0	32.4	32.5	25.1	
Education level (%)							<0.001
Primary school and below	28.2	23.8	25.5	26.2	30.0	35.3	
Middle school	36.1	31.6	35.0	38.3	37.1	38.6	
High school and above	35.7	44.6	39.5	35.5	32.9	26.1	
Residence (%)							0.185
Rural	65.2	62.0	63.9	65.3	67.2	67.7	
Urban	34.8	38.0	36.1	34.7	32.8	32.3	
Urbanicity index	72.0 ± 17.2	72.0 ± 17.5	72.7 ± 17.2	72.1 ± 16.8	72.2 ± 17.3	70.8 ± 17.3	0.376
Smoking (%)							0.018
Former/non-smoker	98.5	97.2	99.4	98.2	98.4	99.2	
Current smoker	1.5	2.8	0.6	1.8	1.6	0.8	
Alcohol drinking (%)							0.521
Former/non-drinker	92.4	92.4	92.3	92.5	91.2	93.9	
Current drinker	7.6	7.6	7.7	7.5	8.8	6.1	
Sleep duration (%)							0.397
6~9 h	84.4	84.8	82.8	83.4	84.6	86.6	
<6/>9 h	15.6	15.2	17.2	16.6	15.4	13.4	
Physical activity (MET h/week)	173.7 ± 160.3	171.9 ± 157.1	182.4 ± 164.9	171.5 ± 155.5	177.0 ± 169.3	165.7 ± 153.9	0.328
Dietary intake							
TEI (kcal/d)	1,824.8 ± 639.9	1,821.9 ± 644.5	1,803.1 ± 596.5	1,835.8 ± 657.4	1,837.0 ± 667.9	1,825.6 ± 631.4	0.978
Fat (% of total energy)	34.5 ± 12.3	33.7 ± 12.2	34.7 ± 12.0	34.9 ± 12.1	34.8 ± 12.8	34.5 ± 12.2	0.228
Fiber (g/d)	11.6 ± 7.6	11.6 ± 7.3	11.7 ± 8.7	11.6 ± 7.5	11.6 ± 7.3	11.6 ± 6.9	0.921
Calcium (mg/d)	334.8 ± 185.7	325.0 ± 187.9	339.2 ± 184.5	336.4 ± 171.6	331.3 ± 187.1	341.9 ± 196.7	0.166
Magnesium (mg/d)	250.5 ± 113.6	245.0 ± 100.0	247.1 ± 105.7	249.1 ± 120.5	253.3 ± 111.8	257.9 ± 127.8	0.565
Vitamin C (mg/d)	76.5 ± 154.6	70.3 ± 119.2	72.0 ± 151.4	87.3 ± 234.3	68.9 ± 59.2	84.2 ± 155.5	0.070

trunk BF% and risk of T2D in Chinese adults stratified by sex are also shown in Table 2.

After adjusting for all potential confounders, males in the third (21.1–24.0%), fourth (24.0–27.3%), and fifth (≥ 27.3%) quintiles of total BF% showed 2.03 (95% CI 1.09–3.79), 2.56 (95%CI 1.46–4.48), and 2.16 (95%CI 1.22–3.82) times the risk of T2D as compared with those in the lowest quintile (< 17.2%, p-trend < 0.001). In females, the RR (95% CI) for risk of T2D was1.92 (1.14, 3.24), when comparing the highest (≥ 38.6%) with the lowest (<27.6%) quintile (p-trend = 0.014). For trunk BF% of males, RRs (95%CI) of T2D were 2.46 (1.40, 4.31) and 2.02 (1.18, 3.45) in the fourth (25.5–29.4%) and fifth (≥ 29.4%) quintiles, respectively, as compared with the lowest quintile (<17.2%, p-trend = 0.001). For females, as compared the lowest quintile of trunk BF% (<25.4%), the RRs (95%CI) were 1.99 (1.06, 3.73) and 2.58 (1.45, 4.60) for risk of T2D in the fourth and fifth quintiles, respectively (p-trend = 0.001).

Cut-off points of total and trunk BF% for risk of type 2 diabetes

The ROC curves and the AUCs of the total and trunk BF% in relation to the risk of T2D were plotted and calculated to identity the values of total and trunk BF% that best predicted T2D risk (Figure 2). After adjusting for potential confounders, the AUCs for total BF% were 0.656 and 0.709, and the AUCs for trunk BF% were 0.659 and 0.714, respectively, in males and females.

The Youden's indices indicated that the optimal cut-off values of total and trunk BF% were 21.9% (sensitivity: 0.796;

TABLE 2 Risk ratio (95% CI) of type 2 diabetes across the quintiles of the total and trunk BF% among Chinese adults aged 18–65, CHNS[a].

		Number of case/subjects	Model 1	Model 2	Model 3
Male					
Total BF%					
Q1	<17.2%	15/493	1	1	1
Q2	17.2–21.1%	15/499	0.97 (0.47, 2.01)	0.91 (0.44, 1.89)	0.92 (0.44, 1.94)
Q3	21.1–24.0%	31/486	2.10 (1.11, 3.96)[b]	2.00 (1.08, 3.73)[b]	2.03 (1.09, 3.79)[b]
Q4	24.0–27.3%	41/496	2.71 (1.52, 4.82)[c]	2.60 (1.48, 4.55)[c]	2.56 (1.46, 4.48)[c]
Q5	≥27.3%	35/497	2.31 (1.29, 4.11)[c]	2.17 (1.23, 3.84)[c]	2.16 (1.22, 3.82)[c]
p trend			<0.001	<0.001	<0.001
Trunk BF%					
Q1	<17.2%	16/494	1	1	1
Q2	17.2–21.9%	21/501	1.26 (0.67, 2.38)	0.17 (0.62, 2.21)	1.18 (0.62, 2.22)
Q3	21.9–25.5%	21/481	1.32 (0.69, 2.53)	1.24 (0.66, 2.33)	1.25 (0.66, 2.34)
Q4	25.5–29.4%	43/500	2.66 (1.49, 4.75)[c]	2.51 (1.43, 4.41)[c]	2.46 (1.40, 4.31)[c]
Q5	≥29.4%	36/495	2.23 (1.30, 3.81)[c]	2.03 (1.19, 3.49)[b]	2.02 (1.18, 3.45)[b]
p trend			<0.001	<0.001	0.001
Female					
Total BF%					
Q1	<27.6%	16/618	1	1	1
Q2	27.6–31.6%	29/623	1.81 (1.00, 3.28)	1.59 (0.88, 2.87)	1.55 (0.86, 2.77)
Q3	31.6–34.9%	22/626	1.36 (0.71, 2.58)	1.08 (0.57, 2.03)	1.07 (0.57, 2.01)
Q4	34.9–38.6%	33/637	1.99 (1.08, 3.63)[b]	1.56 (0.85, 2.84)	1.56 (0.86, 2.85)
Q5	≥38.6%	45/620	2.81 (1.66, 4.77)[c]	1.97 (1.16, 3.35)[b]	1.92 (1.14, 3.24)[b]
p trend			<0.001	0.012	0.014
Trunk BF%					
Q1	<25.4%	13/625	1	1	1
Q2	25.4–30.5%	27/620	2.12 (1.12, 4.01)[b]	1.90 (1.01, 3.60)[b]	1.83 (0.98, 3.44)
Q3	30.5–34.4%	23/634	1.73 (0.89, 3.35)	1.48 (0.77, 2.83)	1.43 (0.75, 2.74)
Q4	34.4–39.0%	33/625	2.54 (1.34, 4.78)[c]	2.01 (1.07, 3.77)[b]	1.99 (1.06, 3.73)[b]
Q5	≥39.0%	49/620	3.81 (2.13, 6.82)[c]	2.71 (1.51, 4.86)[c]	2.58 (1.45, 4.60)[c]
p trend			<0.001	0.001	0.001

[a] A two-level mixed-effects Poisson regression with robust (sandwich) estimation of variance, taking community as the second level, and individual as the first level. Model 1 adjusted for no covariates. Model 2 adjusted for age, income level (categorical), education level (categorical), urbanized index, residence (categorical), smoking (categorical), alcohol drinking (categorical), physical activity and sleep duration (categorical). Model 3 additionally adjusted for TEI, percentage of total energy comes from total energy, dietary fiber, calcium, magnesium, and vitamin C intake.

[b] $p < 0.05$, [c] $p < 0.01$. *p* trend was examined by assigning the median value of each quantile as a continuous variable.

specificity: 0.449) and 25.2% (sensitivity: 0.788; specificity: 0.464) for males, and 36.7% (sensitivity: 0.676; specificity: 0.638) and 30.3% (sensitivity: 0.703; specificity: 0.632) for females, respectively. The shortest distance from the corner showed 13.2% (sensitivity: 0.555; specificity: 0.656) and 29.5% (sensitivity: 0.642; specificity: 0.583) for males, and 36.7% (sensitivity: 0.676; specificity: 0.638) and 23.4% (sensitivity: 0.697; specificity: 0.637) for females, respectively (Table 3).

Discussion

In this longitudinal prospective cohort study, we observed that males with total BF% more than 21.1% (≥ quintile 3) and trunk BF% more than 25.5% (≥ quintile 4), and females with total BF% more than 38.6% (quintile 5) and trunk BF% more than 34.4% (≥ quintile 4) had the significantly increased risk for T2D as compared with the subjects in quintile 1 group. Moreover, the optimal cut-off points determined by Youden's index and the shortest distance from the corner were different. For BF%, increased sensitivity for risk of T2D may promote physical activity and healthy lifestyle, whereas relatively wide margin for false positives may not lead to inappropriate treatment or serious physical, mental and financial burden. Therefore, it may be appropriate to choose the Youden's index to determine the optimal cut-off points which tend to comprehensively reflect the total ability of a screening test to detect patients or non-patients. The cut-off values of total and

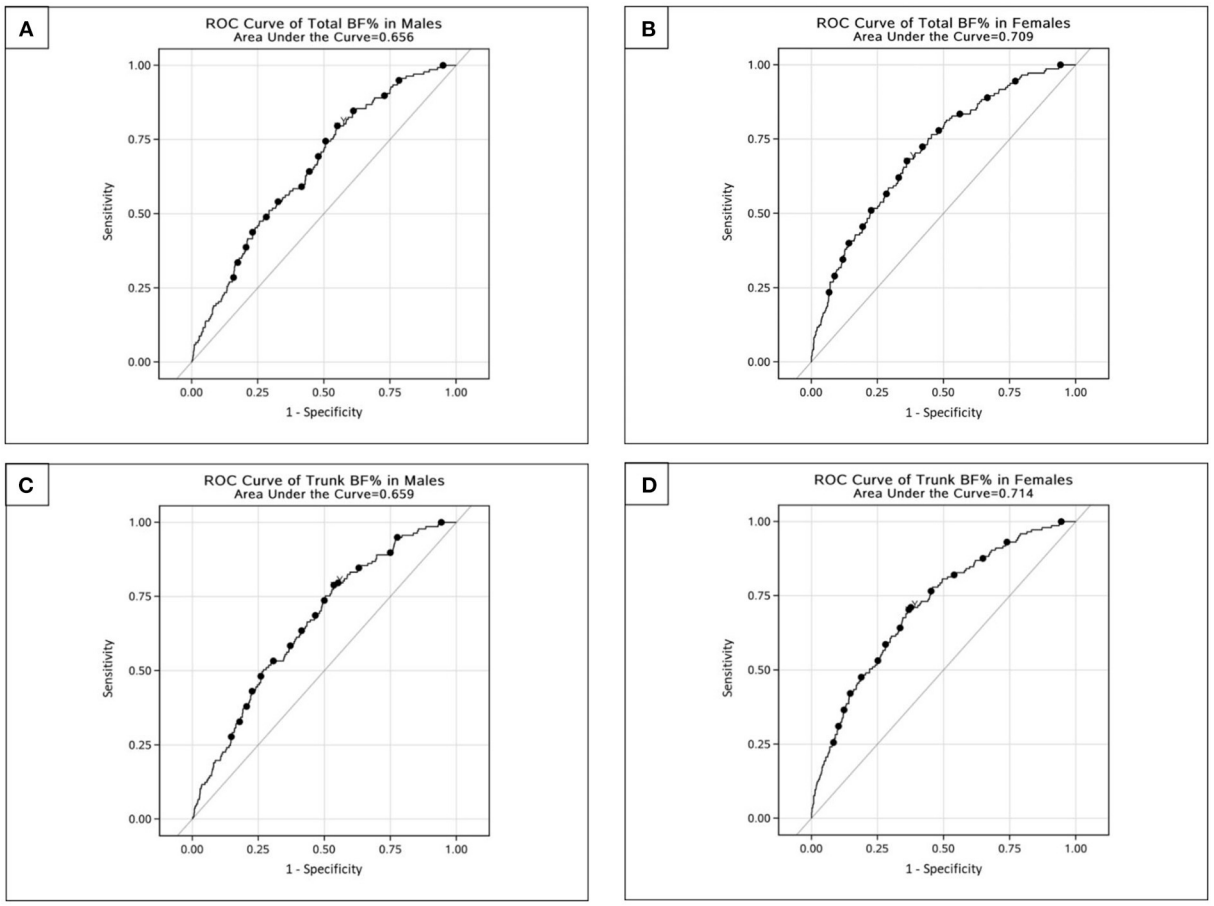

FIGURE 2
ROC curves for total and trunk BF% related to risk of type 2 diabetes among Chinese adults. **(A)** ROC curve of total BF% in males. **(B)** ROC curve of total BF% in females. **(C)** ROC curve of trunk BF% in males. **(D)** ROC curve of trunk BF% in females.

trunk BF% were 21.9% (sensitivity: 0.796; specificity: 0.449) and 25.2% (sensitivity: 0.788; specificity: 0.464) for males and 36.7% (sensitivity: 0.676; specificity: 0.638) and 30.3% (sensitivity: 0.703; specificity: 0.632) for females, respectively, based on the Youden's index.

Epidemiological evidence supported that body fat was significantly associated with the risk of obesity-related T2D, but the cut-off values varied in the different populations. Macek et al. (14) cross-sectionally identified the optimal BF% cut-off points for diabetes were 25.5% for males and 40.0% for females based on a sample of 4,735 Polish adults aged 45–64 years old. Zhu et al. (30) reported that the BF% cut-off values were 29.1% for males and 37.2% for females in Caucasians, and 28.3 and 37.1% for male and female African Americans aged 20 years and older using the 1988–1994 NHANES data. The cut-off points from our study were lower than those from aforementioned European and American studies. The ethnicity, to some extents, explains the disparity of body fat between Chinese and White populations, but previous studies also indicated that Asians had relatively higher body fat percentage which predisposed them to prediabetes and diabetes at the given BMI compared to other ethnic groups (16, 31).

Further, the BF% cut-off points in the present study were slightly different from those in other Asian populations. For example, among 10, 774 middle-aged Japanese males (mean age: 47.4 ± 5.7 years), the BF% value for detecting participants with diabetes risk was estimated to be 23.2% (15). In a study of 41, 088 Korean adults aged 18–92 years, the optimal cut-off points were 21.0% for males and 37.0% for females to predict the risk of obesity-related cardiovascular disease (13). In the Chinese population, Jia et al. (32) used the 2007–2008 CNDMS data of 23,769 participants aged 20 or older to evaluate the optimal BF% cut-off values, the results showed that the value was 24.5% in males and 35.7% in females with the diabetes being the endpoints. Among 3,961 subjects aged 30–70 years of Shanghai Diabetes Studies, the cut-off points for the detecting people with

TABLE 3 The appropriate cut-off points of total and trunk BF% for risk of type 2 diabetes among Chinese adults aged 18–65, CHNS.

	Cut-off (%)	Sensitivity	Specificity
Male		Youden's index	
Total BF%	21.9	0.796	0.449
Trunk BF%	25.2	0.788	0.464
Female			
Total BF%	36.7	0.676	0.638
Trunk BF%	30.3	0.703	0.632
Male		The shortest distance from the corner	
Total BF%	13.2	0.555	0.656
Trunk BF%	29.5	0.642	0.583
Female			
Total BF%	36.7	0.676	0.638
Trunk BF%	23.4	0.697	0.637

risk of T2D were 25.0 and 35.0% for males and females, respectively (33). The optimal total BF% cut-off points in our study were lower in males and higher in females than those reported by the existing studies, as well as others (34, 35). These variations might result from disparity in ages, socio-economic status, dietary culture and eating behavior, lifestyle factors of study population. Also they may be due to methodologies controlling for potential confounders for the ROC curves in our study and different methods to determine the optimal cut-off values, and the outcomes across the studies.

In present study, total BF% level above 21.1% (quintile 3) was significantly associated with the increased risk of T2D in males, while females only showed the significant association between the fifth quintile (38.6%) and the risk of T2D. The results were consistent with a Korean study (36), and suggested sex difference in the influence of BF% on the pathogenesis of T2D. The hormonal difference between males and females greatly impacts body fat distribution, with more lean mass in males and higher fat mass in females for a given BMI (37). It was also reported that the fat was mainly distributed in the trunk for males, while the fat tended to be deposited in the limbs and hips for females, especially in the lower body (38). These above-mentioned findings in body composition, in conjunction with the diversities in culture, lifestyle, environment, socioeconomic status, and energy metabolism may be accounted for differences between males and females in risk of T2D (39).

In 2015–2017, the estimated prevalence of diabetes was 11.2% (95% CI: 10.5–11.9%) among adults in mainland China, which was higher among adults aged 50 and older and among males (40). Previous Chinese cohort studies found that subjects with excess BF% were more likely to have an increased risk of developing diabetes, regardless of the BMI status, and suggested that maintaining normal body fat was meaningful to diabetes prevention (10, 41). However, elevated BF% is not included in conditions recommended for screening T2D in the Chinese guideline (20). Of note, a cross-sectional study conducted by Ruan et al. with 85 T2D patients in China, indicated that reducing body fat was an important adjuvant therapy to improve glycemic control among T2D patients with high body fat (42). Therefore, cost-effective actions to maintain appropriate body fat levels and preventing diabetes, such as specific nutrition education, clinical application of BF% based on sex, and lifestyle intervention included targeting diet and physical activity, are meaningful to take to reduce the disease burden related to T2D in the Chinses healthcare setting.

The major strengths of this study include the use of the CHNS 2015–2018 with a large national population-based study sample, longitudinal assessment of risk ratios for T2D according to quintiles of BF%, and to determine the optimal cut-off values of total and trunk BF% for males and females, respectively. Our study directly shows the etiological role of exposure due to the large-scale prospective cohort study, and provides a more precise effect estimate given many advantages of multilevel mixed-effect modeling instead of traditional regression analyses (43). Moreover, we regarded socio-demographic, lifestyle and dietary variables as confounding factors to justify potential bias. However, several limitations of our study should be considered. First, strict inclusion criteria for the study subjects might reduce the representativeness and generalizability of these findings. Second, BF% measured by BIA is affected by individual and environmental factors such as age, BMI, time of measurement, and physical activity (44, 45), and BIA prediction equations vary by population and device used (15). Although our study used the same type of body composition analyzer validated for Chinese and measured BF% under controlled conditions, the current findings might not apply to other populations.

Conclusion

In conclusion, the present study showed that the risk of T2D significantly increased over specific level of total and trunk BF% in both Chinese males ($\geq$ 21.1 and $\geq$ 25.5%, respectively) and females ($\geq$ 38.6 and $\geq$ 34.4%, respectively). The optimal cut-off values of total and trunk BF% for prediction of T2D risk were determined to be 21.9 and 25.2% for males, and 36.7 and 30.3% for females, respectively. These findings are valuable for suitable modification of body fat percentage based on T2D for Chinese population due to the prospective nature and contribute to the development and implementation of public health actions to further improve the disease burden related to T2D in the Chinese healthcare system.

Data availability statement

The original contributions presented in the study are included in the article/Supplementary material, further inquiries can be directed to the corresponding authors.

Ethics statement

The studies involving human participants were reviewed and approved by National Institute for Nutrition and Health, Chinese Center for Disease Control and Prevention. The patients/participants provided their written informed consent to participate in this study.

Author contributions

Conceptualization and methodology: SZ, ZW, and HJ. Formal analysis and writing-original draft preparation: SZ. Writing-review and editing: ZW, XJ, and JZ. Investigation: HJ, LW, XJ, and JZ. Statistical expertise: HW and BZ. Project administration: BZ and GQ. All authors read and approved the final manuscript.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fpubh.2022.1032130/full#supplementary-material

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A multilevel analysis of improved drinking water sources and sanitation facilities in Ethiopia: Using 2019 Ethiopia mini demographic and health survey

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Background: Access to water, sanitation, and hygiene is an important element for communicable disease control including the existing COVID-19 pandemic. This is due to the growing water demand and decreasing water availability, because of shrinking resources, increased urbanization, and pollution. This problem is higher, particularly among least developed countries like Ethiopia. This study, therefore, aimed at investigating the level of improved water sources and sanitation as well as their predictors in Ethiopia using EMDHS-2019.

Method: Mini Ethiopian Demographic and Health Surveys 2019 database survey was used in this study. Data collection took place over 3 months, from 21 March 2019 to 28 June 2019. A total of 9,150 households were selected for the sample, of which 8,794 were engaged. Among involved households, 8,663 were successfully interviewed at a response rate of 99%. The dependent variables measured in this study were improved drinking water sources and sanitation facilities. Due to the nested nature of DHS data, multilevel binary logistic regression analysis was done using Stata-16.

Results: The majority (72.62%) of household heads were men, and 69.47% of participants were from rural areas. Close to half (47.65%) of study participants did not have any form of formal education, while the lowest proportion (9.89%) of them had higher education. Approximately 71.74 and 27.45% of the households have accessed improved water sources and sanitation, respectively. Based on the final model results, wealth index, educational status, and having a television individual-level variables while community-level poverty, community-level education, community-level media exposure, and place of residence were statistically significant predictors of getting improved water source and sanitation.

Conclusion: The level of access to improved water sources is moderate but it lacks progress, while access to improved sanitation was lower. Based on these findings, great improvements should be made in providing access to an improved water source and sanitation facilities in Ethiopia. Based on these findings, great improvements should be made in providing access to improved water source and sanitation facilities in Ethiopia.

KEYWORDS

access, improved water sources, improved sanitation, EDHS, Ethiopia

Background

The World Health Organization and United Nations Children's Fund (WHO/UNICEF) Joint Monitoring Programme for Water Supply, Sanitation and Hygiene (JMP) produces internationally comparable estimates of countrywide, regional, and worldwide development on drinking water, sanitation, and hygiene (WASH) and is accountable for international monitoring of the Sustainable Development Goal (SDG) targets related to WASH (1). SDG-6 aims to "ensure availability and sustainable management of water and sanitation for all and includes targets for universal access to safe drinking water (6.1), sanitation, and hygiene" (6.2) (2). Access to water, sanitation, and hygiene is an important element for communicable disease control (3), including the existing COVID-19 outbreak (4).

Globally, 1.8 billion people gained access to at least basic water services between 2000 and 2017. Despite this, in 2017, 2.2 billion individuals still lacked access to properly managed drinking water, 4.2 billion lacked safely managed sanitation, and 3 billion lacked basic hand washing facilities internationally (5). According to WHO/UNICEF, 2017 report only 24% of the rural population and 44% of the urban population have access to sanitation facilities in sub-Saharan African countries (6). This is due to the growing water demand and decreasing water availability, because of shrinking resources, urbanization, and pollution (7).

Insufficient drinking water, sanitation, and hygiene (WASH) are key predictors of numerous wide-ranging disease burdens, focusing primarily on diarrheal diseases in low-income settings (8). More than 50 pathogens that are accountable for diarrheal illness, schistosomiasis, and soil-transmitted helminth infections, communicated due to unsatisfactory sanitation (9). As well as access to safe water, sanitation and hygienic situations have a vital role in protecting human health from emerging and re-emerging disease outbreaks, including the existing COVID-19 pandemic (10).

At the same time, household water demand is estimated to increase significantly over the period 2010–2050 in all the globe except for Western Europe (7). The more carefully planned out drinking water and sanitation are in a country's national development goals, the more important these issues are likely to be to that nation's policymakers (2). However, the accessibility of water is endangered by climate change, population growth, changes in demographic characteristics, and urbanization (11).

Previous research has shown that in Africa some of the factors associated with access to improved household water sources include the place of residence, wealth status (12–14), education, ethnicity, access to electricity, gender, water collection time, and the number of rooms in a household (15). Due to these problems, the goals of the sustainability Development Strategy in achieving universal access to safe water and sanitation are still extremely far from its anticipated target, particularly among least-developed countries like Ethiopia (12). Every 5 years, DHS is conducted in Ethiopia which includes water and sanitation. According to research conducted in 2020 by Water.org, only 42% of the population in Ethiopia has access to clean water; of which, only 11% of that amount has access to sufficient sanitation services (16).

Continuous studies are needed until the occurrence of inequalities toward WASH is reduced and warrants sustainable development in unindustrialized nations. This could be used as evidence for any concerned body targeting that would possibly involve investment and resources to advance their access to water and sanitation to reach worldwide access by 2030. Practically, still there is a higher prevalence of open defecation and intermittent water supply in the towns and rural areas of the country. Even though there are few of these studies have done using such representative data to examine the magnitude and determinants of access to improved drinking water sources and sanitation, there is a need to do more research, especially during the time of COVID-19. Examining such patterns is helpful evidence for health-related policy makers, Non-governmental Organizations (NGOs), and all other stakeholders responsible for WASH. Therefore, this study was aimed at investigating the level of improved water and sanitation as well as their predictors in Ethiopia using EMDHS-2019.

Methods

Study setting and data source

This study was done in Ethiopia, which is the second-largest number population next to Nigeria in Africa. Mini Ethiopian Demographic and Health Surveys 2019 (EDHS-2019) database survey was used in this study. MEDH survey was conducted in nine geographical regions (Tigray, Afar, Amhara, Oromia, Somali, Benishangul-Gumuz, Southern Nations Nationalities and Peoples Region (SNNPR), Gambella, and Harari) and two administrative cities (Addis Ababa and Dire Dawa) of the country. This MEDHS Survey is a nationwide representative population-based survey with large sample sizes. EDHS data are open source and can be retrieved on the DHS website (https://dhsprogram.com/Data/terms-of-use.cfm).

The 2019 EMDHS sample was a two-stage stratified cluster sample, sampling weights were calculated based on sampling probabilities separately for each sampling stage and for each cluster. In the first stage, a total of 305 EAs (93 in urban areas and 212 in rural areas) were selected with probability proportional to EA size (based on the 2019 EPHC frame) and with independent selection in each sampling stratum. In the second stage of selection, a fixed number of 30 households per cluster were selected with an equal probability of systematic selection from the newly created household listing. EPHI investigators, an ICF technical specialist, an advisor, and representatives from other organizations, including CSA, FMoH, the World Bank, and USAID, supported the data collection. Data collection took place over a three-month period, from 21 March 2019 to 28 June 2019. A total of 9,150 households were selected for the sample, of which 8,794 were engaged. Among involved households, 8,663 were successfully interviewed at a response rate of 99%.

Study variables

Outcome variables

Improved water sources were defined as water from piped water, boreholes or tube wells, protected dug wells, protected springs, rainwater, and packaged or delivered water (12, 17, 18). While access sanitation facilities, such as flush/pour flush to piped sewer systems, septic tanks, or pit latrines, were defined as improved sanitation facilities, ventilated improved pit latrines, composting toilets, or pit latrines with slabs were also included (12, 17, 18).

The dependent variables measured in this study were improved drinking water sources and improved sanitation facilities. Unimproved water sources or sanitation facilities were represented

as dichotomous variables, with "1" representing "improved" and "0" representing "unimproved", respectively, for both water sources and sanitation.

Predictor variables

Individual-level variables

Sex of household head (male or female), wealth index (poor, middle, and rich), educational status, and having television and radio were individual predictor variables. The poorest and poor were coined as "poor", middle as "middle" whereas rich and richest, were categorized as "rich".

Community-level variables

Community-level education, the place of residence (urban/rural), community-level media exposure, region [(recoded as pastoralist region (Benishangul, Somali, Gambella, and Afar), Semi-pastoralist (Oromia, SNNPR), Agrarian (Amhara and Tigray) and City administration (Addis Ababa, Dire Dawa, and Harari)], community-level educational attainment, community-level poverty, and community-level media exposure were community-level variables. All the variables were selected using previous related literature review (19–22).

Data quality assurance

For data quality assurance purposes, the 2019 EMDHS pretest containing in-class training, biomarker training, and field exercise days was done. The field exercise was conducted in clusters around Adama, which were not included in the 2019 EMDHS sample. A debriefing session was held with the pretest field staff, and adjustments to the questionnaires were done based on lessons drawn from the field practice. Trained collectors, supervisors, field editors, interviewers, secondary editors, and reserve interviewers were engaged to correct the limitation of the tool during the pretest.

Operational definition

Improved water on premises

Having access to improved water sources located at 0 min from the point of use (23, 24).

Basic water service

Having access to an improved water source located between 1 and 30 min, for a roundtrip (23–25).

Limited water service

Having access to improved water sources located farther than 30 min for a round trip (23–25).

Unimproved water sources

This refers to all unimproved water sources irrespective of the collection time (23, 24).

Improved sanitation

Confirm hygienic separation of human feces from human contact using flush/pour flush to the piped sewer system, septic tank, pit latrine, ventilated improved pit (VIP) latrine, pit latrine with slab, and composting toilet (26).

Data analysis

Due to the nested nature of DHS data, multilevel binary logistic regression analysis was done. Descriptive analysis was employed to examine the frequency, percentage, mean, and standard deviation of the variables of interest. Subgroup analysis was done by splitting the data by geographic location, educational status, and wealth status of the participant to make comparisons among subgroups in the data. Bivariable and multivariable multilevel binary logistic regression was utilized to evaluate associations between outcome variables and independent variables because the outcome variables were dichotomous (improved and unimproved). Independent individual variables in bivariable analysis with a p-value <0.2 were included in multivariable multilevel logistic regression analysis. Then 95% confidence interval (CI) was employed, and a p-value <0.05 was used for testing statistical significance in multivariable logistic regression analysis.

Before undertaking the logistic regression, the variables were tested for multicollinearity using the method of forward and backward correlation. Considering that the EDHS survey used complex sampling, sampling weights were applied to each analysis in order to adjust the variances in the probability of sample selection.

Four models comprising important variables were built-in in this study. These models were run individually and aimed at accessing improved water sources and improved sanitation services. Model 0 (Null model) was fitted without independent variables to test random variability in the intercept and to estimate the mean odds ratio (MOR), intra-class correlation coefficient (ICC), and proportion change in variance (PCV). Model II measured individual-level variable effects on the outcome variables. Model III evaluated the effects of community-level factors on dependent variables. Model IV (final model) is used to determine the effects of individual and community-level variables instantaneously dependent variables.

ICC, MOR, and PCV were calculated as follows:

$$ICC = \frac{Va}{Va + (\pi^2)/3} = \frac{Va}{(Va + 3.29)}$$

where Va is the area-level variance.

PCV $= \frac{Vc-Vl}{Vc}^{*}100\%$, where Vc was the variance of the lowest model, and Vl was the variance of the model with more predictor variables.

MOR $= \exp\left[\sqrt{(2^{*}Va)}^{*}0.6745\right] \approx \exp(0.95\sqrt{Va})$, where VA is the area-level variance.

Deviance Information Criterion (DIC), Akaike's information criterion (AIC), Bayesian's information criterion (BIC), and likelihood ratio were used to select the fitted model, and the model with a low information criterion value was an appropriate model. Based on these values, the final (model with individual and community-related variables) model has the smallest information criterion value among the model considered; therefore, the full model was best fitted. AOR

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TABLE 1 Socio-demographic characteristics of study participants.

Variables	Categories	Frequency (%)	Drinking water sources	
Individual-level variables			Unimproved	Improved
Age of household head	<30	2,520 (29.09)	685 (7.91)	1,835 (21.18)
	30–40	2,287 (26.40)	675 (7.79)	1,612 (18.61)
	41–51	1,717 (19.82)	462 (5.33)	1,255 (14.49)
	>51	2,139 (24.69)	626 (7.23)	1,513 (17.47)
Minimum = 15, Maximum = 98, Std. Dev.= 16.49423				
Sex of household	Male	6,291 (72.62)	1,862 (21.49)	4,429 (51.13)
	Female	2,372 (27.38)	586 (6.76)	1,786 (20.62)
Educational status	No education	4,128 (47.65)	1,542 (17.80)	2,586 (29.85)
	Primary	2,715 (31.34)	693 (8.00)	2,022 (23.34)
	Second	963 (11.12)	136 (1.57)	827 (9.55)
	Higher	857 (9.89)	77 (0.89)	780 (9.00)
Wealth index	Poor	3,498 (40.38)	1,729 (19.96)	1,769 (20.42)
	Middle	1,285 (14.83)	379 (4.37)	906 (10.46)
	Rich	3,880 (44.79)	340 (3.92)	3,540 (40.86)
Having radio	No	6,170 (71.22)	2,012 (23.23)	4,158 (48.00)
	Yes	2,493 (28.78)	436 (5.03)	2,057 (23.74)
Having Television	No	6,679 (77.10)	2,380 (27.47)	4,299 (49.62)
	Yes	1,984 (22.90)	68 (0.78)	1,916 (22.12)
Community-level variables				
Media exposure	Unexposed	5,195 (59.97)	1,969 (22.73)	3,226 (37.24)
	Exposed	3,468 (40.03)	479 (5.53)	2,989 (34.50)
Level of education	Lower	4,308 (49.73)	1,808 (20.87)	2,500 (28.86)
	Higher	4,355 (50.27)	2,448 (28.26)	6,215 (71.74)
Poverty	Higher	4,276 (49.36)	1,902 (21.96)	2,374 (27.40)
	Lower	4,387 (50.64)	546 (6.30)	3,841 (44.34)
Residence	Urban	2,645 (30.53)	149 (1.72)	2,496 (28.81)
	Rural	6,018 (69.47)	2,299 (26.54)	3,719 (42.93)
Region	Tigray	714 (8.24)	189 (2.18)	525 (6.06)
	Afar	664 (7.66)	344 (3.97)	320 (3.69)
	Amhara	1,007 (11.62)	369 (4.26)	638 (7.36)
	Oromia	1,018 (11.75)	360 (4.16)	658 (7.60)
	Somali	657 (7.58)	355 (4.10)	302 (3.49)
	Benishangul-Gumuz	734 (8.47)	110 (1.27)	624 (7.20)
	SNNPR	1,017 (11.74)	336 (3.88)	681 (7.86)
	Gambela	693 (8.00)	182 (2.10)	511 (5.90)
	Harari	719 (8.30)	81 (0.94)	638 (7.36)
	Addis Ababa	702 (8.10)	10 (0.12)	692 (7.99)
	Dire Dawa	738 (8.52)	112 (1.29)	626 (7.23)

with a 95% confidence interval in the multivariable model was used to select variables that have a statistically significant association with improved water sources and sanitation. All the data analyses were done using STATA Version 16.0 software.

Ethical considerations

The data were accessed and utilized with the Central Statistical Agency of Ethiopia's prior permission. The first authors registered for dataset access and wrote the research topic, as well as

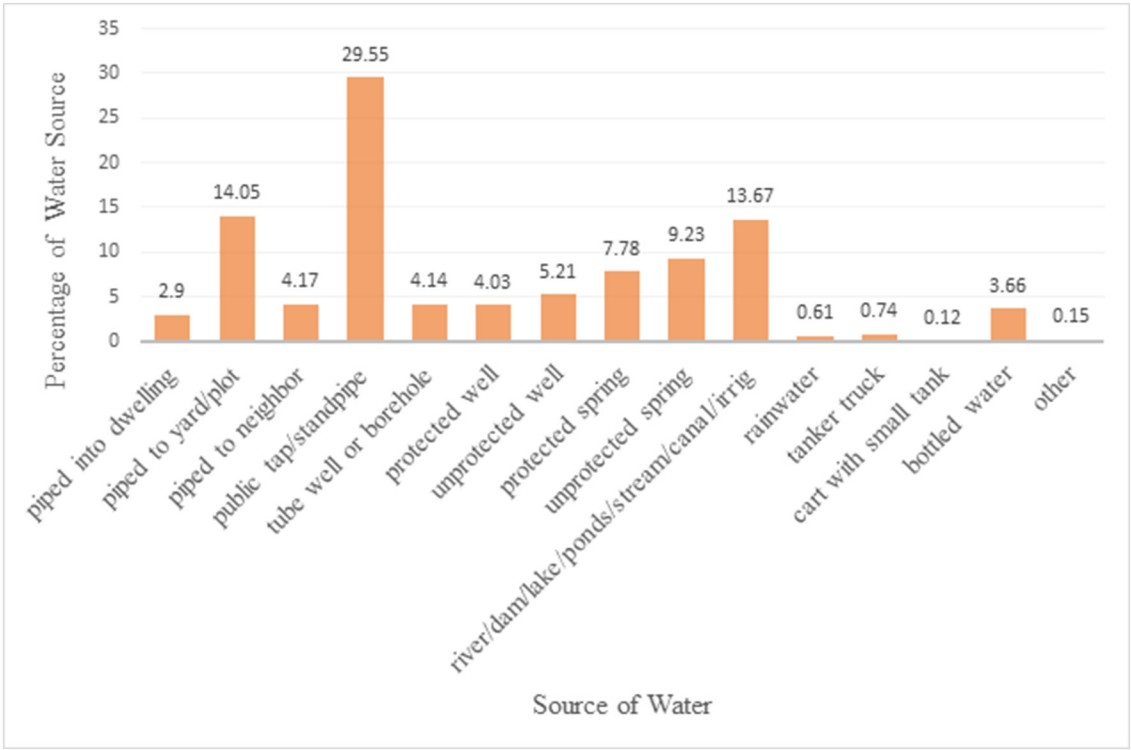

FIGURE 1
Proportion of different water sources based on the EMDHS 2019 datasets.

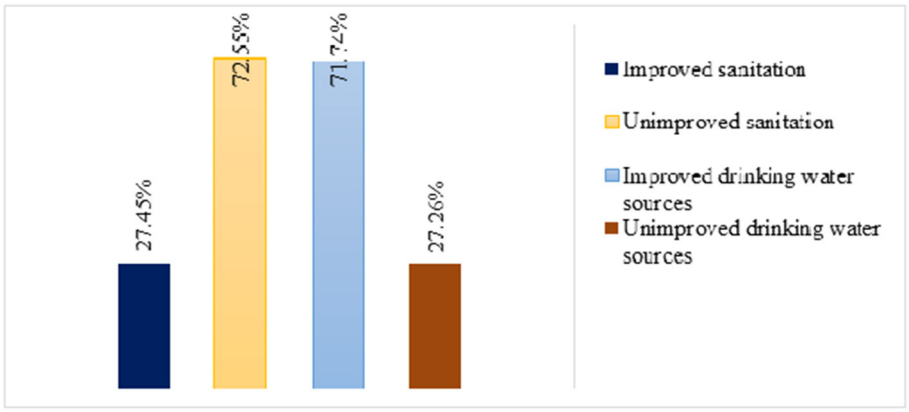

FIGURE 2
Level of improved water source and sanitation in Ethiopia from EMDHS 2019 Datasets.

its significance on the website. Then, we accessed the datasets at the website https://dhsprogram.com/Data/terms-of-use.cfm. The downloaded data were used simply for this study. As with all EDHS data, EMDHS 2019 data also were preserved as trustworthy of the participants in the survey.

Results

Descriptive statistics on socio-demographic characteristics

A total of 8,663 participants were involved in this study. The majority (72.62%) of household heads were men. Approximately 6,018 (69.47%) of the participants lived in a rural area. The highest proportion (47.65%) of educational status was no education, while the lowest (9.89%) was higher education. Nearly, 30% (30.53%) of study participants lived in urban. More than three-quarters (77.10%) of the included study subjects had no television (Table 1).

Drinking water sources and sanitation

Figure 1 shows different types of water reported from EMDHS-2019 in the most recent survey in the country. Among the water source categories, public tap/stand pipe was the largest proportion (29.55%) followed by piped to yard/plot (14.05%) (Figure 1).

The aforementioned water sources in Figure 1 are categorized as improved and unimproved water sources. Then, the overall national level of improved drinking water sources was 71.74% [95% CI = (70.78%−72.68%)]. While improved sanitation was 27.45% [95% CI = (26.52%−28.40%)], and unimproved sanitation was 72.55% [95% CI = (71.60%−73.48%)] (Figure 2).

According to the WHO, there are three categories of improved water service: enhanced on premises, basic water service, and limited water service. In this study, only few (0.68%) of the participants accessed improved on premises services (Figure 3).

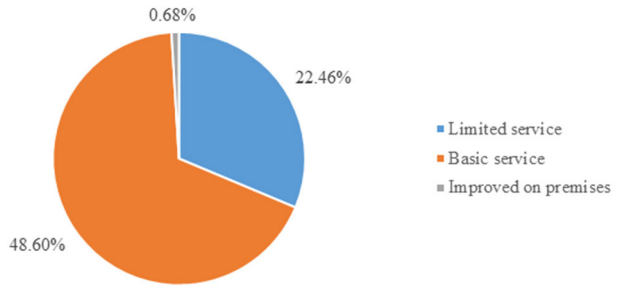

FIGURE 3
Improved water source classification based on time and improved source type in Ethiopia from EMDHS 2019 Datasets.

Subgroup analysis of improved water and sanitation

Figure 4 indicated that the subgroup analysis of the pattern for subgroup differences showed that there was a statistically substantial subgroup consequence based on wealth status ($p < 0.001$). The range of variation was from 42.52 to 96.89% between the poorest and the richest participants in accessing water from improved water sources (Figure 4).

Geographical location was another factor considered in the subgroup analysis. The outcomes of this subgroup analysis revealed a statistically important subgroup consequence ($p < 0.001$), which implies that the country's topography significantly changes the country's access to water from improved sources (Figure 5).

Figure 6 indicates that there was a great heterogeneity among households in using improved water sources due to their variation in educational status ($p = 0.001$). This indicated that households owned by more educated people are more likely to have a variety of inputs and are more likely to use public assets such as piped water sources.

The poorest lacks the monetary income to access improved sanitation facilities that support healthy lives. In this finding, there was great variation in having improved sanitation facilities among households due to their difference in wealth status ($p = 0.001$) (Figure 7).

Figure 8 indicates that there was a great heterogeneity among households in using improved sanitation facilities due to their variation in areas where they live ($p = 0.001$). In more civilized areas, households are more likely to own various contributions and are

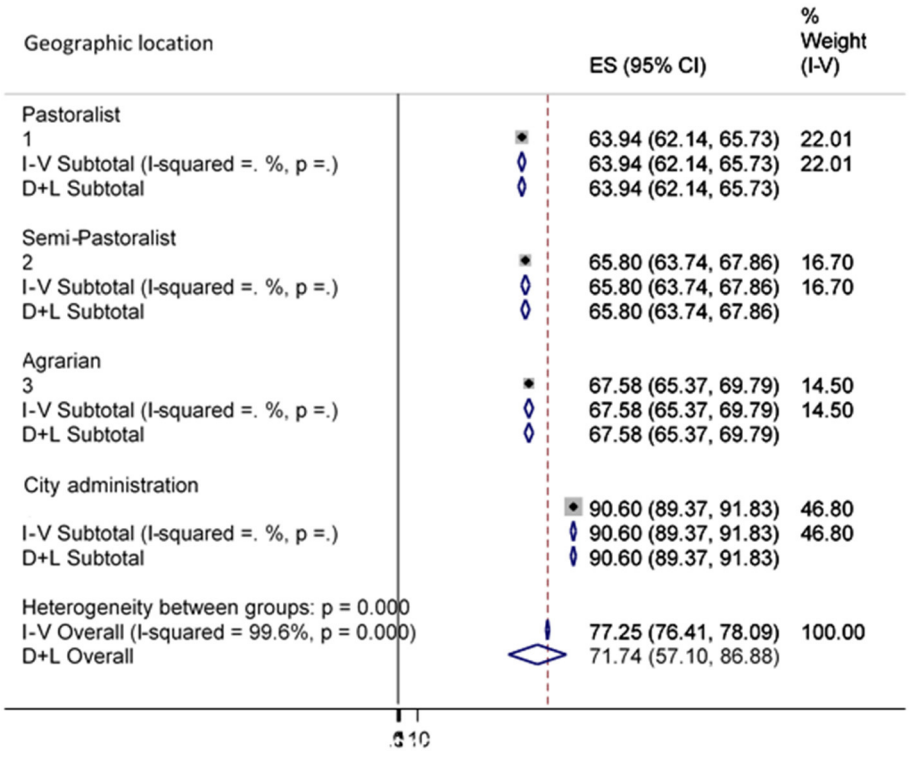

FIGURE 4
Sub-group analysis of improved water source by wealth status.

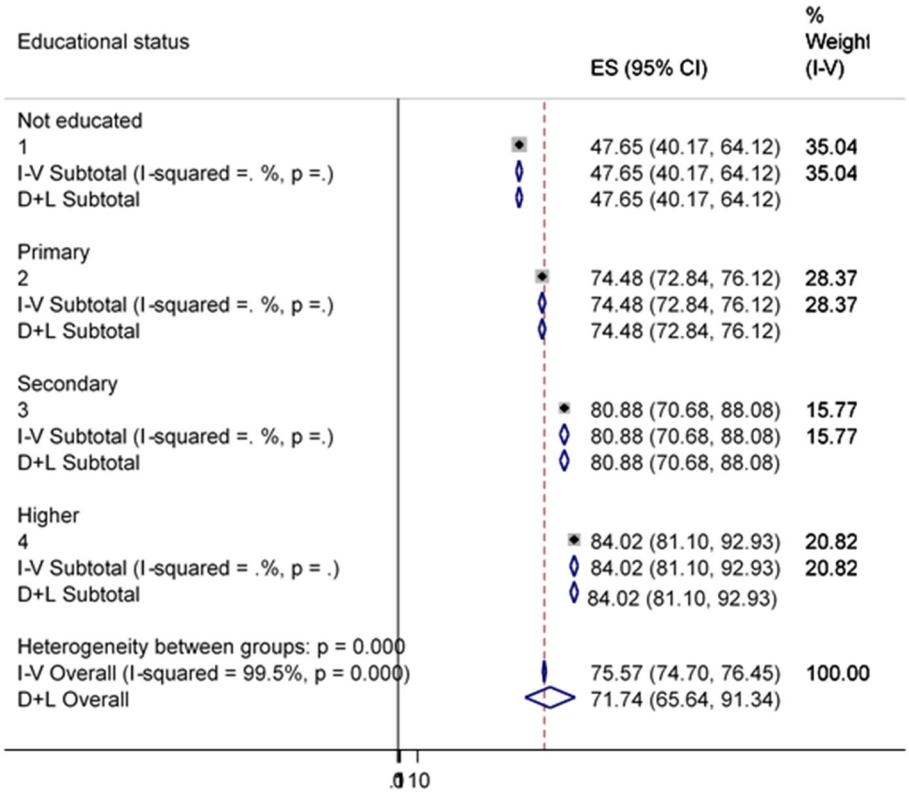

FIGURE 5
Sub-group analysis of improved water source by Geographic location.

more likely to benefit from them in public as long as they possess things like health education.

There was a significant degree of heterogeneity among households in having improved sanitation facilities due to educational status differences ($p = 0.001$) (Figure 9).

Imputation and specificity

Water source and available sanitation contain no soft missing (.) values, and the imputation variable is complete, finally, imputing nothing. Therefore, there was no influence of missing data on our conclusion of the results.

On the one hand, sensitivity, specificity, positive predictive value, and negative predictive value of the model were 84.41, 44.49, 79.42, and 52.92%, respectively. On the other hand, the sensitivity, specificity, positive predictive value, and negative predictive value of the model in sanitation were 65.01, 93.03, 77.92, and 87.54%, respectively (Table 2).

The receiver operating characteristic (ROC) curve is a diagnostic method, shown as a graph that is used to assess the presentation of a binary logistic regression classification method. In this study, correctly classifying the water sources as improved and unimproved was 73.13%, and the area under the ROC curve was 0.7783 (Figure 10).

Whereas, correctly classifying sanitation as improved and unimproved was 85.34%%, and the area under the ROC curve was 0.8726 (Figure 11).

Determinants of household drinking water sources in Ethiopia using multilevel logistic regression analysis

Based on the final model results, wealth index, educational status, and having a television in individual-level variables, while community-level poverty, community-level education, community-level media exposure, and place of residence were statistically significant predictors of getting improved water sources.

The odds of getting water from an improved water source among the households with a middle level of wealth are 2.20 [AOR = 2.20; 95% CI (1.76–2.76)] times higher as compared with poor households. Whereas, the rich had a chance of 3.78 [AOR = 3.78; 95% CI (2.90–4.93)] times more likely to get water from an improved water source as compared with poor households.

The probability of getting water from an improved source among the households that had television was 1.85 [AOR = 1.85; 95% CI (1.49–2.95)] times more likely as compared with households that had no television.

Households in urban areas were 9.40 [AOR = 9.40; 95% CI (3.19–27.73)] times more likely to get water from improved sources as compared with rural area households.

The odds of getting water from an improved water source were 4.54 [OR = 4.54: 95% CI (1.95–10.53)] times more likely in households with higher education compared to households with a lower level of education.

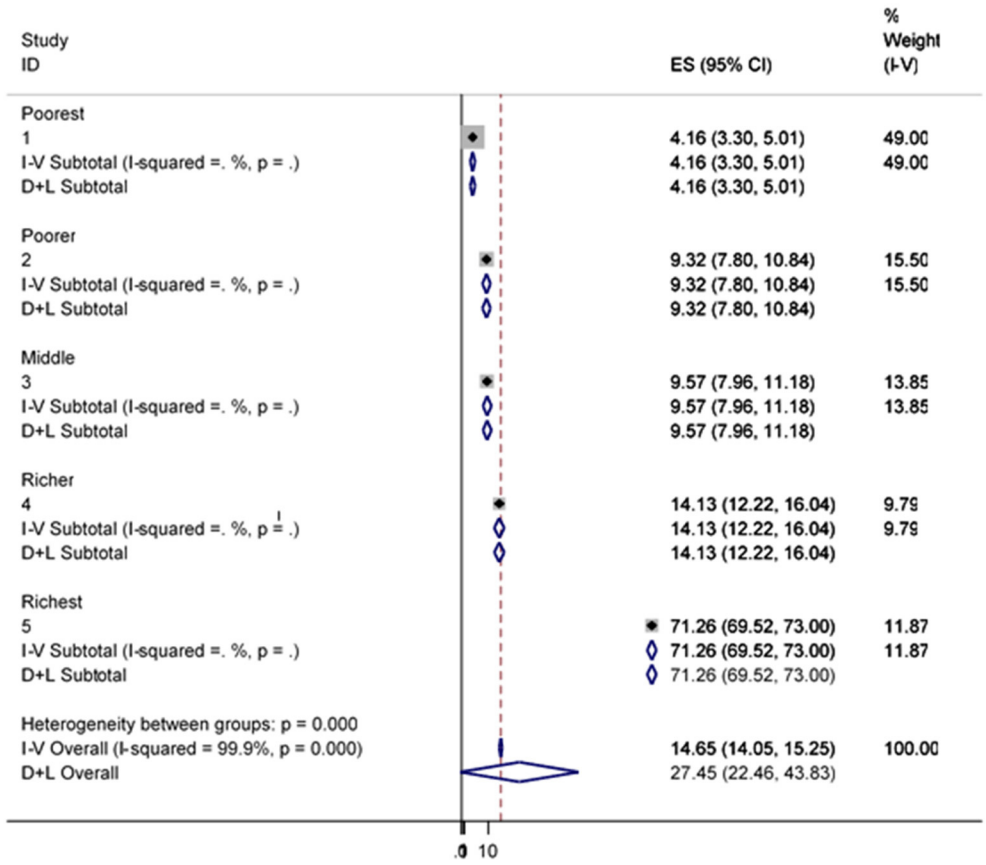

FIGURE 6
Sub-group analysis of improved water source by educational status.

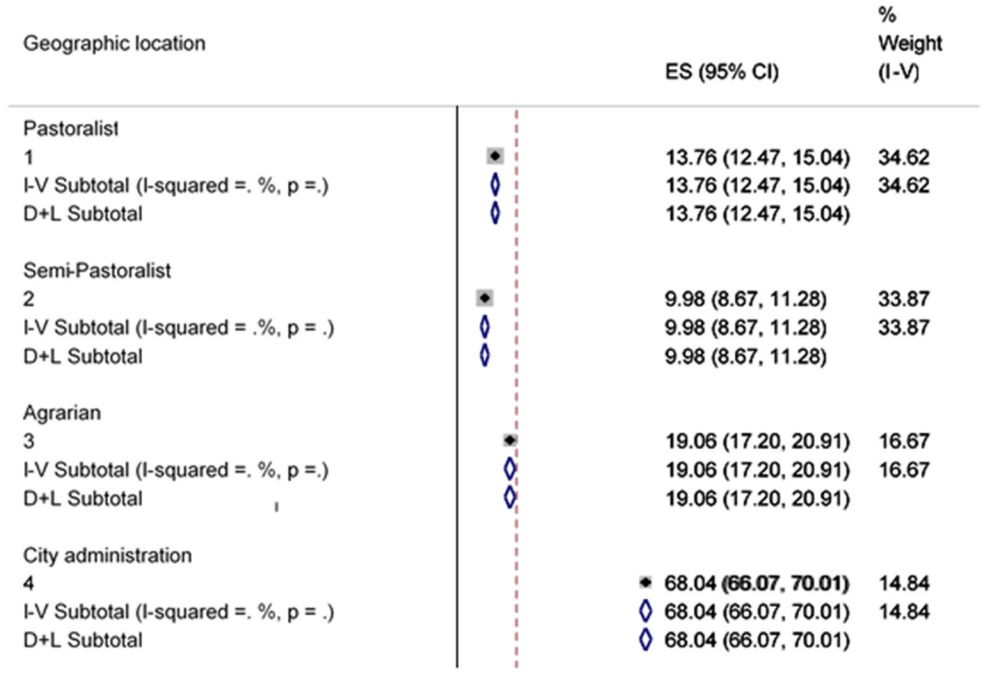

FIGURE 7
Sub-group analysis of improved sanitation facility by Wealth status.

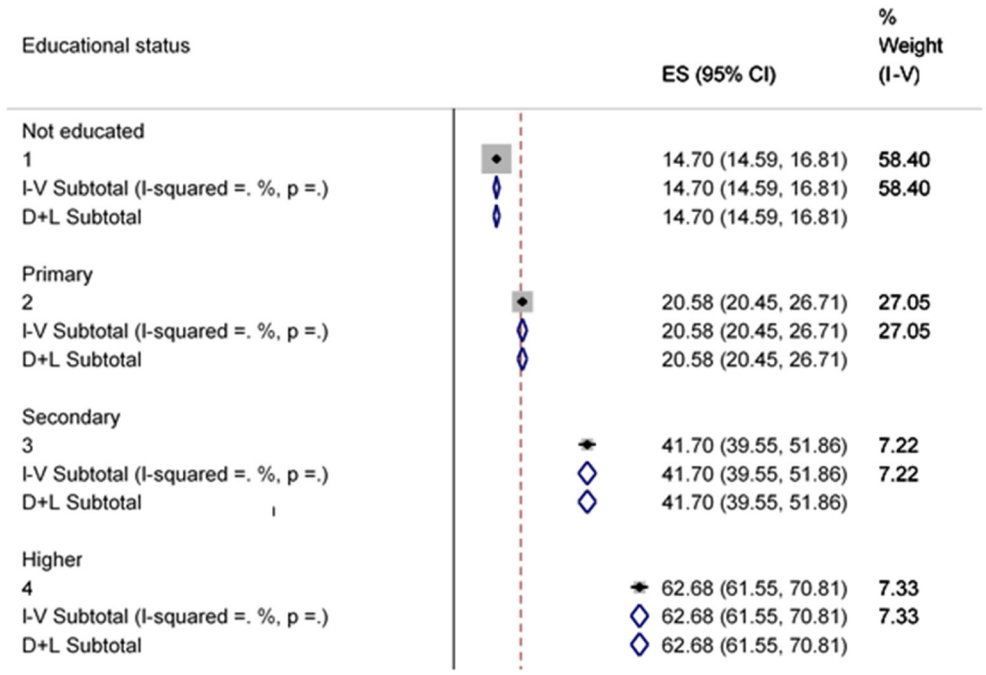

FIGURE 8
Sub-group analysis of improved sanitation facility by geographic location.

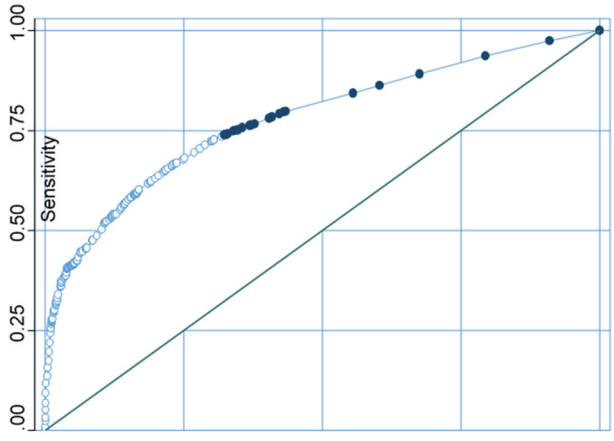

FIGURE 9
Sub-group analysis of improved sanitation facility by educational status.

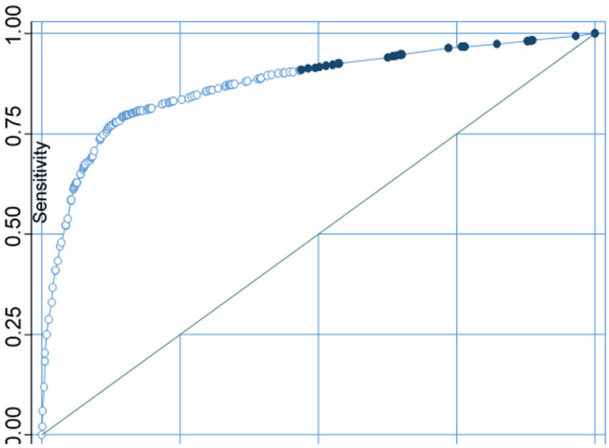

FIGURE 10
Sensitivity analysis curve of water sources.

The other predictor variable was community-level poverty. Households at lower-level poverty were 2.58 [OR = 2.58: 95% CI (1.10–6.03)] times more chance of getting water from improved sources compared to households with a higher level of poverty (Table 3).

Measures of variation and model fit statistics

As shown in Table 3, the Null model indicated that there was substantial variation in getting water from the improved source across households due to their differences in the cluster. More than 80% (85.53%) of variation in getting water from the improved source is endorsed due to cluster variation. The MOR value from the Null model indicated that the median increased odds ratio of getting an improved water source if an individual moves to another area with an improved water source is available. The second model (Model II) revealed that the maximum PCV (34.61%) variations in getting water from the improved sources were due to individual-level factors. Model III showed the lowest values of AIC, DIC, and the large value of LLR (Table 4). Therefore, the final model was the best-fitted model.

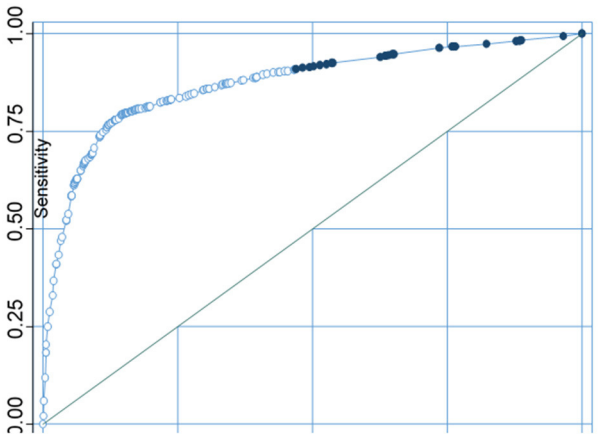

FIGURE 11
Sensitivity analysis curve of sanitation facilities.

TABLE 2 Specificity analysis of unimproved water source and sanitation facilities.

Classified	Unimproved water source		
	True	False	Total
Correct	5,246	1,359	6,605
Incorrect	969	1,089	2,058
Total	6,215	2,448	8,663
	Unimproved sanitation		
	True	False	Total
Correct	1,546	438	1,984
Incorrect	832	5,847	6,679
Total	2,378	6,285	8,663

Factors associated with household improved sanitation in Ethiopia using multilevel logistic regression analysis

The final model results indicated that wealth index, educational status, and having television were individual-level variables, while community-level poverty, community-level education, community-level media exposure, place of residence, and region were community-level variables significantly associated factors with improved sanitation.

The odds of accessing improved sanitation among the households with a middle level of wealth were 1.86 [AOR = 1.86; 95% CI (1.38–2.49)] more times higher as compared with poor households.

While the odds of accessing improved sanitation among the households rich were 3.53 [AOR = 3.53; 95% CI (2.62–4.76)] times higher as compared with poor households.

The likelihood of having improved sanitation among the households with having a television was 2.31 [AOR = 2.31; 95% CI (1.49–2.95)] times more likely as compared with counterparts households that had no television.

Households in urban areas had the chance of accessing improved sanitation at 6.63 [AOR = 6.63; 95% CI (3.76–11.70)] times more likely compared to households in rural areas.

The odds of accessing improved sanitation are 2.43 [OR = 2.43: 95% CI (1.85–3.18)] times more likely in households with higher education compared to households with a lower level of education.

The other predictor variable was community-level poverty. Households in lower-level poverty were 2.32 [OR = 2.32: 95% CI (2.15–4.00)] times more chance of accessing improved sanitation compared to households with a higher level of poverty (Table 5).

Discussion

Accessing waters from an improved source was moderate while accessing improved sanitation facilities was low.

Nearly 70% (71.74 %) of households' accessed drinking water from improved sources. This value was lower than the finding of the study on the world's population, which showed 74% of the households had access to a safe water source (27). This finding was similar to the study done in Benin in which 71.75% of the households accessed improved drinking water facilities (22). While this finding is higher than the studies done in Ethiopia (69.94%) (19), Zambian (64.5%) (28), Nepal (46.0%) (17), and Vietnam (64.6%) (29). In contrast, this finding is lower than the studies done in Ghana (88%) (20) Chi Linh, Viet Nam (86.5%) (30), and Minority Ethnic People in Vietnam (98.0%) (29). This difference could be due to the variation of study participants in socio-cultural, number of study participants, study design, period, and study setting. The largest proportion (48.60%) of improved water sources was basic water service, while the lowest (0.68%) was improved water on the premises due to the time taken for round trip. This might be due to the low expense for the development of the water sources as a possible, which enable improved water source for the community at large (25, 31).

More than one-fourth (27.45%) of the households accessed improved sanitation in Ethiopia. This finding was lower than the findings from Zambian (74.6%) (28), Kandahar City, Afghanistan (85.7%) (32), and a Rural Area of Haryana, North India (84.8%) (33). However, this finding was higher than the study done in Ghana (14% 0.06) (34). This variation may be attributed due to the difference in awareness in the study population, the government focuses on health policy, availability and accessibility of the services, economical differences in the countries, study design, study period, and sample size.

This evidence showed that the magnitudes of the households with access to the improved water source (from 69.94 to 71.74%) and improved sanitation facilities (from 25.36 to 27.45%) are nearly the same when compared with the previous EDHS 2016. However, in order to achieve worldwide access to at least basic drinking water and sanitation, it is expected to increase by many times the existing rates of improvement (3). Accordingly, reaching the SDG's aim to achieve Goal 6 Ensure access to water and sanitation for all by 2030 (35) will be challenging.

The final model results showed that wealth index, educational status, and having a television are individual-level variables, while community-level poverty, community-level education, community-level media exposure, and place of residence were statistically significant predictors of getting improved water sources and sanitation.

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TABLE 3 Multilevel regression analysis improved water source predictors variables in Ethiopia, EMDHS 2019.

Variables	Model 0	Model I AOR (95% CI)	Model II AOR (95% CI)	Model III AOR (95% CI)
Household head sex				
Female		1.183 (0.98, 1.43)		1.15 (0.95, 1.39)
Male		1		1
Wealth index				
Poor		1		1
Middle		2.34 (1.86, 2.92)*		2.20 (1.76, 2.76)*
Rich		4.52 (3.48, 5.88)*		3.78 (2.90, 4.93)*
Educational status				
No education		1		1
Primary		1.74 (1.56, 1.94)*		1.52 (1.14, 1.834)*
Secondary		3.63 (2.99, 4.39)*		3.05 (2.32, 3.65)*
Higher		6.04 (4.74, 7.70)*		5.25 (3.87, 6.82)*
Having television				
No		1		1
Yes		2.07 (1.40, 3.08)*		1.85 (1.49, 2.95)*
Having radio				
No		1		1
Yes		0.95 (0.78, 1.16)		1.17 (0.59, 2.31)
Residence				
Rural			1	1
Urban			12.85 (4.41, 37.42)*	9.40 (3.19, 27.73)*
Media exposure				
Unexposed			1	1
Exposed			1.40 (1.17, 1.69)*	1.20 (1.25, 1.63)*
Community-level education				
Lower education			1	1
Higher education			5.14 (2.22, 11.94)*	4.54 (1.95, 10.53)*
Community-level poverty				
Higher			1	1
Lower			4.77 (2.06, 11.09)*	2.58 (1.10, 6.03)*
Region				
Pastoralist			1	1
Semi Pastoralist			0.35 (0.14, 1.88)	0.28 (0.13, 1.62)
Agrarian			1.07 (0.41, 2.80)	0.95 (0.36, 2.47)
City administration			2.23 (0.69, 7.23)	1.89 (0.58, 6.21)
VIF		1.73	1.45	2.81

1, Reference; *$p < 0.001$; VIF, variance inflation factor.

Wealth index and community level of poverty were statistical predictor variables of the type of water sources used at households. The current study revealed that middle and rich in wealth participants were more likely to have improved sources of drinking water services. This might be due to the increase in household income, households tend to use built improved sources of drinking water, whereas poor households are likely to stick with unimproved water sources. The household wealth index played a vigorous role in the achievement and utilization of improved toilet facilities because of the association

TABLE 4 Measures of variation and model fitness for both improved water source and sanitation in Ethiopia.

Parameters	Empty Model 0	Model I	Model II	Model III
Measures of variations for water sources				
MOR	3.83	3.24	2.92	2.89
PCV	Reference	34.61%	19.55%	2.54%
ICC	0.8455	0.7816	0.7422	0.7373
Variance				
Model fitness test statistics for water sources				
AIC	5,535.568	5,365.221	5,393.423	**5,287.067**
BIC	5,549.702	5,414.689	5,457.024	**5,386.002**
Log likelihood	−2,765.7842	−2,675.6105	−2,687.7113	**−2,629.5334**
Measures of variations for sanitation				
MOR	2.95	2.05	1.45	1.59
PCV	Reference	54.47%	50.19%	6.10%
ICC	0.7566	0.5860151	0.4135	0.7373
Model fitness test statistics for sanitation				
AIC	5,867.791	5,552.671	5,542.386	**5,381.411**
BIC	5,881.925	5,623.339	5,605.988	**5,501.547**
Log likelihood	−2,931.8957	−2,766.3355	−2,762.1931	**−2,673.7055**

AIC, Akaike's information criterion; BIC, Bayesian's information criterion; ICC, intra-class correlation coefficient; PCV, variance partition coefficients.
Bold values = model fit.

between household wealth and access to improved wellbeing (36, 37).

The sex of the household head was not associated to have access to improved drinking water sources and sanitation. This finding contradicted with other previous studies (38–41).

The odds of accessing improved water sources and sanitation among households found in urban areas were more likely than in rural areas. This finding was supported by other previous studies (13, 28, 42–44). On average, households in urban areas access a higher level of improved sanitation service provision (20.48%) than households in rural areas (6.97%). The possible explanation for this variation might be the community in urban areas has a greater chance of accessing infrastructures, skilled labor, and technological resources, which enable improved sources of water and sanitation services.

Another socio-demographic significant factor was the education status and community level of education of the participant. The odds of getting water from improved sources and sanitation were increased among participants as their education level increased. This finding was consistent with a previous similar study that indicated that individuals with higher education need further levels of human resources and have a better developed empathy for the importance of access to safe drinking water to their health and wellbeing (27). This finding was supported by other previous studies (12, 20, 21). This association might be due to educated families possibly becoming informed on the benefits of using improved sources of drinking water. Alternatively, access to education is an important medium for indorsing consciousness toward using an improved source of water for better health.

Households who had television and community exposure to the media were more likely to have access to improved water sources and improved sanitation facilities in comparison with the households of their counterparts none exposed to media. A possible explanation for this could be continuous and informative media exposure might create possible health problems that could occur due to improved sanitation.

There were differences in accessing improved sanitation among regions. Households from the city administration were more likely compared to those from the pastoralists regions to have access to improved and improved sanitation services. This finding was supported by other previous similar studies (45). This variation might be due to that city administrations are with governmental organizations, have enough infrastructure for sanitation, and are aware of the community in sanitation. All these could lead to the households having improved sanitation for safeguarding their health and to create aesthetically attractive cities compared to pastoralist regions.

For both water source and type of sanitation facilities, model evaluation results points were found at the upper left junction of the ROC. A point projected by a pinpointing test falling into the area above the sloping represents a good investigative grouping, else a bad calculation. Thus, the models used in this study were appropriate for the sensitivity test.

Conclusion

Generally, the level of accessing improved water sources is moderate but it lacks progress. While access improved, sanitation

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TABLE 5 Multilevel regression analysis improved sanitation predictors variables in Ethiopia, EMDHS 2019.

Variables	Model 0	Model I AOR (95% CI)	Model II AOR (95% CI)	Model III AOR (95% CI)
Household head sex				
Female		0.99 (0.98, 1.19)		0.94 (0.79, 1.12)
Male		1		1
Wealth index				
Poor		1		1
Middle		2.5 (1.52, 2.77)*		1.86 (1.38, 2.49)*
Rich		5.12 (3.83, 6.85)*		3.53 (2.62, 4.76)*
Educational status				
No education		1		1
Primary		1.08 (0.90, 1.29)*		1.04 (0.87, 1.25)
Secondary		1.31 (1.03, 1.68)*		1.23 (0.96, 1.57)
Higher		2.52 (1.92, 3.31)*		2.43 (1.85, 3.18)*
Having television				
No		1		1
Yes		2.54 (2.05, 3.16)*		2.31 (1.72, 3.10)*
Having radio				
No		1		1
Yes		1.13 (0.96, 1.34)		1.30 (1.00, 1.70)
Residence				
Rural			1	1
Urban			9.78 (5.50, 17.37)*	6.63 (3.76, 11.70)*
Media exposure				
Unexposed			1	1
Exposed			1.86 (1.57, 2.21)*	0.81 (0.57, 1.13)*
Community-level education				
Lower education			1	1
Higher education			1.86 (1.07, 3.09)*	2.30 (1.31, 5.53)*
Community-level poverty				
Higher			1	1
Lower			2.03 (1.19, 3.45)*	2.32 (2.15, 4.00)*
Region				
Pastoralist			1	1
Semi Pastoralist			0.54 (0.30, 0.99)*	0.55 (0.30, 1.00)
Agrarian			1.91 (1.05, 3.46)*	1.74 (0.97, 3.12)
City administration			6.09 (3.31, 11.20)*	5.02 (2.76, 9.12)*
VIF		1.35	1.56	2.65

1, Reference; *$p < 0.001$.

was lower. Wealth index, educational status, having television, community-level poverty, community-level education, community-level media exposure, and place of residence were statistically significant predictors of getting improved water source and sanitation. Based on these findings, great improvements should be made in providing access to the improved water source and sanitation facilities in Ethiopia. This can be done by creating awareness in a community of improved water sources and sanitation, through poverty reduction, an endowment to the poorest households, and communicating educator messages

to the community using communication mediums. The finding of this study recommends that further efforts had better be made to escalate to get improved water sources and sanitation services among households found in rural areas. Health-related policy makers, local health administrators, NGOs, health extension workers, and all other stakeholders should work together in order to reduce health problems with WASH and to reach SDG's 6 goals.

Data availability statement

Publicly available datasets were analyzed in this study. This data can be found here: https://dhsprogram.com/Data/terms-of-use.cfm.

Author contributions

Data curation and methodology: JA and ME. Formal analysis and software: JA. Investigation and review and editing: JA, EA, AM, and ME. Validation and visualization: JA, EA, and ME. Writing: JA and AM. All authors contributed to the article and approved the submitted version.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Association between urinary nickel with obesity status in adults: A cross-sectional study

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Objectives: The prevalence of obesity is on the rise and is connected to numerous factors. However, the relationship between obesity and nickel has never been investigated. Our study aimed to explore the association between urinary nickel and obesity Status in adults.

Methods: From the 2017–2018 National Health and Nutrition Examination Surveys (NHANES), 1,705 participants ≥18 years of age were enrolled. To explore further the relationship among urinary nickel, body mass index (BMI), and waist circumference(WC), Weighted multivariate linear regression analyses and further subgroup analyzes were conducted.

Results: Urinary nickel does not correlate with BMI level but positively correlates with WC. In the subgroup analyzed according to sex, Urinary nickel has a positive correlation with BMI and WC in males but has a negative correlation in females. Secondary stratification analysis according to sex and race, Urinary nickel positively correlates with BMI in White males. It also positively correlates with WC in both White and Black males.

Conclusions: A correlation was found between urinary nickel levels and BMI and WC in adult males. Adult men, especially those already obese, may need to reduce nickel exposure.

KEYWORDS

urinary nickel, obesity, body mass index, waist circumference, NHANES

Introduction

Nickel occupies the 28th spot in the periodic table. It is a brutal metal found naturally in air, water, and soil (1). Nickel is vital for microorganisms, plants, animals, and humans (2). Nickel deficiency can cause growth retardation and fecundity decline, impairment of specific senses, reduced iron absorption, and alteration of essential enzymes in animal tissues and organs, leading to various clinical changes (3–5). However, specific toxicity and carcinogenic properties are connected with excessive nickel. In humans, numerous health issues, including contact dermatitis, cardiovascular conditions, and lung and nasal cancer, can result from prolonged exposure to nickel (6). Nickel exposure most commonly occurs through respiratory inhalation (7), food and water intake (8), and skin absorption (9). With the extensive use of nickel-containing products in daily life (10), especially in medical devices (11), great attention is paid to nickel-related health issues.

Globally, obesity has become a severe public health issue (12). Research shows that 70% of American and 50% of Chinese adults are overweight or obese (13, 14). There are several diseases associated with obesity, such as hypertension (15), malignant tumors (16), and diabetes (17). Several investigations conducted during the COVID-19 pandemic also revealed that obese people with COVID-19 infection had much greater rates of severe illness and fatality than normal persons (18, 19). Since obesity constitutes a significant threat to health, a more profound knowledge of relevant factors of obesity is necessary. Recent studies have confirmed a correlation between urinary nickel and the prevalence of diabetes and high blood pressure (20, 21). As everyone knows, diabetes and high blood pressure are closely related to obesity, but the relationship between urinary nickel and obesity status is unclear (22).

Body mass index (BMI) can effectively assess the state of health, has the characteristics of simple, feasible, and non-invasive (23, 24), and is an essential indicator for the diagnosis of obesity. Waist circumference (WC) has become increasingly crucial in predicting death and morbidity in recent years, and the combination of WC and BMI has been emphasized in diagnosing obesity (25, 26). In this study, we conducted a cross-sectional study to explore the relationship between urinary nickel and obesity index (BMI and WC). To our knowledge, this is the first study to examine the relationship between obesity status and nickel exposure, which is of great significance to this field.

Materials and methods

Study population

In this study, our subjects included adults (≥18 years) from NHANES during 2017–2018. Study participants had 1,705 people after eliminating those with missing data regarding urinary nickel, BMI, or WC. The selection process is depicted in Figure 1.

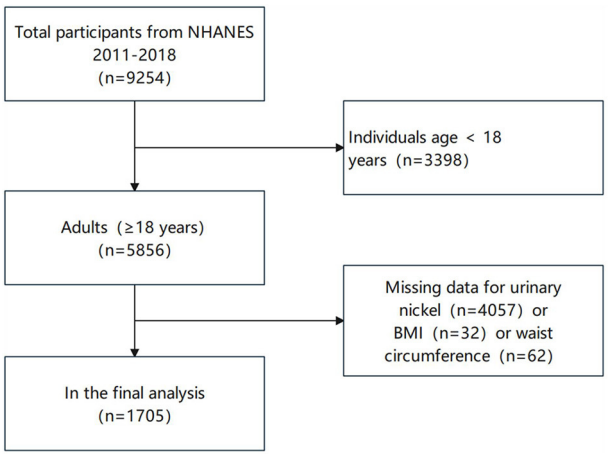

FIGURE 1
Flow chart for selecting participants.

Ethics statement

Participation in the study was voluntary, and the National Center for Health Statistics Research Ethics Review Board approved this study's conduct. To protect everyone's privacy, NHANES will anonymize collected data before making it public as public data. We agree to follow all guidelines for using NHANES data for research purposes and comply with all applicable standards and laws.

Urinary nickel, BMI, and WC

It was decided to collect a single spot urine sample and store it at ≤-20°C for long-term or short-term analysis stored at 2–8°C before analysis. Inductively coupled plasma mass spectrometry (ICP-MS) was employed to determine nickel levels in urine, which is a susceptible technique that can measure multiple elements at low concentrations. To be brief, ICPs operate with argon flows passing through an atomizer and spray chamber to process urine samples. The sample vaporizes at a high temperature, dissociates the ionized gas, and then the ions reach the ion detector. Finally, the isotope ratio of the elements is measured. A urinary nickel concentration of 0.31 mg/L is considered a detection limit of detection. More detailed laboratory procedure manuals are shown on the NHANES's website (27).

This study measured the weight, height, and WC of adults 18 and older using standardized methods. Physical examination measured BMI and WC. BMI is calculated by dividing the square of a person's weight (in kilograms) by their height (in meters), and precise measurements could well be acquired using standard digital scales and rulers. Medical professionals measured the subjects' WC with a flexible ruler. According to the WHO-recommended measurement method, the subject's feet were separated by 25–30 cm. The measurer placed the measuring tape around the abdomen in a circle at the midpoint of the line connecting the anterior superior iliac crest and the lower border of the 12th rib, close to the soft tissue, but without compression, and measured at the end of exhalation and before inspiration. When the WC and BMI are both normal, it is not obesity; when the BMI is normal, but the WC of men is ≥94 cm, and that of women is ≥80 cm, it is central obesity; when the WC is standard, but the BMI is ≥30 kg/m^2, it is defined as peripheral obesity; when the BMI and WC are both above normal, it is defined as mixed obesity (28, 29).

Covariates

The information on age, race, the ratio of family income to poverty, and the educational level of the participants was obtained through the questionnaire. To ensure the quality of the questionnaire and data collection, questionnaires are developed in advance by professional surveyors and released. Data is received by trained medical staff at a mobile medical examination center. Qualified laboratory specialists collected and processed blood samples at the mobile medical examination center. The following parameters will be evaluated: total cholesterol, triglycerides, glycohemoglobin, blood urea nitrogen, serum creatinine, serum

uric acid, and total protein. Hispanic, Mexican American, Non-Hispanic White, Non-Hispanic Black, and Other Race were classified. Education levels below high school, high school, and higher education were ranked based on their education level. The NHANES website (www.cdc.gov/nchs/nhanes/) provides public access to the data from this survey.

Statistical analysis

This study used statistical software R (version 3.4.4) to conduct all statistical analyzes. Per National Center for Health Statistic (NCHS) recommendations, samples were weighted according to NHANES (30). An analysis of the associations between urinary nickel, BMI, and WC was conducted using weighted linear regression. In this study, we built three regression models. No adjustments had been made to Model 1: race, gender, and age adjustment were made in Model 2. A complete adjustment was made to Model 3 for all Covariates. To further explore the relationship between urinary nickel, BMI, and WC, Weighted multivariate linear regression analyses and subgroup analyzes were conducted. A p-value of <0.05 determined statistical significance.

Results

Description of participant characteristics

The statistical characteristics of the study population are displayed in Table 1. A total of 1,705 adults participated in this study. In different groups of urinary nickel (quartiles,

TABLE 1 Weighted characteristics of the study sample.

Urinary nickel	Total	Q1	Q2	Q3	Q4	*P*-value
Gender (%)						0.217
Male	48.61	46.87	49.79	52.12	45.36	
Female	51.39	53.13	50.21	47.88	54.64	
Age (years)	47.04 ± 17.54	46.83 ± 15.62	46.92 ± 17.75	46.43 ± 18.07	48.16 ± 18.86	0.554
Race/ethnicity (%)						0.010
Mexican American	9.16	8.96	9.68	7.93	10.19	
Other Hispanic	6.37	7.34	5.34	6.57	6.14	
Non-Hispanic White	62.64	63.46	66.64	64.25	54.84	
Non-Hispanic Black	11.32	8.6	10.18	13.12	14.11	
Other race	10.52	11.63	8.17	8.13	14.71	
Education level (%)						0.007
Less than high school	11.21	9.73	9.05	12.82	13.87	
High school	27.79	23.85	27.97	32.46	27.21	
More than high school	61.01	66.43	62.97	54.73	58.92	
Ratio of family income to poverty (%)	2.97 ± 1.58	3.29 ± 1.56	2.99 ± 1.59	2.86 ± 1.59	2.67 ± 1.50	<0.001
Total cholesterol (mmol/L)	4.83 ± 1.00	4.94 ± 1.03	4.93 ± 1.00	4.74 ± 1.00	4.68 ± 0.93	<0.001
Triglyceride (mmol/L)	1.59 ± 1.32	1.51 ± 0.94	1.62 ± 1.37	1.59 ± 1.00	1.63 ± 1.88	0.476
Glycohemoglobin (%)	5.67 ± 0.90	5.57 ± 0.68	5.68 ± 1.00	5.72 ± 0.97	5.71 ± 0.93	0.046
Blood urea nitrogen (mmol/L)	5.32 ± 1.84	5.03 ± 1.64	5.22 ± 1.82	5.51 ± 1.76	5.59 ± 2.12	<0.001
Serum creatinine (umol/L)	77.29 ± 23.18	76.29 ± 18.12	76.33 ± 25.40	78.60 ± 18.98	78.21 ± 29.52	0.313
Serum uric acid (umol/L)	319.33 ± 80.89	311.22 ± 79.13	316.48 ± 80.56	326.22 ± 83.56	325.23 ± 79.22	0.016
Total protein (g/L)	71.09 ± 4.21	71.28 ± 3.86	71.53 ± 4.08	70.66 ± 4.42	70.81 ± 4.47	0.008
Body mass index (kg/m^2)		29.14 ± 6.67	29.73 ± 6.70	30.24 ± 7.26	30.28 ± 7.16	0.053
Waist circumference (cm)		99.09 ± 16.03	100.60 ± 16.44	102.24 ± 18.33	102.51 ± 18.38	0.012
Obesity status						0.026
No obesity	23.78	24.50	26.59	20.34	23.41	
Central obesity	32.08	36.89	29.28	33.79	27.39	
Peripheral obesity	0.04	0	0	0.17	0	
Mixed obesity	44.09	38.61	44.14	45.70	49.19	

Continuous variables are presented as Mean ± SD. A weighted linear regression model was used to calculate the *P*-value. A categorical variable is shown as a percentage, and Chi-square test was used to calculate the *P*-value.

Q1-Q4), gender, age, triglycerides, serum creatinine, and BMI were not statistically significant. In contrast, race/ethnicity, education level, the ratio of family income to poverty, total cholesterol, glycohemoglobin, blood urea nitrogen, serum uric acid, total protein, WC, and obesity status were statistically significant. The main types of obese people are mixed and central obesity.

Covariable selection

As shown in Table 2, we select covariates by univariate analysis. When the outcome index is BMI, the age, race/ethnicity, education level, ratio of family income to poverty, total cholesterol, triglyceride, glycohemoglobin, and serum uric acid were select as covariable. When the outcome index is WC, the age, gender, race/ethnicity, education level, blood urea nitrogen, serum creatinine, total cholesterol, triglyceride, glycohemoglobin, serum uric acid, and total protein were select as covariable.

TABLE 2 Univariate analysis to select covariates.

Urinary nickel	Body mass index (kg/m^2) β (95% CI), *P*	Waist circumference (cm) β (95% CI), *P*
Gender		
Male	Reference	Reference
Female	0.43 (−0.23, 1.09)	−4.13 (−5.76, −2.50)***
Age	0.03 (0.01, 0.05)**	0.21 (0.16, 0.25)***
Race/ethnicity		
Mexican American	Reference	Reference
Other Hispanic	−1.39 (−3.08, 0.31)	−3.37 (-7.58, 0.83)
Non-Hispanic White	−0.95 (−2.11, 0.21)	0.57 (−2.31, 3.46)
Non-Hispanic Black	0.10 (−1.36, 1.56)	−0.90 (−4.52, 2.73)
Other race	−3.08 (−4.56, −1.60)***	−6.71 (-10.39, −3.03)***
Education leve		
Less than high school	Reference	Reference
High school	1.94 (0.77, 3.10)**	4.20 (1.30, 7.10)**
More than high school	1.09 (0.02, 2.16)*	1.55 (−1.11, 4.22)
Ratio of family income to poverty	−0.21 (−0.42, −0.00)*	−0.08 (−0.60, 0.44)
Total cholesterol	0.45 (0.12, 0.78)**	1.48 (0.66, 2.30)***
Triglyceride	0.96 (0.71, 1.21)***	3.00 (2.39, 3.60)***
Glycohemoglobin	1.97 (1.62, 2.33)***	5.96 (5.09, 6.82)***
Blood urea nitrogen	0.16 (−0.02, 0.33)	0.91 (0.47, 1.35)***
Serum creatinine	0.00 (−0.01, 0.02)	0.06 (0.03, 0.10)***
Serum uric acid	0.02 (0.02, 0.03)***	0.07 (0.06, 0.08)***
Total protein (g/L)	−0.04 (−0.12, 0.04)	−0.23 (−0.43, −0.04)*

*$P < 0.05$, **$P < 0.01$, ***$P < 0.001$.

Association between urinary nickel and BMI

Table 3 shows the association between urinary nickel and BMI based on multivariate regression analysis. In all three models, no significant associations were found. However, stratified by sex, all three models (model 1: 0.3520, 0.0604–0.6436; model 2: 0.3278, 0.0398–0.6159; model 3: 0.2965, 0.0302–0.5628) revealed a positive association for males, *P* for trend of three models was, respectively, 0.001, 0.003, and 0.010. As a result of secondary stratification based on sex and race, urinary nickel had a positive correlation with BMI in White males (Table 4).

Association between urinary nickel and WC

When exploring the association between urinary nickel and WC, we found a positive association in all their models(model 1:0.4894,0.0486–0.9302; model 2:0.4938,0.0679–0.9197; model 3: 0.4110 0.0221–0.7999). However, stratified by sex, the positive association was only found in three male models (model 1: 1.3408, 0.5525–2.1290; model 2: 1.2004, 0.4511–1.9498; model 3:1.1111, 0.4156–1.8066), with a significant *P* for trend of three models ($P < 0.001$, $P < 0.001$, $P = 0.001$) (Table 5). Secondary stratification analysis according to sex and race, urinary nickel has a positive correlation with WC in both White and Black males (Table 6).

Association among BMI, WC, and urinary nickel stratified simultaneously by gender and obesity status

As shown in Table 7, when stratified simultaneously according to gender and obesity status, BMI was positively correlated with no obesity in women (0.2161, 0.0325–0.3998), while WC was positively correlated with mixed obesity in Men(1.2598, 0.4131–2.1065).

Discussion

In this study, urinary nickel was evaluated concerning obesity status in the general population. Our results prove that urinary nickel positively correlates with BMI and WC among adult males but not females. In previous studies, heavy metal pollution is a significant cause of chronic inflammation and oxidative stress, of which nickel occupies a large part (31). Chronic inflammation and oxidative stress can destroy the normal function of cells by interacting. The effects lead to symptoms such as weight gain or loss, decreased libido, physical pain, and emotional disorder, which pose a significant threat to health and lead to chronic inflammatory diseases, including obesity, diabetes, and cancer (32).

Several studies support our findings. Pokorska-Niewiada et al. showed that trace element disturbances, including nickel, can increase body mass index and contribute to endocrine disorders (33). A study from Spain found that the trace element nickel in fat is the highest, highlighting the potential role of nickel in obesity and obesity-related diseases (34). Another study from Turkey directly

TABLE 3 Association between urinary nickel (ug/L) and body mass index (kg/m^2).

Exposure	Model 1, β (95% CI)	Model 2, β (95% CI)	Model 3, β (95% CI)
urinary nickel (ug/L)	0.1595 (−0.0177, 0.3367)	0.1477 (−0.0283, 0.3237)	0.1243 (−0.0401, 0.2887)
Stratified by sex			
Male	0.3520 (0.0604, 0.6436)*	0.3278 (0.0398, 0.6159)*	0.2965 (0.0302, 0.5628)*
Quintiles of urinary nickel (ug/L)			
Q1	Reference	Reference	Reference
Q2	−0.0948 (−1.2117, 1.0222)	−0.1758 (−1.2797, 0.9281)	−0.3285 (−1.3483, 0.6913)
Q3	1.3909 (0.2707, 2.5111)*	1.2587 (0.1515, 2.3658)*	0.8434 (−0.1902, 1.8770)
Q4	1.5478 (0.3398, 2.7558)*	1.3839 (0.1870, 2.5808)*	1.1568 (0.0419, 2.2717)*
P for trend	0.001	0.003	0.010
Female	0.0827 (−0.1494, 0.3147)	0.1457 (−0.0304, 0.3217)	0.1032 (−0.0586, 0.2649)
Quintiles of urinary nickel (ug/L)			
Q1	Reference	Reference	Reference
Q2	1.2854 (−0.1095, 2.6803)	1.1684 (−0.2166, 2.5534)	0.4477 (−0.7946, 1.6901)
Q3	0.8190 (−0.6159, 2.2540)	0.6397 (−0.7870, 2.0664)	−0.1152 (−1.4114, 1.1809)
Q4	0.7937 (−0.6458, 2.2331)	0.6915 (−0.7433, 2.1263)	−0.4843 (−1.8212, 0.8527)
P for trend	0.361	0.452	0.337

Model 1: A covariate adjustment was not made.
Model 2: Adjustments were made for age and race.
Model 3: The variables related to BMI found by univariate analysis in Table 2 were adjusted.
The stratification variable is not taken into account when analyzing subgroups.
*$P < 0.05$.

TABLE 4 Association between urinary nickel (ug/L) and body mass index (kg/m^2) stratified by sex and race.

Exposure	Model 1, β (95% CI)	Model 2, β (95% CI)	Model 3, β (95% CI)
Male			
Mexican American	0.1834 (−0.5061, 0.8729)	0.1909 (−0.5014, 0.8831)	0.0914 (−0.5979, 0.7807)
Other Hispanic	−0.9798 (−2.3609, 0.4013)	−0.7445 (−1.9756, 0.4866)	−0.3927 (−1.5063, 0.7209)
Non-Hispanic White	0.6654 (0.1423, 1.1885)*	0.5915 (0.0678, 1.1152)*	0.5613 (0.0690, 1.0536)*
Non-Hispanic Black	0.8468 (0.0592, 1.6344)*	0.8986 (0.1172, 1.6800)*	0.6527 (−0.0070, 1.3124)
Other race	−0.2210 (−0.6036, 0.1615)	−0.1839 (−0.5650, 0.1972)	−0.1725 (−0.5243, 0.1793)
Female			
Mexican American	−0.1073 (−1.0488, 0.8341)	−0.1348 (−1.0795, 0.8099)	−0.2352 (−1.1617, 0.6912)
Other Hispanic	0.8725 (0.0364, 1.7085)*	0.8999 (0.0438, 1.7559)*	0.8061 (−0.0006, 1.6129)
Non-Hispanic White	0.0135 (−0.4620, 0.4890)	0.0107 (−0.4644, 0.4859)	−0.0921 (−0.5169, 0.3327)
Non-Hispanic Black	0.0424 (−0.2492, 0.3339)	0.0420 (−0.2503, 0.3342)	0.0981 (−0.1701, 0.3663)
Other race	0.0178 (−0.7519, 0.7876)	0.0101 (−0.7613, 0.7815)	−0.2663 (−1.0007, 0.4681)

Model 1: A covariate adjustment was not made.
Model 2: Adjustments were made for age.
Model 3: The variables related to BMI found by univariate analysis in Table 2 were adjusted.
The stratification variable is not taken into account when analyzing subgroups.
*$P < 0.05$.

shows a positive correlation between BMI and nickel (35). The results of Yang et al. proved that men exposed to nickel were more prone to dyslipidemia and BMI ≥ 25 (36). In addition, when Cortés et al. studied the relationship between heavy metal exposure and chronic disease development in Chile, introducing BMI as a variable would confuse the relationship between IL-6 and nickel and increase the impact on individual inflammatory states by 40% at the same time. This study indirectly proves that nickel levels in the urine will affect BMI (37), it indirectly proves that nickel levels in the urine will affect BMI.

TABLE 5 Association between urinary nickel (ug/L) and waist circumference (cm).

Exposure	Model 1, β (95% CI)	Model 2, β (95% CI)	Model 3, β (95% CI)
urinary nickel (ug/L)	0.4894 (0.0486, 0.9302)*	0.4938 (0.0679, 0.9197)*	0.4110 (0.0221, 0.7999)*
Stratified by sex			
Male	1.3408 (0.5525, 2.1290)***	1.2004 (0.4511, 1.9498)**	1.1111 (0.4156, 1.8066)**
Quintiles of urinary nickel (ug/L)			
Q1	reference	reference	reference
Q2	−0.1665 (−3.1852, 2.8523)	−0.0903 (−2.9614, 2.7808)	−0.6539 (−3.3052, 1.9975)
Q3	4.5665 (1.5389, 7.5942)**	4.1480 (1.2684, 7.0276)**	3.0214 (0.3213, 5.7214)*
Q4	5.1487 (1.8838, 8.4135)**	4.5536 (1.4405, 7.6667)**	3.8424 (0.9421, 6.7428)**
P for trend	<0.001	<0.001	0.001
Female	0.1958 (−0.3429, 0.7344)	0.1702 (−0.3608, 0.7012)	0.0899 (−0.3783, 0.5581)
Quintiles of urinary nickel (ug/L)			
Q1	Reference	Reference	Reference
Q2	2.9783 (−0.2597, 6.2162)	2.4638 (−0.7253, 5.6529)	1.1570 (−1.6489, 3.9628)
Q3	1.2207 (−2.1101, 4.5516)	1.1005 (−2.1844, 4.3854)	−0.5545 (−3.4614, 2.3524)
Q4	2.0712 (−1.2701, 5.4125)	2.0569 (−1.2468, 5.3606)	−0.2818 (−3.2284, 2.6648)
P for trend	0.368	0.337	0.631

Model 1: A covariate adjustment was not made.
Model 2: Adjustments were made for age, sex, and race.
Model 3: The variables related to WC found by univariate analysis in Table 2 were adjusted.
The stratification variable is not taken into account when analyzing subgroups.
*$P < 0.05$, **$P < 0.01$, ***$P < 0.001$.

TABLE 6 Association between urinary nickel (ug/L) and waist circumference (cm) stratified by sex and race.

Exposure	Model 1, β (95% CI)	Model 2, β (95% CI)	Model 3, β (95% CI)
Male			
Mexican American	0.6573 (−1.0528, 2.3674)	0.7223 (−0.9737, 2.4182)	0.4985 (−1.1143, 2.1112)
Other Hispanic	−2.1927 (−5.9489, 1.5634)	−1.4216 (−4.5441, 1.7009)	−1.0170 (−3.7059, 1.6720)
Non-Hispanic White	2.4342 (0.9895, 3.8789)**	1.9892 (0.5901, 3.3883)**	1.8745 (0.5521, 3.1969)**
Non-Hispanic Black	2.4826 (0.3981, 4.5671)*	2.7612 (0.7748, 4.7475)**	2.4318 (0.7041, 4.1594)**
Other race	−0.2967 (−1.2383, 0.6449)	−0.3579 (−1.3019, 0.5861)	−0.3177 (−1.2222, 0.5868)
Female			
Mexican American	-−0.2682 (−2.2340, 1.6976)	−0.4061 (−2.3483, 1.5360)	−0.2736 (−2.1989, 1.6517)
Other Hispanic	1.8316 (−0.0582, 3.7213)	2.1629 (0.2657, 4.0600)*	1.7983 (−0.0158, 3.6124)
Non-Hispanic White	−0.1163 (−1.2535, 1.0210)	−0.1326 (−1.2543, 0.9892)	−0.3736 (−1.3291, 0.5819)
Non-Hispanic Black	0.3575 (−0.2903, 1.0052)	0.3620 (−0.2849, 1.0089)	0.4896 (−0.0956, 1.0749)
Other race	−0.2080 (−1.9376, 1.5216)	−0.1975 (−1.9321, 1.5372)	−0.6288 (−2.3407, 1.0830)

Model 1: A covariate adjustment was not made.
Model 2: Adjustments were made for age.
Model 3: The variables related to WC found by univariate analysis in Table 2 were adjusted.
The stratification variable is not taken into account when analyzing subgroups.
*$P < 0.05$, **$P < 0.01$.

When subgroup analyzes were performed, we found that urinary nickel was independently and positively associated with BMI and WC in adult men. Numerous prior research had shown that nickel exposure damages male reproductive organs, which is strongly connected to oxidative stress, DNA damage, and hormonal imbalance (38–40). One study found gender differences in the inflammatory response of mice to the lung after nickel exposure, with the male being more susceptible to acute pneumonia and subchronic lung inflammation than females by a mechanism that induces increased neutrophil by CXCL1 and IL-6/STAT3 signaling

TABLE 7 Association among body mass index (kg/m^2), waist circumference (cm), and urinary nickel (ug/L) stratified simultaneously by gender and obesity status.

Body mass index (kg/m^2)	Male	Female
Stratified by obsity status		
No obesity	−0.0107 (−0.2064, 0.1850)	0.2161 (0.0325, 0.3998)*
Central obesity	−0.1511 (−0.3176, 0.0154)	0.0204 (−0.1501, 0.1909)
Peripheral obesity	-	-
Mixed obesity	0.2849 (−0.0328, 0.6026)	−0.1046 (−0.3184, 0.1092)
Waist circumference (cm)		
Stratified by obesity status (%)		
No obesity	−0.0453 (−0.4763, 0.3857)	0.1861 (−0.2251, 0.5973)
Central obesity	0.3738 (−0.1659, 0.9134)	−0.0108 (−0.5076, 0.4861)
Peripheral obesity	-	-
Mixed obesity	1.2598 (0.4131, 2.1065)**	−0.0434 (−0.4833, 0.3965)

The variables related to BMI or WC found by univariate analysis in Table 2 were adjusted. The stratification variable is not taken into account when analyzing subgroups. *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$.

pathways and enhanced monocyte infiltration by CXCL1 and CCL2 in male (41). At present, we have not found any other strong evidence for the reason for gender difference related to this study, and we suspect that the reason for the difference may be related to the differences in hormone levels, eating habits, and work stress between men and women. Large-sample prospective studies may be needed to explore this problem.

The precise mechanism of nickel exposure in BMI and WC is still unclear, but we try to clarify it from the following aspects. Firstly, in the hypothalamus, nickel exposure harms neurological function. As a result, hypothalamic neurons degenerate, paraventricular and supraoptic nuclei are reduced, and myeloperoxidase activity, nitric oxide increase, tumor necrosis factor-α and interleukin-1β of factors that promote inflammation ascend, which will affect the endocrine axis and might lead to hormonal imbalances (42, 43); Secondly, there is the possibility that nickel can affect the hypothalamic-pituitary-thyroid axis, causing abnormal thyroid activity (44). Finally, nickel disrupts the function of insulin β cells, resulting in abnormal glucose and lipid metabolism and affecting body weight (45, 46). Nickel exposure has also been linked to diabetes in some studies (20, 47).

Heavy metal contamination is everywhere—vegetables, seafood, meat and poultry, water sources, and household products are all at risk of exceeding heavy metal levels. Long-term nickel exposure causes irreparable harm to human system functioning, yet using nickel-related items in the medical, commercial, and industrial sectors continues to grow fast. The national legislature should reinforce and enhance the pertinent laws and regulations to minimize heavy metal contamination. Our study demonstrated a significant association between nickel exposure and BMI and WC in males, and men with long-term nickel exposure must pay particular attention to this health risk. In addition, the mechanism through which nickel exposure lowers male sperm quality is conclusive, and men with reproductive needs should avoid nickel-related industries. We appeal to the public to reduce exposure to heavy metals, especially nickel.

As a result of the large sample size, valid subgroup analyses were possible. However, some limitations need attention. In terms of screening for overweight and obesity, BMI and WC are highly specific, but they are less sensitive when used to identify adiposity due to their inability to discern fat distribution accurately; higher visceral fat is far more harmful than more fat in areas such as the thighs, and therefore may incorrectly classify a person as unhealthy or at a high-risk category for disease (48). Likewise, a higher BMI may also be induced by increased muscle mass, which may not always indicate obesity (49). Additionally, these two indicators do not account for a multiplicity of characteristics like gender and age. It is well-known that men and women have varying quantities of muscle, which might alter the final indicator findings. Individuals with a high percentage of body fat may create more angiotensin and aldosterone, while muscle does not (50).

Conclusions

In adult males, both BMI and WC were positively associated with urinary nickel. It is essential for adult men, especially those who are already obese, to reduce their nickel exposure. With the continued growth of nickel applications, nickel-related research will be expanded in the future, and our study may give suggestions for future studies in some specific aspects. Meanwhile, there is a need for further research to understand how urinary nickel might influence BMI and WC.

Data availability statement

The original contributions presented in the study are publicly available. This data can be found here: www.cdc.gov/nchs/nhanes/.

Ethics statement

Participation in the study was voluntary, and National Center for Health Statistics Research Ethics Review Board approved the study's conduct. To protect everyone's privacy, NHANES will anonymize collected data before making it public as public data. It is our agreement to follow all guidelines for using NHANES data for research purposes, as well as comply with all applicable standards and laws. The patients/participants provided their written informed consent to participate in this study.

Author contributions

Conceptualization: H-LL, D-LL, and S-FC. Methodology and Writing—review and editing: G-XW. Software: B-LH. Formal analysis: G-XW and B-LH. Writing—original draft preparation: B-LH. Visualization: J-TL and Z-BF. Supervision: L-YF, H-XZ,

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Monitoring residues of pesticides in food in Brazil: A multiscale analysis of the main contaminants, dietary cancer risk estimative and mechanisms associated

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Introduction: Pesticides pose a risk for cancer development and progression. People are continuously exposed to such substances by several routes, including daily intake of contaminated food and water, especially in countries that are highly pesticide consumers and have very permissive legislation about pesticide contamination as Brazil. This work investigated the relationship among pesticides, food contamination, and dietary cancer risk.

Methods: Analyzed two social reports from the Brazilian Government: the Program for Analysis of Residues of Pesticides in Food (PARA) and The National Program for Control of Waste and Contaminants (PNCRC).

Results and discussion: First, we characterized the main pesticide residues detected over the maximum limits allowed by legislation or those prohibited for use in food samples analyzed across the country. Based on this list, we estimated the dietary cancer risks for some of the selected pesticides. Finally, we searched for data about dietary cancer risks and carcinogenic mechanisms of each pesticide. We also provided a critical analysis concerning the pesticide scenario in Brazil, aiming to discuss the food contamination levels observed from a geographical, political, and public health perspective. Exposures to pesticides in Brazil violate a range of human rights when food and water for human consumption are contaminated.

KEYWORDS

pesticide, food intake, cancer risk, environmental exposure, Brazil

Introduction

Pesticides are a large and heterogeneous group of chemicals used primarily to destroy, repel, or mitigate insects, small animals, weeds, and other undesirable organisms. Chemically these substances are categorized as organochlorines, organophosphates, carbamates, pyrethroids, neonicotinoids, and phenylpyrazoles.

Most of them are considered persistent organic pollutants that can accumulate in the ecosystem and remain in the environment for considerable periods due to their lipophilic characteristic and long half-life (1–3).

Once in the environment, such pesticides can reach the human body through the daily ingestion of contaminated food and drinking water. This exposure may harm humans since these substances are associated with disease development. Neurodegenerative disease (4), respiratory pathologies (5), metabolic disorders (6), reproductive dysfunction (7, 8), and cancer (9, 10) has been linked to pesticides.

In countries whose economy is based on agriculture, this contamination poses a public health issue. In this context, Brazil is at the top of the world's biggest pesticide consumers (11) altogether to China and the United States. Agribusiness is one of the essential activities for the Brazilian economy. Expanding Brazil's export share has been one of the main objectives guiding the Ministry of Agriculture, Livestock, and Supply (MAPA) work (12).

Nonetheless, these active ingredients are not restricted to the production of agricultural commodities. They are commonly found in horticulture and fruit growing, as observed from the reports of the Program for the Analysis of Residues of Pesticides in Food (PARA), coordinated by the National Health Surveillance Agency (ANVISA). This monitoring investigates pesticide residues in food, observing their compliance with the Maximum Residue Limits—MRL allowed and the presence of active ingredients not authorized for a particular crop or banned in the country.

PARA is the most extensive study regarding monitoring the presence of pesticides in foods of plant origin in Brazil, as it has national coverage and all sample analyses are carried out by specialized laboratories. The program is essential, considering that from the results, it is possible to assess the scenario of irregularities and health risks in a country that consumes many pesticides. The activities of PARA began in 2001, and the main goal is to evaluate the levels of pesticide residues that reached the consumer's table. Since then, PARA has coordinated jointly with municipal and state health surveillance agencies and state public health laboratories (13).

Therefore, despite the pivotal role of fruits and vegetables in nutrition and preventing chronic diseases, consuming contaminated food may have critical consequences. As conventional food cultivation uses many pesticides, it poses a chronic risk for cancer development, for example, due to its carcinogenic potential and frequent presence over the maximum residual limits. Studies have developed tools to estimate the dietary cancer index that allows evaluation of the impact of acute and chronic consumption of pesticide-contaminated food on cancer risk (14).

Little information on the cancer risk attributable to food intake is available worldwide, and conflicting results have been reported (15–19). Also, more information is needed concerning the food-derived pesticide-attributable risks for large-scale populations, as in Brazil. In the present study, we investigated literature data about the relationship between food and risk and carcinogenic pathways, considering the main pesticides described in the last Brazilian PARA report. Further, we estimated the Pesticide Residue Index (PRSI) and revised the major mechanisms enrolled regarding its impact on cancer.

Methods

This study aims to comprehend the multiscale relationship between food contamination by pesticides and the cancer risk attributable to its ingestion. Therefore, it comprises three main parts:

1. The analysis of the pesticide food contamination data from the Brazilian PARA Report.
2. The estimative of the dietary cancer risk related to PARA reported food pesticide contamination.
3. A systematic analysis of literature concerning the consequences of this pesticide exposure.

The number of detections of active ingredients reported in PARA and the concentration detected in mg/kg in the vegetable samples were analyzed. From these data, samples that showed some pesticide concentrations were selected, and then the median per crop was applied. The percentage of pesticide residue detection in samples considered satisfactory by the Vegetal PNCRC was consulted in SDA Ordinance No. 448 of November 17, 2021, published in the Official Gazette of the Federal Government (20).

To assess the Pesticide Residue Index (PRSI), which represents the pesticide residues in a single serving, we used the original equation for Theoretical Maximum Daily Intake (TMDI) (Equation 1). Through some minor changes in the TMDI equation, a second equation was generated and applied to food samples to achieve the PRSI. The comparison between both equations showed us the specific foods and pesticides in these samples have higher than recommended pesticide residues.

For the systematic review of literature, data were obtained from studies available in three critical databases (PubMed, Google Scholar, and Web of Science) on pesticide exposure and its correlation with carcinogenesis. We restricted our search to articles published from 2012 to 2022. We used a combination of the following words in the title and abstract: pesticides, cancer, tumor, and carcinogenesis. Four authors reviewed titles, article abstracts to classify eligible articles, and full text if necessary. All of the included pieces were written and published in English. Animal, *in vitro*, cross-sectional, case-control, cohort, and ecological studies were included.

Results

Results of monitoring pesticides residues in food in Brazil: PARA report analysis

Aiming to understand the picture of food contamination in Brazil, we evaluated the results from the PARA report. The first cycle of the program comprised the period between 2001 and 2007 and analyzed nine types of products. The data showed that foods such as strawberries, tomato, and lettuce had the highest levels of unsatisfactory samples, reaching ∼50% of sampling by culture. From 2008 onwards, the amount of food analyzed increased each year, reaching 36 different products in the cycle from 2017 to 2020, although, so far, only the first cycle of 14 varieties has been published (Table 1).

Sampling carried out between 2010 and 2018, on average, showed that 63% of the food samples contained some pesticide residue, indicating that most of the food consumed in Brazil has traces of

TABLE 1 Historical overview of the sampling of in natura foods carried out in PARA (2001–2018).

PARA report, year	Number of vegetables analyzed	Varieties analyzed	Total samples analyzed
2001/2007	9	Lettuce, banana, potato, carrot, orange, apple, papaya, strawberry and tomato.	7,321
2008	17	Lettuce, banana, potato, carrot, orange, apple, papaya, strawberry, tomato, pineapple, rice, onion, beans, mango, bell pepper, cabbage and grapes.	1,773
2009	20	Lettuce, banana, potato, carrot, orange, apple, papaya, strawberry, tomato, pineapple, rice, onion, beans, mango, bell pepper, cabbage, grapes, kale, beet and cucumber.	3,130
2010	18	Lettuce, potato, carrot, orange, apple, papaya, strawberry, tomato, pineapple, rice, onion, beans, mango, bell pepper, cabbage, kale, beet and cucumber.	2,488
2011/2012	15	Papaya, cucumber, bell pepper, pineapple, zucchini, lettuce, rice, beans, carrots, orange, apple, corn (cornmeal), strawberry, tomato and grape.	4,690
2013/2015	25	Papaya, banana, mango, cucumber, bell pepper, pineapple, zucchini, beet, potato, onion, cabbage, lettuce, cabbage, rice, beans, carrot, guava, orange, apple, wheat (flour), corn (cornmeal), cassava (flour), strawberry, tomato and grape.	12,051
2017/2018, 1st cycle	14	Bell pepper, guava, carrot, tomato, lettuce, grape, beetroot, orange, pineapple, mango, chayote, sweet potato, garlic and rice.	4,616
2019/2020, 2nd cycle	22	Not published	Not published

active ingredients due to the spraying of these products. Of this percentage, 27%, on average, are considered unsatisfactory due to the risk they pose to human health. Furthermore, most samples are deemed inadequate because detected pesticides were unauthorized for the crop, which endangers farmers directly exposed to these products and food consumers (21).

The most recent report in Brazil about PARA (released in 2019) deals with the first phase of the 2017–2018 cycle. This cycle analyzed 4,616 samples and searched up to 270 active ingredients of pesticides. Residues of 122 different active ingredients were detected in the samples analyzed, resulting in a total of 8,270 detections.

The most detected pesticides were the insecticide imidacloprid (713 detections) and the fungicides tebuconazole (570 detections) and carbendazim (526 detections) (Figure 1A). Imidacloprid is among the 10 most commercialized pesticides in Brazil (22) and has been associated with the death of bees (23). For this reason, is prohibited in the European Union (24). Carbendazim has been banned in the United States and the European Union for more than a decade, in association with cancer and fetal malformations.

The foods that presented the highest number of unsatisfactory samples were: peppers (81.9%), guava (42.4%), carrots (39.6%), and tomato (34.8%). Of the total monitored, 41 samples from the 2017–2018 cycle (0.89%) had a potential acute health risk; of this amount, 27 were orange (Figure 2). In addition, 2.9% of the samples, corresponding to 134 units, had 10 or more active ingredients in the same food (13).

Despite the advances, the number of samples analyzed in Brazil seems to be less than ideal, given that throughout PARA (2001–2018), 36,069 samples were analyzed, which represents a little more than a third of what was analyzed in the European Union, only in the year 2018. Another point is that Brazil has been much more permissive about the established MRLs and the pesticides that are used in the national territory, which have been banned for years in European Union countries, as is the case of carbendazim, chlorpyrifos, and acephate, which represents a framework of environmental injustice (25).

Another essential element presented in the reports is the multi-exposure; that is, the consumer, when eating, may be ingesting more than one pesticide at a time. This risk of combined action is not yet estimated in Brazil, but methodologies and pilot studies already exist in the European Union and the United States to guarantee consumer safety (26). This, therefore, is a crucial point for the improvement of PARA.

In addition to PARA, another program has been monitoring pesticide residues in plant samples in Brazil since 2008: the National Program for Control of Waste and Contaminants (PNCRC Vegetal), carried out by the Ministry of Agriculture, Livestock, and Supply (MAPA). The PNCRC/Vegetal has the function of monitoring the quality and safety of products of plant origin produced and consumed throughout the national territory concerning the occurrence of pesticide residues and chemical, physical and biological contaminants. Products of plant origin intended for the domestic and export markets are monitored. MAPA has carried out the PNCRC/Vegetal since 2008, and samples are preferably collected at processing establishments and/or packers, wholesalers, and supply centers. MAPA reports show that on average, 56% of the samples analyzed between 2015 and 2020 had some level of residue (27).

The most recent report, from 2020, shows pesticide residues in 67.17% of the total samples, highlighting the high frequency of residue detection for the following foods: cowpea, grapes, peppers, and wheat flour, which had more than 85% of the samples with the presence of pesticides (Figure 1B). Among the most detected active ingredients are carbendazim, chlorpyrifos, and acephate (20).

Dietary cancer risk estimative attributable to PARA reported pesticides residues in food

Based on the residues described in Tables 2, 3, we calculated the dietary cancer risk attributable to food contamination. The equation

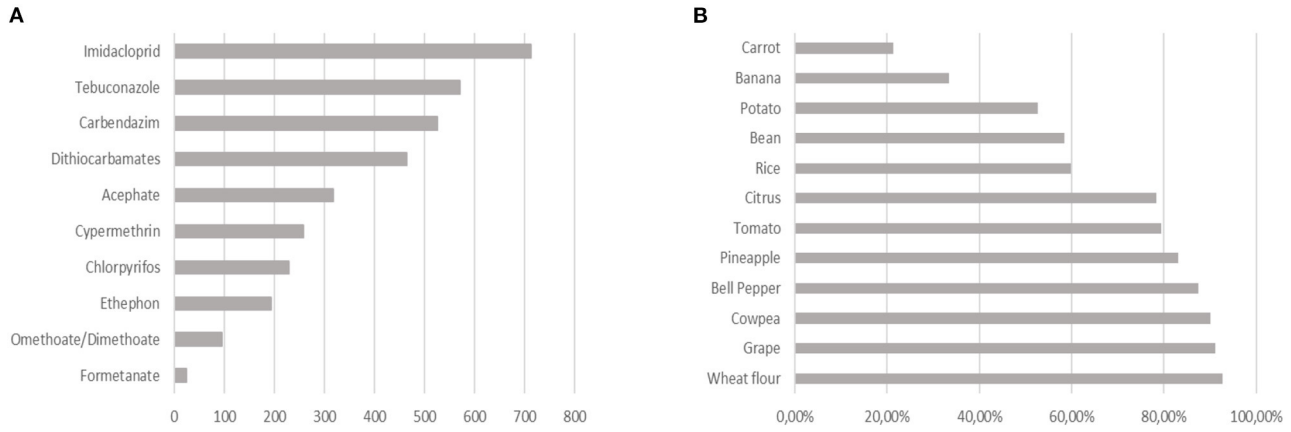

FIGURE 1
Recognition of pesticide residues according to PARA. **(A)** Main pesticide residues detected in PARA 2017–2018: total detected by active ingredient. **(B)** Percentage of pesticide residue detection about the parameters analyzed in the satisfactory samples of the PNCRC Vegetal 2020.

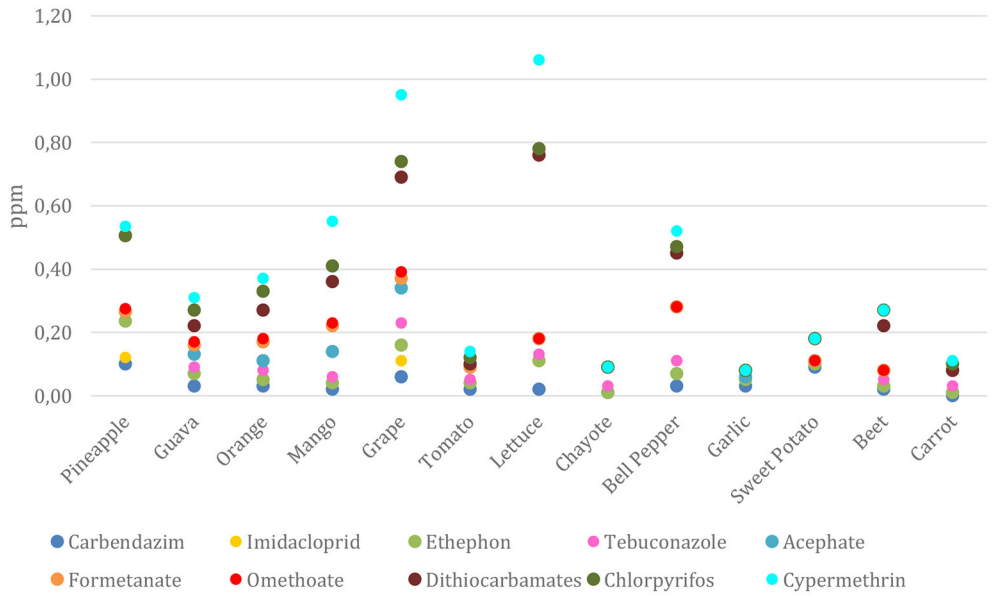

FIGURE 2
Main pesticide residues detected in PARA 2017–2018: median of values detected per culture (mg/kg).

for the Theoretical Maximum Daily Intake (TMDI) (Equation 1) considers the Maximum Residue Limits (MRL) (28) to establish the highest level of pesticides legally tolerated on food or feed crops. For MRL, we only considered the compounds authorized for use in Brazil since the current guidelines of the National Health Surveillance Agency (Anvisa) do not cover unauthorized products. The daily intake of any particular pesticide residue in a given food is obtained by multiplying the residue level in the food (MRL) by the amount consumed (F).

Equation 1. Original equation:

$$TMDI = MRL \times F$$

TMDI = Theoretical Maximum Daily Intake;
MRL = Maximum Residue Limits (in ppm or $mg.kg^{-1}$); and
F = Recommended food serving size (in mg).

We evaluated both the TMDI and the Pesticide Residue Sample Index (PRSI) for 44 pesticides applied in crops in 2019 and 33 pesticides used in 2020 (Tables 4, 5). PRSI is an adaptation of the original equation (Equation 1) by replacing MRL with accurate pesticide measurements (PR) from all available crops to identify pesticide contamination (PRSI, Equation 2) in food and/or food crops in different Brazilian regions.

Equation 2. The equation to identify accurate pesticide contamination:

$$PRSI = PR \times F$$

PRSI = Pesticide Residue Sample Index;
PR = Pesticide residues measured in agricultural crops of different Brazilian regions; and

TABLE 2 Pesticide active substances percentages applied over the limit in agricultural crops according with TMDI and PRSI values in 2019.

Crop	Pesticide	MLR	PR	F	TMDI	PRSI	TMDI × PRSI	% Over the limit (median)
Banana	Trifloxystrobin	0.3	0.59	0.13	0.039	0.0767	0.0377	96.6
	Carbendazim	0.5	1.665	0.13	0.065	0.2457	0.1807	278
	Cyazofamid	0.2	1.64	0.12	0.024	0.1968	0.1728	720
	Metalaxyl-M	0.5	0.99	0.12	0.06	0.1188	0.0588	98
	Pyraclostrobin	1	3.5	0.12	0.12	0.42	0.3	250
	Bifenthrin	0.02	0.06	0.086	0.00172	0.00516	0.00344	200
Black bean	Glyphosate	0.05	0.28	0.08	0.004	0.0224	0.0184	460
	Glufosinate	0.05	0.41	0.08	0.004	0.0256	0.0216	540
Papaya	Carbendazim	0.5	1.24	0.16	0.08	0.1984	0.1184	148
	Trifloxystrobin	0.05	0.13	0.16	0.008	0.0208	0.0128	160
Melon	Cypermethrin	0.02	0.04	0.23	0.0012	0.0069	0.0057	275
	Thiamethoxam	0.1	0.145	0.24	0.024	0.0348	0.0108	45
	Carbendazim	0.5	1.6	0.24	0.12	0.384	0.264	220
	Fenpyroximate	0.1	0.3	0.06	0.006	0.018	0.012	200
Soy bean	Glyphosate	10	23.75	0.043	0.43	1.02125	0.59125	137.5
	Cypermethrin	0.05	0.335	0.043	0.00215	0.014405	0.012255	570
Tomato	Bifenthrin	0.02	0.04	0.08	0.0016	0.0032	0.0016	100
	Acephate		0.09254	0.08	0.0016	0.026902	0.0253016	1581.35
	Cyromazine	0.03	0.07	0.08	0.0024	0.0056	0.0032	133.3
Grape	Dimethomorph	2	2.75	0.0992	0.1984	0.2728	0.0744	37.5

F = Recommended food serving size (in mg), according to the Dietary Guidelines for the Brazilian population (77).

Upon the TMDI equation, we could identify values that were used for comparison with PRSI results in food or food crops potentially consumed by the Brazilian population. To estimate food consumption (F), we used the Dietary Guidelines for the Brazilian people, established by the Brazilian Health Ministry (77), in which food serving portions are recommended. The data on the PR found in the crops were evaluated for each pesticide-active substance.

For comparison purposes, we observed the difference between the TMDI and PRSI from several crops in Brazil in 2019 and 2020. This difference is demonstrated in a percentage higher than the limit tolerated (% higher than the tolerated column, Tables 2, 3). We found that in 2019, all 46 crops analyzed had higher active substance residues than advocated by law, and 38 of these crops displayed 100% more residues than tolerated. The numbers are even worse for 2020 crops, as from 104 crops evaluated, 102 showed 100% more residues than allowed. These findings should be awareness-raising, as several food crops in Brazil were found with increased pesticide residues, often overpassing 1,000%.

It emphasizes the adverse effects that public policies demeaning can have, as Brazil is going against the world and has been increasing the number of legally pesticide-active substances in the country. The draft bill PL 6299/2022 (78)—more widely known as the "poison package," is an example, already approved by the parliament and waiting to be voted on by the senate (According to the project PL 6299/2002). The demeaning of these public policies not just impacts the overuse of these compounds but also the indiscriminate application of them, as we observe a lot of active substances applied in Brazilian crops are banned for crop use, according to the country's legislation (Supplementary Tables S1, S2).

One example of the importance of sound policies related to pesticides is the latest European Food Safety Authority report, where more than 88.000 food samples produced in 2020 were analyzed, and 94.9% of the samples were within legally permitted levels (79).

Therefore, the dietary risk assessment analysis suggests that the food commodities analyzed are unlikely to concern consumers' health.

The most widely used chemical herbicide is N-(phosphonomethyl) glycine, commonly known as glyphosate (80). In our database, we observed glyphosate as the most used pesticide, applied in eight different regions in 2019 and 43 crops from other areas in 2020, followed by glufosinate. Glyphosate was also the main pesticide used irregularly, i.e., above the maximum allowed residue levels. Economically, this herbicide is popularly sold under the name of Roundup. As a broad-spectrum herbicide, it is used in agriculture and forestry, representing one of the most important chemical compounds in use since its release. Although it is less bioavailable than other herbicides, glyphosate residue levels may represent a risk to consumers depending on several factors, such as the application technique, water quality, and environmental conditions (81).

Glyphosate is considered "safe" because neither its active substance nor its primary degradation product,

TABLE 3 Pesticide active substances percentages applied over the limit in agricultural crops according with TMDI and PRSI values in 2020.

Crop	Pesticide	MLR	Result	C	TMDI	PRSI	TMDI × PRSI	% over the limit (median)
Pinneapple	Carbendazim	0.5	2.078985	0.13	0.065	0.27026805	0.20526805	315.8
Potatoes	Acephate	0.1	0.24916	0.2025	0.02025	0.0504549	0.0302049	149.16
	Imidacloprid	0.05	0.10035	0.2025	0.010125	0.02032088	0.010195875	100.7
	Methamidophos	0.01	0.02216	0.2025	0.002025	0.0044874	0.0024624	121.6
Black bean	Glyphosate	0.05	0.44	0.08	0.004	0.0352	0.0312	780
	Acephate	0.02	0.03	0.08	0.0016	0.0024	0.0008	50
	Glufosinate	0.05	0.18	0.08	0.004	0.018	0.014	350
Cowpea bean	Glyphosate	0.01	0.94	0.048	0.00048	0.05856	0.07152	14900
	AMPA (Glyphosate metabolite)	0.01	0.115	0.048	0.00048	0.00552	0.00528	1100
	Acephate	0.02	0.025	0.048	0.00096	0.0012	0.00024	25
	Glufosinate	0.05	0.33	0.048	0.0024	0.01584	0.01344	560
	Flutriafol	0.2	0.41636	0.06	0.012	0.0249816	0.0129816	108
	Fenpropatrina	0.2	0.280045	0.06	0.012	0.0331209	0.0211209	176
	Chlorfenapyr	0.3	1.55	0.06	0.018	0.093	0.075	416.6
	Cypermethrin	0.02	0.05886	0.06	0.0012	0.0046104	0.0034104	284.2
Tomato	Acephate	0.02	0.13673	0.08	0.0016	0.0109384	0.0093384	583.65
	Bifenthrin	0.02	0.04778	0.08	0.0016	0.0038224	0.0022224	138.9
	Lambda-cyhalothrin	0.05	0.118095	0.08	0.004	0.0094476	0.0054476	136.19
	Cyazofamid	0.5	1.1673	0.0992	0.0496	0.11579616	0.06619616	133.46

aminomethylphosphonic acid (AMPA), is associated with any known adverse effect on human health. However, there is a controversy in the literature regarding its carcinogenic potential, as some studies describe its potential to cause endocrine and/or microbiome disruption (80, 82, 83). Besides, glyphosate exposure can also induce epigenetic modulation, such as decreasing global DNA methylation and promoting histone modification, as reviewed elsewhere (84).

Carbendazim (methyl 2-benzimidazolecarbamate) was also detected above the maximum allowed residual level in different crops in 2019 (pineapple, lettuce, papaya, and pear) and 2020 (pineapple). The Brazilian National Health Surveillance Agency (Anvisa) decided to ban the use of carbendazim in 2022, as it was considered carcinogenic (85). Carbendazim is a systemic fungicide that inhibits microtubule polymerization in cells by acting with β-tubulin (86). This inhibition disrupts the microtubule assembly and leads to impaired segregation of chromosomes during cell division, inducing mitotic arrest (87). Organophosphorous pesticide exposure can cause severe systemic and central nervous system disturbances, primarily associated with inhibiting acetylcholinesterase activity (88). Acephate (O, S-dimethyl-acetyl-phosphoramidothioate) and methamidophos (O,S-dimethyl phosphoramidothioate) are two of the most common and efficient OPs used in agriculture. Acephate is classified as a class II "moderately hazardous" pesticide, and methamidophos is classified as a class Ib "highly hazardous" pesticide (89). Acephate is prohibited in tomato crops but was detected in several samples evaluated. Methamidophos is the toxic metabolite of acephate (90). Despite being banned from Brazil since 2012, we observed contamination above the limits of methamidophos in potato crops in 2020. Both active substances have their use restricted or prohibited in the European Union due to their harmful potential, however, we still found residues of these substances, even under restriction or prohibition by law, in food, water, and crops in Brazil.

Cancer risk evidences for PARA and PNCRC reported pesticides residues

Literature screening

Concerning the literature review, we conducted a literature search using the R software (91) with the bibliometrix package (92) to check the terms "pesticides" and "cancer" and "tumor" and "carcinogenesis" in the PubMed database. The search retrieved 174 articles that met our criteria. These terms are appearing more and more in high-impact journals that are devoted to toxicology or cancer studies (Supplementary Figure S1A). Furthermore, an increase in the number of publications dealing with "cancer and pesticides" is evident in recent years (Supplementary Figure S1B).

Brazil is in the top 10 when we look at the countries with higher article production in the last 10 years; the publication rate is increasing in all countries. The USA and China are the countries with the most significant number of publications. Brazil, in this ranking, occupies ninth place with 43 articles available in the PubMed database (Supplementary Figure S2A). Also, a large number of collaborations

TABLE 4 Mechanisms associated with human carcinogenesis following exposure to pesticides.

Pesticide	Type of cancer	Associated mechanism	Exposure	References
Acephate	Retinoblastoma	–	Prenatal exposure to pesticides in individuals living near application areas.	(29)
Acephate	Testicular germ cell tumors (TGCT)	Endocrine disruptor	Fetal exposure to agricultural endocrine disrupting pesticides.	(30)
Acetamiprid	Liver cancer	–	The presence of acetamiprid in blood samples was detected in the liver cancer group. The blood concentration of a-fetoprotein was higher in both control and cancer groups, showing the risk of developing liver cancer after exposure to acetamiprid.	(31)
Aminomethylphosphonic acid (AMPA)	Breast cancer	–	Exposure to AMPA was evaluated in healthy postmenopausal women and women with breast cancer. The AMPA levels found in the excretion of women with cancer vs. controls were 38% higher.	(32)
Carbendazim	–	–	Possibility of developing cancer after exposure (estimated risk >1) in four areas of Spain (Alzira, Burriana, Benicarló and Benifaió) in babies.	(33)
Carbofuran	Prostate cancer	Men carrying the homozygous wild-type TT genotype at two correlated CDK7 SNPs, rs11744596 and rs2932778, were at increased risk of developing prostate cancer after exposure to carbofuran.	–	(34)
Chlorpyrifos (organophosphate)	Colorectal	–	There was an increased risk of developing cancer and occupational, environmental and food exposure to the insecticide chlorpyrifos.	(35)
Chlorpyrifos	Prostate cancer	–	Men exposed to pesticides and who have the polymorphism in the CYP1A1 enzyme are at greater risk of developing prostate cancer.	(36)
Chlorpyrifos	Breast cancer	–	Women exposed to chlorpyrifos were three times more likely to develop breast cancer when compared to the other pesticides analyzed.	(37)
Chlorpyrifos	Lung cancer	–	Increased risk of developing lung cancer in occupationally exposed individuals.	(38)
Chlorpyrifos	Kidney cancer	–	High risk for the development of renal tumors in occupationally exposed individuals.	(39)
Clothianidin (Neonicotinóides)	Liver cancer	Alters cell growth.	Environmental	(31)
Methyl-Kresoxim		–	Environmental exposure increases the susceptibility to develop astrocytoma.	(40)
Dimethoate	Prostate cancer	–	Increased risk of developing the disease when there is environmental/occupational exposure.	(41)
Dimethoate	Meduloblastoma	–	Higherchances of presenting the disease when mothers were exposed to the environment during pregnancy.	(40)
Fipronil	Bladder cancer	–	Environmental	(42)
Phosmet	Acute lymphoblastic leukemia	–	Pesticide exposure during pregnancy due to residential proximity to agricultural applications may increase childhood ALL risk.	(43)
Glyfosate	Non-Hodgkin lymphoma	–	gbh exposure is associated with increased risk of NHL in humans	(44, 45)
Glyfosate	Acute myeloid leukemia	Users in the highest exposure quartile had an increased risk of acute myeloid leukemia (AML) compared to never users.	Occupational exposure	(46)
Glyfosate	–	B-cell lymphoma was positively associated with phenoxy herbicides and the organophosphate herbicide glyphosate.	Occupational exposure	(47)

(Continued)

TABLE 4 (Continued)

Pesticide	Type of cancer	Associated mechanism	Exposure	References
Imazalil	Breast cancer	Positive association between dietary exposure and risk of postmenopausal breast cancer was found specifically among overweight and obese women.	Dietary exposure	(18)
Omethoate	–	It can lead to changes in telomere length in workers exposed to the presence of polymorphism in the GSTM1 gene can also influence telomere length.	Occupational exposure	(48)
Omethoate	–	Alteration in p53 and p21 expression levels and may be related to telomere length changes induced by omethoate.	Occupational exposure	(48)
Permethrin	Leukemia	It can cause rearrangements and breaks in genes associated with leukemia in adults and children.	Chronic exposure	(49)
Permethrin	Multiple myeloma	It was observed that there is a high prevalence of its precursor monoclonal gammopathy of undetermined significance, in farmers who use it.	Occupational exposure	(50)
Permethrin	Multiple myeloma	Occupational exposure to Permethrin is associated with an increased risk of developing multiple myeloma.	Occupational exposure	(51)
Permethrin	Leukemia		Mothers who had occupational/daily contact with pesticides during pregnancy may be associated with an increased risk of developing acute leukemia in children occupational exposure.	(52)
Permethrin	–	Decreased telomere length associated with some pesticides including Permethrin.	Occupational exposure	(53)
Permethrin	Lymphoblastic leukemia	Several pesticides have been evaluated for their association with the risk and development of lymphoblastic leukemia in children. there was no association with Permethrin.	Environmental exposure	(54)
Permethrin	Multiple myeloma	Change in hematological parameters in Permethrin applicators.	Occupational exposure	(55)
Permethrin	Non-Hodgkin lymphoma	There was no association between occupational exposure to pyrethroids and non-Hodgkin's lymphoma.	Occupational exposure	(56)
Permethrin	–	Classified pesticides that are potentially carcinogenic by the USEPA and used in large volume.	–	(57)
Propiconazole	Central nervous system tumor	–	A study carried out with mothers who lived in rural areas showed a high risk for medulloblastoma.	(40)
Thiamethoxam	Liver cancer	–	The results showed that exposure through diet increases the chances of liver cancer prevalence.	(31)

between different countries to study the topic in question is striking, thus evidencing the concern with the relation between pesticides and cancer (Supplementary Figure S2B).

The trend topics are diverse, but the terms "exposure," "cancer," "human," "pesticides," and "carcinogenesis" are highlighted. Besides, there is a direct link among each other, meaning the co-occurrence of those terms. These terms were searched for in the titles of the articles (Supplementary Figure S3).

Selected data are discussed in the following topics.

Human exposure data

Most of the pesticides reported in PARA and PNCRC are classified by IARC as possibly, potentially or proven carcinogenic (Supplementary Table S3), the most used pesticides as active

TABLE 5 Mechanisms associated with *in vitro* carcinogenesis following exposure to pesticides.

Pesticide	Cell lineage	Mechanism	References
Acetamiprid	4T1 breast cancer cells	Acetamiprid induced dose-dependent 4T1 breast cancer cell proliferation, migration, and estrogen receptor interaction.	(58)
Cyfluthrin	H295R human adrenocortical carcinoma cells	Cyfluthrin increased E2 (estradiol) expression	(59)
Cypermethrin	BG-1 ovarian cancer cell	Cypermethrin induced the growth of the ovarian cancer cell line BG-1 and up-regulated cyclin D1 expression.	(60)
Chlorpyrifos	MCF-7 and MDA-MB-231 breast cancer cell lines	Increases cell division by activating the estrogen receptor (ERα).	(61)
Chlorpyrifos	MCF-7 breast cancer cell line	Stimulates angiogenesis progressing to breast cancer	(62)
Chlorpyrifos	Breast cancer cell lines MCF-7 and MDA-MB-231	Increases migration, invasion, phosphorylation	(63)
Chlorpyrifos	A549cell andNCI-H1299 Lung cancer cell	Generates oxidative stress, activates Nrf2 promoting cancer cell survival	(64)
Clothianidin	SH-SY5Y human neuroblastoma cells	Increase cell growth; alters calcium influx; alter gene expression	(65)
Glyfosate	T47D breast cancer cells	Glyphosate promoted the growth of T47D cells *via* estrogen receptors, activation of the ERE (estrogen response element), and, altered estrogen receptors by increasing the expression ratio of ERα and ERβ.	(66)
Glyfosate	MCF-7 and MDA-MB-231 breast cancer cell lines	Low concentration of Roundup dysregulated in both lineages, 11 canonical pathways, the most important being cell cycle repair and DNA damage repair pathways, and alterations in metabolism that can alter mitochondrial oxygen consumption, increase ROS levels, induce hypoxia, cause accumulation of mutations.	(67)
Imidacloprid	Hs578t breast cancer cell lines	Increases CYP19 expression, a key aromatase in estrogen biosynthesis.	(68)
Imazalil	HepG2 cells—human hepatocellular carcinoma	Increased levels of cell proliferation markers, Ki-67 positive nuclei and mcm2 mRNA	(69)
Omethoate	FaDu cell of head and neck cancer	Activation of the Akt/GSK-3β/cyclin D1 pathway, leading to the proliferation of pharyngeal cancer cells.	(70)
Permethrin	K562 cells (chronic myeloid leukemia)	Permethrin induces aneuploidy and structural alterations in the IGH and KMT2A genes, causing fusion of the ETV6-RUNX1 gene in peripheral blood mononuclear cells. It has also been shown to induce fusion of the ETV6-RUNX1 and IGH-BCL2 genes in K562 cells.	(71)
Permethrin	Peripheral blood mononuclear cells	The pesticide at low concentrations induces aberrations in the KMT2A and IGH genes, detected in the interphase and metaphase phases.	(49)
Thiamethoxam	Adenocarcinoma cells (H295R)	Exposure to neonicotinoid pesticides could increase the concentration of the CYP19 enzyme in adenocarcinoma cells (H295R), which cause cell proliferation in breast cancer.	(72)
Thiamethoxam	H295R adrenocortical carcinoma cells	The pesticide induces CYP19 aromatase enzyme activity, increased estradiol and estrone production, CYP3A7 enzyme expression and inhibited estriol in H295R cells.	(73)
Triethanolamine/ trifloxystrobin	SH-SY5Y neuroblastoma cells	It has been observed to cause inhibition of mitochondrial oxidative respiration and to alter the levels of various lipids in neuronal cells.	(74)
Triflumuron	HCT116 Colon Cancer cells	It induces the generation of reactive oxygen species, followed by lipid peroxidation, and an increase in malondialdehyde, it also activates antioxidant enzymes (oxidative stress).	(75)
Triflumuron	HepG2 liver cancer cells	It demonstrated dose-response agonistic activities of HIF-1α at non-cytotoxic concentrations, stimulation of cell migration and invasion.	(76)

4T1, mouse breast cancer cells; A549, human lung adenocarcinoma cells; BG-1, human ovarian cancer cells; FaDu, human hypopharyngeal cancer cells; H295R, human adrenal corticocarcinoma cells; HCT116, human colorectal carcinoma cells; HepG2, human hepatocarcinoma cells; Hs578t, human breast cancer cells; K562, human immortalized myelogenous leukemia cells; MCF-7, human breast cancer cells; MDA-MB-231, human breast cancer cells; NCI-H1299, human lung adenocarcinoma cells; SH-SY5Y, human neuroblastoma cells; T47D, human breast cancer cells.

ingredients and basic formula, chemical group, class, agricultural use, classification according to EPA, classification according to IARC, and classification according to WHO (89).

The mechanism of action of pesticides on target-specific pests is well-established in the literature (93–95), but the action of these compounds on human health is a reasonable investigation and needs to be elucidated. The association with human diseases, including cancer, when exposed to pesticides is already well-established. Still, the mechanisms by which these compounds are responsible for human carcinogenesis must be better understood.

The carcinogenic process can occur gradually, taking several years for a single cancer cell to develop and give rise to a tumor. For

TABLE 6 Mechanisms associated with carcinogenesis *in vivo* after exposure to pesticides.

Pesticide	Type of cancer	Mechanism	Exposition	References
Cypermethrin	Liver cancer	Cypermethrin treatment suppressed LPS-induced M1 macrophage polarization and promoted a switch to M2 macrophage status. Furthermore, cypermethrin induced metastasis of lung cancer cells in both studies.	–	(113)
Cyproconazole	Liver cancer	Treatment with propiconazole induced liver cell proliferation in an *in vivo* model. Furthermore, TGF-β was overexpressed after treatment.	–	(114)
Cyproconazole	Liver cancer	Cyproconazole induced mild and duration-dependent hepatic hypertrophy in constitutive androstane receptor knockout (CARKO) mice.	–	(115)
Glyfosate	Multiple myeloma	Glyphosate induces monoclonal gammopathy of undetermined significance and promotes disease progression to MM.	Orally	(116)
Glyfosate	Liver cancer	Glyphosate promoted genetic modulation in female Sprague-Dawley rats. There was alteration in the expression of hepatic genes, DNA damage and activation of the TP53 gene.	Orally	(69)
Imazalil	Liver cancer	Imazalil activates the PXR receptor and induces hepatocyte proliferation.	Orally *in vitro*	(117)
Metidathione	Liver	Metidathion increases the incidence of liver toxicity, in addition to increasing neoplasms in male mice.	7 and 28 day exhibitions	(118)
Permethrin	Liver cancer	Permethrin induces a significant increase in hepatocellular neoplasms.	–	(119)
Pyraclostrobin	–	Pyraclostrobin induces elevated levels of hydrogen peroxide, 2, malondialdehyde (MDA) and reactive oxygen species (ROS).	–	(120)
Pyraclostrobin	–	Interaction with pro-apoptotic (Bax), apoptotic (Caspase-3, Caspase-8 and Caspase-9), pro-inflammatory (NFκB), cancer (CYP2E1) and cell regulatory (p53) genes and decreased anti-inflammatory gene expression apoptotic (Bcl-2).	–	(121)
Propiconazole	Liver cancer	Inhibition of CYP450 enzymes, genetic alterations caused by the increase of ROS and promotion of cell proliferation, alterations in DNA directly or indirectly by the action of ROS, promotes proliferation and loss of function of tumor suppressor genes.	–	(122)
Propiconazole	Liver cancer	Propiconazole can induce tumors by a mechanism dependent on constitutive androstane receptors (CAR).	–	(123)
Propiconazole	Liver cancer	Propiconazole affects CYP450, Glutathione S transferase and increases oxidative stress.	Diet	(114)
Propiconazole	Liver cancer	Propiconazole induces an increase in ROS and alters the expression of antioxidant enzymes (SOD, CAT, GST).	Environmental	(124)
Propiconazole	Liver cancer	Propiconazole activates CAR/RXR, P450 metabolism, hepatic hypertrophy-glutathione depletion, LPS/IL-1-mediated inhibition of RXR, and NRF2-mediated oxidative stress pathways.	–	(125)
Propiconazole	Liver cancer	Increased endogenous DNA adducts (carcinogenic DNA-binding molecule) and increased cell proliferation.	Diet	(126)
Propiconazole	Liver cancer	This pesticide activates the CAR receptor and leads to increased liver weight and hepatocyte proliferation.	–	(127)
Thiamethoxam		A study carried out on Drosophila evaluated the pro-mutagenic potential of this pesticide at high concentrations	–	(128)

tumor development, the cell goes through several phases of growth and adaptation, which can be synthesized in three stages: initiation, promotion, and progression (96).

Initiation is the first phase of tumor development. At this stage, the initiator molecules (carcinogenic) meet the cellular microenvironment and lead to DNA damage, which is not adequately repaired, thus establishing mutations. The greater the exposure to these initiator molecules, the greater the risk of tumor development (97). Promotion is the second phase, affecting cells that have already started (mutants). Promoting agents have the role of increasing the proliferative rate, creating a more significant number of mutation-bearing cells. Promoting agents do not directly affect DNA but cell receptors, leading to the alteration of signaling pathways and increased cell proliferation. Promoters can be further divided into two categories: specific promoters, which interact with receptors on target cells, and non-specific promoters, which alter gene expression without the involvement of a known receptor. Promoters do not lead to the formation of tumors alone; they only increase the cellular expansion of cells already initiated, thus leading to the formation of tumors (98).

The third and final phase of carcinogenesis is cell progression. This phase is associated with changes in the cell genotype, an increase in the rate of proliferation, invasive and metastatic capacity, biochemical (glycolytic pathway and oxidative phosphorylation), and morphological changes (99). At this level of development, tumor formation is irreversible.

IARC has been assessing the carcinogenic risk of pesticides to humans and has critically evaluated monographs on individual chemicals, classifying them into risk cancer categories (IARC53). Carcinogenic risk means the probability that an agent will lead to cancer (neoplasm or tumor) in humans exposed to it. Carcinogen denotes an agent or mixture capable of increasing the incidence of malignant neoplasms. Assessment of carcinogenicity is based on evidence from epidemiological studies, depending on variability over time and location of mixtures, processes, occupations, and industries.

Human exposure to these compounds occurs acutely or chronically and can occur through the skin, respiratory and oral routes, food, water, or accidental ingestion (Table 4) (2, 6, 152). In addition, people may be in direct contact with pesticides during the preparation and use of pesticides and/or indirectly through breathing residual concentrations in the air or exposure to residues found on surfaces, food, and dust (100). Children are vulnerable to pesticides because of their physiological and behavioral differences compared to adults, such as hand-to-mouth exposure (101).

Considering the pesticides described in the PARA and PNRCN reports, we bring some information about their cancer-related effects. Parental occupational exposure to pesticides, such as permethrin, acephate, phosmet, and propiconazole, cause changes in their germ cells. It has been related to an increased risk of developing cancer in childhood, such as acute lymphoblastic leukemia, retinoblastoma, central nervous system tumors, and cell tumor testicular germ cells in adolescence (29, 30, 43, 49, 52). A study carried out by Lombardi et al. (40) related dimethoate and propiconazole to an increased risk of developing medulloblastoma in children whose mothers were exposed to the action of these pesticides during pregnancy (40).

The risk intensifies when the mother is exposed during pregnancy and in the first years of the child's life through contamination by air, dust, and clothes used by the parents when applying pesticides, food, and even breast milk. Exposure does not need to be high or extended because their physiological characteristics make them more susceptible to the effects on their body (102). Among these characteristics, the following can be mentioned: absorption through the skin, which is more intense due to the weight/body surface ratio; greater inhalation due to its respiratory rate and ventilation per minute; higher intake of contaminated food and water per body weight compared to adults and incomplete metabolism causing toxicity to the organism (103).

In human studies, aminomethylphosphonic acid (AMPA), chlorpyrifos, and imazalil were positively associated with the risk of breast cancer (18, 32, 37). It is known that some pesticides accumulate in adipose tissue and can act in the body as endocrine disruptors, having estrogenic effects, which is one of the critical factors that contribute to the development of breast cancer. They can influence the synthesis, transport, metabolism, and elimination of estrogen, disrupting the body's normal homeostasis (37).

Organochlorines act as alpha estrogen nuclear receptor agonists, promoting cell proliferation and tumor progression. Other pesticides promote the activation of cytochrome P450 (CYP) member, CYP19 alpha-aromatase enzyme in adipose tissues, indirectly contributing to the increase of estrogen in peripheral tissues and the intratumoral environment (104). Another mechanism would be related to the interaction with aryl hydrocarbon receptors (AhR). This transcription factor regulates enzymes that participate in the metabolism of xenobiotics belonging to the CYP family. This alteration would lead to the accumulation of adducts in the DNA, one of the factors linked to breast carcinogenesis (105).

A positive association was found between chlorpyrifos exposure and lung cancer incidence (38). Other pesticides, such as acetamiprid, clothianidin, and thiamethoxam, were associated with a higher risk of liver cancer (31). In a case-control study carried out with rural workers exposed to pesticides, the tumor biomarkers p53, alpha-fetoprotein, and alpha L-fucosidase were at higher levels when compared to the unexposed control group. Further, the shorter length of the telomeres and decreased telomerase activity were associated with increased DNA damage (106).

There was a link between the increased risk of developing non-Hodgkin's lymphoma and human exposure to glyphosate (44, 45). Several mechanisms triggered by this exposure may contribute to the onset of the disease, such as immunotoxicity, genotoxicity, and hormonal effects (107). Chromosomal aberrations, such as translocations, would be one of the critical effects caused by pesticides in this type of tumor, favoring the overexpression of oncogenes and thus promoting cell proliferation (108).

The use of permethrin has been linked to the occurrence of multiple myeloma (50, 51, 55). Permethrin can act directly on the progression of the disease by having an immunomodulatory effect or, in the same way, lead to monoclonal gammopathy of undetermined significance, which increases the risk of developing multiple myeloma (109). In the study by Shearer et al. (55), a change in myeloid lineage cells was observed, including immature granulocytes and red blood cells. Consequently, the exacerbated presence of immature granulocytes suppressed the antitumor immune response and favored tumor angiogenesis (55).

Individuals exposed to glyphosate, phosmet, and permethrin are more likely to develop leukemia, and the exposure of pregnant women also increases the chances of their children presenting the disease (43, 46, 52). Some pesticides, such as permethrin, can lead to chromosomal rearrangements, which may later inactivate the topoisomerase 2 or cause oxidative stress, promoting breaks in DNA double-strand (49, 110). The presence of polymorphisms in enzymes such as the glutathione S-transferase and CYP450 families alter their normal functioning, compromising the metabolism of xenobiotics, which may also contribute to increased susceptibility to leukemia (111). Chlorpyrifos and carbofuran were associated with an increased risk of prostate cancer in exposed men (34, 36).

Colorectal and renal tumors were associated with a greater chance of developing in workers exposed to the pesticide chlorpyrifos (35, 39). In turn, astrocytoma had a greater chance of occurrence in those exposed to methyl-Kresoxim (40).

Occupational exposure to omethoate demonstrated changes in the length of telomeres (48). The telomeric region of the chromosome is responsible for preventing the degradation of the final portion of chromosomes and end-to-end chromosomal fusion, ensuring genome stability during cell divisions. Changes in this region contribute to several diseases, including cancer, due to oxidative stress and immunotoxicity that generate DNA damage (112).

In vitro and *in vivo* data

Pesticide-induced effects have been the subject of many published *in vitro* and *in vivo* studies aimed at expanding the scientific basis of current risk assessment procedures by allowing a better understanding of the mechanism of chemical-induced toxicity and

its safety levels. These experimental studies show that pesticides alter DNA, leading to mutations and chromosomal aberrations that lead to the development of cancers and other diseases (2). The articles in our search that address *in vitro* and *in vivo* studies used for this review are listed in Tables 5, 6.

Some pesticides act directly on receptor expression and hormone secretion. The secretion of estrogen is one of the main pathways affected, suggesting a higher risk for women exposed to these pesticides. Imidacloprid and thiamethoxam, pesticides from the neonicotinoid class, increase the expression of the aromatase enzyme cytochrome P450 19 (CYP19), the key to the stimulation of estrogen biosynthesis. This increase is directly related to the increased proliferation of cell lines such as breast cancer lineage Hs578t and adenocarcinoma lineage H295R (72). Cyfluthrin, chlorpyrifos, and glyphosate, the most widely used pesticide globally, act similarly. These increase estradiol (E2) synthesis in adrenocortical carcinoma (H295R) and breast cancer (T47D) cells and increase cell proliferation *via* the estrogen receptors ERα and ERβ. These results indicate that even low concentrations and environmental levels of pesticides cause increased estrogen levels, which, at high levels, are related to potential risk factors for developing, especially, breast cancer in women (58, 59, 61, 66).

There are indications from studies in animal models that pesticides also act on androgen receptors and on specific factors that stimulate the development of liver neoplasms, such as cyproconazole and propiconazole, from the class of conazoles. These pesticides act as essential mediators for increased hypertrophy and tumor initiation, the constitutive androstane receptor (CAR). Along with these pesticides, imazalil, permethrin, and methidathion act on the liver. These increase hepatocyte proliferation; imazalil, by increasing the expression of transforming growth factor alpha (TGF-α) and genes of the cytochrome p450 family, such as Cyp3a11, a target of the pregnane X receptor (PXR), which has its expression increased in liver carcinogenesis or other adverse events in the organ. In contrast, methidathion and permethrin stimulate liver cell proliferation in a PXR and CAR receptor-independent manner (114, 115, 118, 119, 122, 123, 125, 127).

Other mechanisms may also be responsible for changes in cell proliferation, such as changes in the cell cycle and in the expression of factors linked to tumor progression. Omethoate and cypermethrin alter the cell cycle of hypopharyngeal carcinoma (FaDu) and ovarian cancer (BG-1) lineages. These pesticides activate the Akt/GSK-3β/cyclin D1 signaling pathway and regulate the cyclin D1 gene, which is responsible for the transition between G1-S phases of the cycle; thus, the cell cycle is, in turn, stimulated, resulting in increased cell proliferation (60, 70). Cypermethrin also promotes, in mice, macrophage class switches from M1 (pro-inflammatory) to M2 (anti-inflammatory) that act by inhibiting effector T cells. This modulation can promote lung tumor progression (113).

The pesticide triflumuron, *via* hypoxia-induced factor 1α (HIF-1α), induces, in hepatocellular carcinoma (HepG2) cells, migration, invasion, and metastasis. Interestingly, this is the first time HIF-α is responsible for promoting these changes in this type of cancer (76). Another factor influenced by pesticide exposure is vascular endothelial growth factor A (VEGF-A). Increased VEGF-A levels in MCF-7 and MDA-MB-231 breast cancer cells increased specific parameters such as angiogenesis, migration, and cell invasion in these cell lines after chlorpyrifos exposure. These findings reinforce the role of angiogenesis in breast cancer progression, and that pesticide exposure contributes to this process (62, 63).

Oxidative stress is one of the mechanisms involved in the process of carcinogenesis already established, according to the literature, in several types of cancer, including childhood leukemias. In this sense, studies have been carried out to understand if there is any influence on pesticide exposure and the generation of oxidative stress. Thus, an investigation conducted with lung cell line A549 observed that the pesticide chlorpyrifos could generate oxidative stress in these cells by activating the NRF2 pathway, a transcription factor. Although NRF2 plays a role in decreasing oxidative stress and inflammation, it has been shown that in some cancers, this factor enables malignant cells to undergo metabolic changes leading to rapid proliferation and, therefore, tumor growth, and this is a possible survival mechanism for tumor cells (64, 129, 130).

Another study involving the pesticide Triflumuron was conducted experimentally in animals and HCT 116 cells. This work aimed to evaluate the genotoxicity of this chemical in the models chosen for the experiment. They observed that triflumuron induced the generation of reactive oxygen species, followed by lipid peroxidation, due to increased levels of malondialdehyde, a pro-oxidative parameter, and activating the antioxidant enzymes, catalase, and superoxide dismutase, in human colon tumor cells (HCT 116). These studies suggest that exposure to these substances, even at low concentrations, can induce oxidative stress, a well-established carcinogenic factor in cancer pathophysiology, including a marker of therapeutic response (75, 129).

Other pesticides have shown pro-carcinogenic effects in animal models. For example, in the zebrafish model, the pesticide pyraclostrobin affected apoptosis-related pathways, cancer, and membrane components, leading to mitochondrial dysfunction and cell apoptosis. It is because it induced the production of reactive oxygen species (ROS) and increased the activity of antioxidant enzymes such as catalase (CAT) and superoxide dismutase (SOD). These findings portend the need for further research into pesticide toxicity in aquatic models (120).

It was also observed once the increase of oxidative parameters, such as MDA, in rats exposed to the insecticide pyraclostrobin and the decrease of antioxidant defenses, DNA damage, and histopathological analysis was also observed in the kidneys and liver of these animals (121).

Among pesticides, glyphosate is a widely used herbicide worldwide. Many researchers aim to understand the relationship of this herbicide with cancer because the product is cytotoxic, even at low concentrations and a short duration of exposure. Stur et al. studying a cell line treated with roundup (composed of glyphosate and surfactants) observed that this compound can induce the production of reactive oxygen species by altering cellular metabolism and mitochondrial oxygen consumption, leading to a sequence of events that culminates in cell death. Oxidative stress is one of the pathways altered by glyphosate, but other pathways suffer interference and are also the target of studies (67, 131).

Exposure to pesticides can also induce the expression of genes involved in carcinogenesis. However, it remains unclear which genes and the mechanism responsible for their triggering, so to elucidate which pathways are

stimulated, both *in vitro* and *in vivo* studies are carried out (132).

For example, studies carried out with glyphosate demonstrated through animal and *in vitro* experiments the pathways related to the development of the investigated cancer. In the case of exposure to small doses of glyphosate (0.05%) *in vitro* in the breast cancer cell line MCF-7 and MDA-MB-231, dysregulation of 11 canonical gene pathways was observed. The most essential included cell cycle and DNA damage repair and accumulating mutations, once again demonstrating the role of pesticides in mutagenicity by generating stress and cell cycle dysregulation (67).

While an experiment was carried out in an animal model, in this case with female Sprague-Dawley rats, a change in the expression of liver genes was observed, reflecting the activation of the TP53 gene due to the damage caused to the DNA. Furthermore, there was a decrease in the expression of miR-30 and an increase in the expression of miR-10. Dysregulation in the expression of microRNAs can alter the expression of target genes and disrupt cellular pathways. DNA base methylation is another modification capable of influencing gene expression, and this mechanism was also changed by glyphosate methylation (69). The work carried out by Wang et al. verified that MYC mice treated with glyphosate showed benign monoclonal gammopathy, anemia, and increased plasma cells in the bone marrow and spleen. Such findings place pesticides as a potential risk factor for developing multiple myeloma and non-Hodgkin lymphoma (116).

In research carried out *in vitro* to analyze the consequences of exposure to permethrin em ETV6-RUNX1 and IGH-BCL2 genes in K562 cells (chronic myeloid leukemia cells), induction was found of breakage and fusion of the damaging genes associated with lymphoma development (71). Furthermore, permethrin exposure induced numerical aberrations frequently observed in the metaphase phase (49).

Other results from *in vitro* and *in vivo* exposure to pesticides evaluated in the present review are shown in Tables 5, 6.

Perspectives and conclusions

Some considerations need to be pointed out about PARA reliability and data validity. Among the positive points of PARA, it should be noted that since its implementation in 2001, the program has been expanded in four dimensions: the number of participating states, number of samples analyzed, types of food analyzed and number of active ingredients researched (21). Although there was no standardization in the presentation of results from the beginning, the reports proved to be more detailed and complete. In the case of the Vegetal PNCRC, there have also been advances, especially from 2019 onwards, when the Ministry of Agriculture, Supply, and Livestock, through inspection actions, began to fine irregularities (133). However, the reports are still strictly technical, issued through ordinances, and not very accessible to the general population.

Considering that Brazil is among the three countries that use pesticides in the world, as well as the significant increase in the number of concessions for registration of pesticides in the country from 2016 onwards (134), official surveillance institutions should pay greater attention to the problem, especially in which refers to the contamination of food by these agrochemicals. The PARA and PNCRC Vegetal methodologies still need to be improved to ensure transparency and transmit greater security to the consumer.

In this sense, in the case of PARA, the number of samples is still low compared to other countries, such as the European Union. Recently, it has involved only 1.38% of Brazilian municipalities, 77 out of 5,568 (13). Another point to be highlighted is that Brazil's two most commercialized active ingredients (glyphosate and 2,4-D), widely used in the production of monocultures, only entered the analysis from 2016 onwards. On the other hand, glyphosate is one of the most detected pesticides in the Vegetal PNCRC, mainly in bean samples (20).

Brazilian researchers have also questioned the fact that the multi-exposure risk assessment is not adopted (135–137) since the reports by PARA and PNCRC Vegetal indicate samples contaminated by more than one active ingredient. Thus, the effects that add up and potentiate should be considered in methodologies for analyzing pesticide residues in food.

It is noteworthy that the publication and dissemination of results could be more problematic in the reports. The focus is on the absence of danger, disregarding that more than half of the total samples have some pesticide residue. Thus, if the sample is considered "satisfactory" for the Brazilian MRLs (which are highly permissive), the impression is that Brazilians are purchasing foods that are perfectly suitable for consumption and are also healthy. It is also not usually publicized that not all active ingredients approved for use in Brazil are monitored. In addition, in 2020, the PARA was suspended due to the COVID-19 pandemic, and no results were released after the 2017–2018 cycle.

It is very important to point out that the exposure to pesticides in Brazil is continuous. It occurs directly for farmers who frequently (138) handle these products and indirectly, through the drift of active ingredients to neighboring areas, as well as the contact of farmers' wives and children with different amounts of pesticides, by having contact with clothing used for work, and even pesticide packaging (139, 140). Children are vulnerable to pesticides because of their physiological and behavioral differences compared to adults, such as hand-to-mouth exposure (101, 141). Urban dwellers are also affected, as the urban water supply and many commercialized foods are already contaminated with pesticides (142).

There is no provision in the Brazilian legislation about the review process of the registration of authorized pesticides, and even today, products banned in other countries are used. Decree No. 4.074/2002 (143) recommends that this review could, in theory, occur at any time, guided by international alerts, new scientific studies, or complaints made by reference institutions under its subsection VI, art. 2. It is also noticed that, even in cases of international alerts, the limited resources available in the agencies or the lawsuits filed by corporations linked to agribusiness, not rarely end up hindering and delaying such reviews, worsening the exposure of the population to pesticides (144).

The MRL is defined as the maximum amount of pesticide residue officially accepted in food as a result of proper application at a specific stage, from its production to consumption, expressed in parts (by weight) of the pesticide or its residues per million parts of food (by weight) (ppm or mg/kg) (145). As for the levels of residues contained in food, they must be below the MRLs, established as references after conducting the necessary toxicological studies. In this context, the issue of maximum residue levels (MRLs) is one of the most relevant for food safety in trade negotiations between countries and companies.

When analyzing the PARA reports, one point that draws attention is that there is a category in which the samples are considered satisfactory when they present pesticide residues within a maximum residue limit pre-established through federal government regulations and the Codex Alimentarius. In general, 30–40% of the samples analyzed in each report fall into this classification. However, setting these limits ends up disregarding essential factors such as the joint action of several chemical compounds acting simultaneously in the human body (146), differences in susceptibility according to age and genetic factors, and the effects of chronic exposure (33).

Pesticide exposures in Brazil violate many human rights of the population. The right to life is potentially violated when pesticides contaminate food and water for human consumption. Bodies become ill (147), and the biodiversity of ecosystems is also threatened.

The Brazilian Constitution provides in its article 225 (148) that everyone has the right to an ecologically balanced environment, an asset for shared use by the people and essential to a healthy quality of life, imposing on the government and the community the duty to defend and preserve it for present and future generations. However, Brazil has adopted a position contrary to several countries that start from the precautionary principle concerning pesticides, such as those belonging to the European Union.

Approximately 80% of the pesticides authorized in Brazil are not permitted for use in at least three countries of the Organization for Economic Cooperation and Development (OECD), including countries with agriculture as an essential economic activity. Australia has 40% of its agricultural territory, a similar condition to Brazil, and no records of 114 active ingredients of pesticides allowed in the Brazilian territory were found. Although Brazil and India have relatively close soil and climate conditions, more than 50% of the pesticides that are registered in the first country are not allowed in the second, and the list of active ingredients of pesticides authorized in Brazil includes examples with recognized toxicity on human health and the environment. It extends to the 279 active chemical ingredients for agricultural use registered in Brazil with their regulatory status in the European Union, the United States, Canada, and Japan, which exposes massive differences. While in the European Union, 136 substances registered in Brazil are approved (143 are not approved), in the United States and Canada, 218 substances are approved. In Japan, 205 active ingredients registered in Brazil are approved (149).

Among the most used pesticides in Brazil, glyphosate stands out. In Brazil's regulations, glyphosate has a maximum residue limit of 1 mg/kg in coffee and sugar cane and 10 mg/kg in soy, corresponding to 10, 20, and 200 times the values allowed in the European Union for the same foods. In the human body, glyphosate is detected in blood, breast milk, and urine, with urinary levels in the general population of 0.16–7.6 μg/L, while in the occupationally exposed population, it is 0.26–73.5 μg/L (150, 151).

The European legislation establishes rules for the use and limits of pesticide residues and practices to be incorporated in the member countries of the European Union to gradually reduce the use of pesticides, as well as the use of alternatives that replace the use of chemicals, aiming to protect human and animal health and the environment. These limits are also extended to countries that intend to export to the European Union.

Currently, Bill 6.299/2002 (78) is being processed in the National Congress, already approved in the House of Representatives, which aims to further relax the legislation on pesticides (152) by facilitating the registration of active ingredients known to be prohibited in other countries, among other serious proposals that favor the indiscriminate use. Among the proposed changes is removing the registration prohibition criteria for potentially carcinogenic agents, toxic to the reproductive system, endocrine disruptors, and teratogenic agents, which are currently similar to the requirements adopted in Europe. With the changes, the use of substances associated with these effects may be permitted, subject to risk assessment. In Europe, there is also pressure on this provision. Still, studies have shown that the supposed economic losses would not be more significant than the health costs, loss of individual quality of life, deaths, and reduced productivity due to absenteeism, among others (153). In addition to its various effects, endocrine disruption indicates prohibition in the European Community. However, this device meets resistance to being fully implemented due to the controversies and doubts produced by the economic sectors to define the criteria for this classification, common strategies regarding the regulation, and use of toxic substances (154).

It is important to note that this bill is being processed even after opposing manifestations of Brazilian official technical bodies (155) such as the Brazilian Institute of Environment and Renewable Natural Resources, the National Health Surveillance Agency, the National Cancer Institute, and the Ministry of Labor.

The observations and recommendations of entities linked mainly to health and the environment were ignored by 2/3 (two-thirds) of the parliamentarians of the House of Representatives, who voted in favor of the continuity of the bill's passage (according to project PL 4166/12). It shows that economic interests, high productivity, and profitability are prioritized to the detriment of the population's quality of life.

Regarding food contamination, there is no direct association in the literature between exposure to pesticides and cancer development. However, pesticides such as glyphosate can cause disruptions in several biological pathways that may be linked to carcinogenesis. Glyphosate was our evaluation's most widely applied active ingredient on crops during 2019 and 2020.

Contact with glyphosate can occur *via* the oral, respiratory (pulmonary), or dermal route (156). The dermal route is the complaint of workers exposed to glyphosate by the absorption route of this element (157). Its accumulation in the body is found mainly in the liver, kidneys, colon, and small intestine, and its excretion happens through about 90% in the feces and within 48h in the urine (156). Importantly, even with many studies already confirmed and still being investigated, the ubiquitous cause of glyphosate and its health safety is of great concern (158).

In 2015, the International Agency for Research on Cancer (IARC), part of the World Health Organization, published its carcinogenicity assessment of glyphosate, concluding that this pesticide would likely be carcinogenic to humans (group 2A) based on limited epidemiological evidence in humans, primarily for non-Hodgkin's lymphoma, and significant evidence of carcinogenicity in animals (159, 160), operating through two critical pathways of known human carcinogens, specifically genotoxicity and oxidative stress induction.

Much research verifies glyphosate use and cancer incidence (46). The IARC evaluation of glyphosate resulted in intense opposition from the pesticide industry and led to many industry-sponsored articles and analyses on this subject (161–169). Notably, two of these studies were conducted in communities that had contact with this

herbicide through aerial spraying, and caused DNA damage (170) and micronuclei (171).

Subsequently, the European Food Safety Authority (162) and the US Environmental Protection Agency (172) also reviewed this issue. They found that glyphosate is probably not carcinogenic in humans. Most pesticide regulatory agencies in other countries have followed their lead, suggesting that data sets and methodological differences partially explain these divergent views. However, this topic is complex and beyond the scope of this article.

Thus, it is imperative to have strict policies regarding these chemicals, following each crop's recommendations in class and the number of chemicals used.

Data availability statement

The original contributions presented in the study are included in the article/Supplementary material, further inquiries can be directed to the corresponding author.

Author contributions

Conceptualization: JB, TF, MO, PL, GB, MC, BG, JS, TS, SG, LZ, and JM. Methodology: GB, MC, TS, and SG. Validation: LZ and JM. Data curation: MO and PL. Writing—original draft preparation: JB, TF, MO, PL, GB, MC, BG, JS, TS, SG, LZ, JM, FR, and CP. Writing—review and editing: JB, TF, FR, and CP. Supervision: FR and CP. Project administration: CP. All authors have read and agreed to the published version of the manuscript.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fpubh.2023.1130893/full#supplementary-material

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Adverse health effects of emerging contaminants on inflammatory bowel disease

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Inflammatory bowel disease (IBD) is becoming increasingly prevalent with the improvement of people's living standards in recent years, especially in urban areas. The emerging environmental contaminant is a newly-proposed concept in the progress of industrialization and modernization, referring to synthetic chemicals that were not noticed or researched before, which may lead to many chronic diseases, including IBD. The emerging contaminants mainly include microplastics, endocrine-disrupting chemicals, chemical herbicides, heavy metals, and persisting organic pollutants. In this review, we summarize the adverse health effect of these emerging contaminants on humans and their relationships with IBD. Therefore, we can better understand the impact of these new emerging contaminants on IBD, minimize their exposures, and lower the future incidence of IBD.

KEYWORDS

inflammatory bowel disease, emerging contaminant (EC), exposome, adverse health effects (AHEs), gut dysbiosis

Introduction

Inflammatory bowel disease (IBD) refers to chronic, relapsing inflammatory disorders of the gastrointestinal tract, and its pathogenesis includes heredity and environmental factors (1). The two major types of IBD are Crohn's disease (CD) and ulcerative colitis (UC) (2). The pathology of IBD involves impairment of the intestinal mucosal barrier, dysbiosis of the gut microenvironment, and the alteration of the gut immune response (3). Chronic abdominal pain and diarrhea are typical symptoms of IBD. Presently no effective treatments can fully cure IBD, and nearly 30% of IBD patients will require surgery within 10 years after their initial diagnosis (4, 5). The global spread of IBD appears to be associated with industrialization and changes in people's diets and environments, and environmental exposures are closely associated with the increased risk of IBD (6).

The new emerging environmental contaminant is a recently coined term that describes exposome to the environment. The emerging environmental contaminants including but are not limited to microplastics (MPs), endocrine-disrupting chemicals (EDCs), chemical herbicides, heavy metals, and persisting organic pollutants (POPs) (7, 8). The variable composition of these exposomes across regions, and the interaction among these exposures may contribute to the heterogeneous nature of the association between emerging environmental contaminants and IBD (9). These exposomes are not commonly monitored in nature, but have the potential to enter the environment and human body, and cause short-term and long-term adverse health effects. In the immediate dietary intake, the contaminants

may cause acute abdominal pain or diarrhea, activating immediate intestinal inflammation (Figure 1). As in long-term exposure, these contaminants will cause chronic diseases like IBD and chronic renal failure, activate a series of chronic inflammation.

In this review, we summarize the current epidemiologic evidence and biological mechanism between new emerging contaminants exposure and the development of IBD. Also, we summarize the common exposure pathways of new emerging contaminants to public generations based on accumulated studies.

Microplastics

Microplastics (MPs) are tiny plastic particles under 5 millimeters in size (10). The primary sources of MPs in human life are plastic bottles, abrasives, and opacifiers (11), and they may be degraded into MPs by various factors like ultraviolet over time (12). The main types of MPs include polyethylene (PE), polypropylene (PP), polystyrene (PS), polyethylene terephthalate (PET), polyvinyl chloride (PVC), Polyurethane (PU), etc. (10, 13) (Table 1).

Human exposures to MPs occur through ingestion, inhalation, and dermal contact (21). MPs exist and exposit in all environments, especially underground and surface water, then the human food chain, and ultimately enter the body through ingestion, which is the major exposure way (11, 13, 22, 23). A recent review has summarized multiple types of food containing MPs, including fruit, vegetables, milk, meat, aquatic food, etc. (12, 24). Besides, the fast spread of takeaway foods accelerates the number of MPs ingested by humans globally, since they are usually packed with plastic products (12). The suggested weekly ingestion range of MPs is within 0.10–5 g/week (5, 7, 8). In a study of a small set of donors, which first measured the mass concentration of the polymetric component of plastic in human blood, the mean of the sum quantifiable concentration of plastic particles in blood was 1.6 μg/ml (25). In human feces, MPs were also detected in the order of 2 MP particles/g (5).

MPs could be detected in the human bloodstream probably due to the absorption of them into the blood by mucosal contact (either ingestion or inhalation) in a size-dependent manner (25). MPs may be transported to organs *via* the bloodstream, causing intestinal toxicity, metabolic disruption, reproductive toxicity, neurotoxicity, immunotoxicity through oxidative stress, apoptosis, and specific pathways, etc. (26). After dietary intake, MPs are usually absorbed in the digestive tract, liver, kidneys, and spleen (27–31). Once deposited, MPs will induce morphological changes, activate inflammatory responses, inhibit cell differentiation, and affect gene expression (27, 32–34). Several clinical trials found that exposure to MPs impairs the gut epithelial barrier, induces intestinal flora alteration, disturbs lipid metabolism, causes oxidative stress and the release of inflammatory factors (15, 35–37). Apart from passive intake, the intestinal tract absorbs MPs through multiple ways, including endocytosis of enterocytes and specialized M cells, paracellular uptake, and active absorption by the intestinal villus (38).

The increased exposure to MPs will also accelerate the pathogenesis of IBD. A recent study revealed that exposure to MPs may impair the antimicrobial capacity of blood clam by reducing plasma inhibition of bacterial growth, humoral immune effector

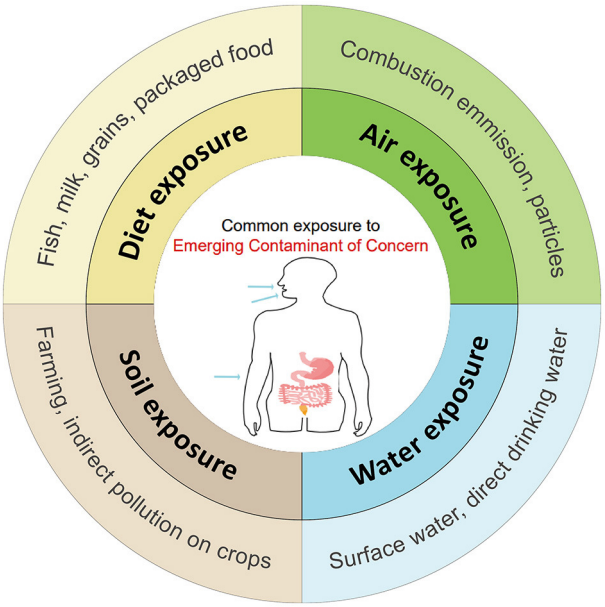

FIGURE 1
The common way and pollutants that general population in exposure of Emerging Contaminants (ECs) through digestive tract, respiratory tract and skin contact.

levels, and chemotactic activity of hemocytes, which showed that MPs imposed significant oxidative stress on hemocytes, causing great immunotoxicity (39).

Researchers found that the average concentrations of MPs in the feces of IBD patients (41.8 items/g dm) were markedly higher than that of healthy people (28.0 items/g dm) (40). In IBD patients, the gut experiences repeating endothelial lesions, which increases the permeability of the intestinal epithelial barrier (41). This means in IBD patients, MPs are more likely to enter the injured intestinal epithelial cells, attach to them, and enhance their translocation to different systems (14, 42). MPs exposure also triggers the over-proliferation of intestinal stem cells, causing the imbalance of colonic epithelial homeostasis, which in turn elevates the occurrence and severity of DSS-induced colitis (43).

MPs will also alter the composition and diversity of intestinal microflora in animal models, which will trigger multiple follow-up effects, such as changing the ability in the differentiation of Treg cells, and activating the signal transduction pathways associated with intestinal mucosal immune function (17, 18, 44–46). In the DSS mouse model, additional exposure to polystyrene microplastics (PS-MPs) can aggravate the severity of colitis (14, 15) by reducing tight junction proteins such as Claudin1 and Occludin, and increasing intestinal permeability (47–49). Correspondingly, TNF-α, IL-1β, and IL-6 increase significantly in the colitis mice exposed to additional PS-MPs, demonstrating their proinflammatory properties (15, 16). After the exposure, the macrophage infiltration will increase in the liver, which further triggers immune cells to release proinflammatory and immune factors like IL-17α and IL-22 (16).

MPs can alter the structure of intestinal microbiota in mice, which may exacerbate intestinal inflammation. Previous studies

TABLE 1 Adverse health effects of microplastics in inflammatory bowel disease.

Contaminant	References	Experiment model/human group	Exposure time/dose	Route of exposure	Source of dietary intake	Impact on the gut	Other health risks
Polystyrene (PS)	Schwarzfischer et al. (14)	Wild type female C57BL/6 mice	0.2 mg/day for 84 days	Dietary exposure	Drinking water	◦ Penetrate the intestinal barrier and accumulate in small intestine, lymphoid organs and liver ◦ Do not affect intestinal health, nor aggravate acute/chronic DSS colitis	/
	Luo et al. (15)	8-week-old male C57BL/6 J mice	0.5/5 μg for 14 days	Oral gavage	Drinking water	◦ ↓Colonic length ◦ ↑Histopathological damage ◦ ↓Mucus secretion ◦ ↑Colon permeability ◦ ↑Colonic inflammatory response	◦ ↑Secondary liver injury associated with inflammatory cell infiltration
	Zheng et al. (16)	male C57 mice	500 μg/L for 28 days	Dietary exposure	Drinking water	◦ ↑Acute colitis and lipid disorders induced by sodium dextran sulfate (DSS) ◦ ↑intestinal permeability	◦ Intensify liver damage in mice with acute colitis ◦ Affect lipid metabolism
	Lu et al. (17)	Five-week-old mice	0.5 and 50 μm polystyrene MP, 100 and 1,000 μg/L, 5 weeks	Dietary exposure	Drinking water	◦ ↓Decrease the secretion of mucin in gut ◦ Induce gut microbiota dysbiosis	◦ Induce hepatic lipid metabolism disorder in mice
Polyethylene (PE)	Li et al. (18)	5-week-old SPF grade male C57BL/6 mice	Respective 6, 60, or 600 μg daily for 5 weeks	Dietary exposure	Special feeds	◦ Induce intestinal dysbacteriosis ◦ ↑Gut microbial species, bacterial abundance and flora diversity ◦ Induce inflammation in intestine ◦ ↑the secretion of serum pro-inflammatory cytokine IL-1α; ◦ ↓the percentage of Th17 and Treg cells among $CD4^+$ cells	/
Most Polypropylene (PP)	Schwabl et al. (19)	8 healthy volunteers aged 33 to 65 from Tokyo	7 days	Dietary exposure	Seafood; Food is usually wrapped, packaged or stored in plastic; Chewing gum	◦ Metastasize to gastrointestinal tissues or other organs and cause harmful effects ◦ Patients with increased intestinal permeability (e.g., IBD patients) may be more susceptible to microparticle absorption and potential damage.	/
	Zhang et al. (20)	26 healthy male students aged 18–25 from Beijing	8 days	Dietary intake	Packaged water, beverages, milk, dairy products, beer	/	◦ Moderate correlation exists between the intake of packaged water and the abundance of microplastics in feces.

"↑" means increased level or concentration; "↓" means decreased level or concentration.

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showed the overgrowth of Staphylococci is related to IBD. Correspondingly, mice treated with MPs exhibited a marked increase in Staphylococcus genus abundance and a decrease in Parabacteroides genus abundance ($P < 0.05$) (18). Li et al. observed increased numbers of gut microbial species, bacterial abundance, and flora diversity in mice treated with a high concentration of MPs (50). An increased abundance of Staphylococcus and Bacteroidetes alongside a decreased abundance of Parabacteroides and Firmicutes are also documented in this research (18). Likewise, Lu et al. found that MPs exposure decreased the abundances of Firmicutes and α-Proteobacteria in the feces of mice, and altered the variety and diversity of gut microbes (17).

To sum up, exposure to MPs, either in the blood or digestive tract, may accelerate the occurrence and development of IBD, and cause serious harm to the human body. Therefore, it is necessary to reduce the amount of dietary intake of MPs, such as reducing the use of bottled water and takeaway food (12, 23). The government also needs to pay attention to more efficient ways to degrade MPs. The potential benefits of reducing the pollution of MPs at sources still deserve further exploration.

Endocrine disruptors

Endocrine disruptors (EDCs) are chemicals that interfere with the hormones in the human body through the endocrine system (51). EDCs can affect the way the body reacts to hormones, and change the gut microenvironment, which may cause immune vulnerability, decreased tolerance to food antigens (52), change in gut microbiota, and the occurrence of IBD (53).

EDCs, including phthalates, flame retardants, pharmaceutical agents, and phenols like bisphenol-A (BPA), ethyl-paraben, and methylparaben (54), are massively produced and used for food containers, personal care products, and other plastic objects (51). EDCs enter the human body mainly through dietary ingestion, inhalation, and dermal uptake, and are mostly bioaccumulated in the adipose tissue (55, 56). Most EDCs are lipophilic; therefore, they can induce microbial dysbiosis, and activate xenobiotic pathways and associated genes, enzymes, and metabolites (Table 2).

BPA is the most common and well-known EDC. It is linked with the active type of IBD. The direct intake of BPA-packaged food rarely causes an immediate intestinal response, as it mainly affects humans through chronic exposure and accumulation. BPA level is significantly increased in the serum of patients with the active period of IBD, compared to patients in the remission period, and regarding the disease phenotype, serum BPA levels were higher in colonic vs. ileal forms (62). It can change the level of microbiota and gut metabolites, increase the incidence of IBD, and accelerate IBD development (57, 59, 62).

BPA may change the metabolic way of gut microbiota and increase the risk of IBD. Many bacteria, such as Bacteroides, Mollicutes, Prevotellaceae, Erysipelotrichaceae, Akkermansia, Methanobrevibacter, and Sutterella, whose proportions increase with exposure to BPA, are associated with different diseases, such as IBD and colorectal cancer (58). Another American research shows BPA can affect the gut microbial composition in an age- and sex-dependent manner (61). In the epidemiological studies, dietary exposure to BPA reduces gut microbial diversity, and in the gut microbiota of CD patients, the population of Proteobacteria increased while Clostridium decreased (59). In the plasma of UC patients, the amount of BPA exposure is observed to have correlation with the altered level of plasma proteins involved in lipid-related metabolic processes and cytokine response, indicating BPA may serve as a biomarker in severe UC (63). BPA exposure may also change the level of gut metabolites, reducing butyric acid and tryptophan, and increasing fecal calprotectin, which indicates a correlation between its exposure and the severity of IBD (57, 62).

Aside from BPA and its analogs, many other EDCs also relate to the development of IBD, such as phthalates, triclosan, and perfluoroalkyl substances (64–66). For example, exposure to the BPA substitute, fluorene-9-bisphenol, can alter gut metabolites in mice and deregulates the sugar and fatty acid metabolisms in the gut (60). Several epidemiological studies have demonstrated the correlation between other EDCs and IBD (62, 66, 67). In a study estimating paraben and phenol exposure, approximately one quarter (25.5%) of all participants in this sub-set reported symptoms of chronic diarrhea, which is a typical complication of IBD (67). Notably, in IBD-specific cases, higher mean concentrations of urinary 4-tert-octylphenol were associated with the increased prevalence of IBD (67).

To reduce the adverse health effects of BPA, in 2015, the European Food Safety Authority (EFSA) reduced the tolerable daily intake (TDI) of BPA from 50 μg/kg body weight (bw)/d to 4 μg/kg bw/d (68). The estimated recommended daily intake of BPA ranges from <1 to 5 μg/kg bw/d (69). To reduce EDCs exposure, it is essential to choose EDCs-free products and not heat food or store hot food in BPA containers marked with the recycling code 3 or 7 (70). From the present situation, it is important to strengthen the food safety policy, and use suitable materials in direct contact with food (56, 71). In the meantime, the necessary action is to reduce waste and use EDCs-free packaging, which may contribute to health improvement in food and reduce the risk of IBD.

Chemical herbicides

Chemical herbicides are herbicides that inhibit the growth of unwanted plants like residential weeds and invasive species (72). Commercially used chemical herbicides can cause substantial mortality of non-target plants and insects. They contaminate soil and reside in water, and may accumulate in the environment over time (Table 3). Glyphosate is the most popular herbicide in America. Its concentration continued to soar in the world, with the level rising from 2 to 430 μg/L in natural water (79). The rising level in water causes severe pollution and threatens food safety.

Chemical herbicides affect humans mainly through intestinal absorption, skin exposure, and inhalation (69). They may enter the body through contaminated food, like crops, fruits, and vegetables (80). In a German survey, the urinary glyphosate concentrations of 3 to 17-year-old children were above the limit of quantification of 0.1 μg/L, and the overall exposure of the young population may relate to their vegetarian diet or consumption of cereals, pulses, or vegetables (81). Much research hasproven the adverse effects of chemical herbicides on non-human beings, such as mice, zebrafish, and livestock.

TABLE 2 Adverse health effect of EDCs in inflammatory bowel disease.

Contaminant	References	Experiment model/Human group	Exposure time/dose	Route of exposure	Source of dietary intake	Impact on the gut	Other health risks
PCBs	Min et al. (57)	Female C57BL/6 mice	5 mg/kg for 6 weeks	Oral gavage	PCB153 dissolved in corn oil	◦ Deteriorate the health of gut microbiota.	◦ Induce obesity, lipid metabolism disorder, and dyslipidemia.
BPA	Javurek et al. (58)	California mice (Peromyscus californium)	BPA (50 mg/kg) or EE (0.1 ppb)	Diet from periconception through weaning	Artificial feeding	◦ Increase gut microbiota proportions ◦ ↑Bacteroides, Prevotellaceae, Sutterella and etc.	◦ Disrupt normal gut flora; ◦ Induce IBD and colorectal cancer.
	Lai et al. (59)	Sixteen 3-week-old male CD-1 mice	Bisphenol A (>98% purity) solution (120 mg/mL)	BPA-water feed	Drinking water	◦ Reduce gut microbiota diversity ◦ Induction of Helicobacteraceae ◦ Reduction of Firmicutes and Clostridia.	◦ Alter the gut microbiota; ◦ Induce IBD.
	Yin et al. (60)	Seven-week-old DSS-induced colitis mouse: ICR mice	BPA and its substitute BHPF, 2 weeks	Dietary exposure	Not mentioned	◦ Induce inflammatory responses ◦ Alter gut metabolites ◦ Deregulate sugar and fatty acid metabolisms.	◦ Change the metabolic way of gut microbiota in IBD.
	Diamante et al. (61)	Eight-week-old female and male C57BL/6J mice, eight breeding pairs	0.005 μg/μL BPA and 0.0015% ethanol;	Exposed during gestation, terminated after 19–21 days	Artificial feeding	◦ Affect fatty acid metabolism, and the gut microbial composition.	◦ Affect the gut microbial composition in an age- and sex-dependent manner.
	Linares et al. (62)	200 CD patients (140 in remission; 60 in active disease)	/	/	Food in EDCs containers; packaged food, seafood.	◦ BPA: higher in colonic vs. ileal forms ◦ Butyrate and tryptophan: lower in exposed patients.	◦ Increased in serum of patients with active disease vs. patients in IBD remission period.

"↑" means increased level or concentration; "↓" means decreased level or concentration.

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TABLE 3 Adverse health effect of chemical herbicides in inflammatory bowel disease.

Contaminant	References	Experiment model/human group	Exposure time/dose	Route of exposure	Source of dietary intake	Impact on the gut	Other health risks
Glyphosate	Tang et al. (73)	Eight-week-old male Sprague rats, weighing 180–220 g	0, 5, 50, and 500 mg/kg of weight glyphosate, for 35 days	Oral gavage	Artificial feeding	◦ ↓The relative of Lactobacillus in small intestine	◦ Induce inflammatory responses ◦ Alter gut microbial composition
	Suppa et al. (74)	Model species Daphnia	1 mg/L glyphosate, corresponding to the MCL of drinking water	Water surroundings	Not mentioned	◦ Induce DNA damage ◦ Alter the gut microbiota ◦ Interfere carbon and fat metabolism	◦ Dysbiosis of gut and its chronic inflammation
	Ding et al. (75)	Six-month-old healthy adult male zebrafish (Danio rerio, AB-wild type)	3.5 mg/L GLY concentration	Water surroundings	Not mentioned	◦ Alter the gut microbiota ◦ ↑Fusobacteria ↓Proteobacteria ◦ ↓Claudin-5, ZO-1, occludin	◦ Gut dysbiosis ◦ Destroy the intestinal mucosal barrier ◦ Enhanced intestinal permeability.
2,4-D	Tu et al. (76)	Specific-pathogen-free (SPF) 8-week-old C57BL/6 male mice	Low-dose 2,4-D exposure: 1 ppm 2,4-D water solution, 13 weeks	Feeding water solution daily	Artificial feeding	◦ Influence the homeostasis of gut microbiome ◦ ↓ Plasma acylcarnitine.	◦ Alter microbiome-related pathways ◦ Disturb gut-host homeostasis ◦ Increase risk of IBD
Combination of 2,4-D, dicamba and glyphosate	Mesnage et al. (77)	Wild-type mES cells (strain B4418)	The mixture of glyphosate, Dicamba and 2,4-D in water.	Water surroundings	/	◦ DNA damage ◦ Oxidative stress ◦ Unfolded protein response	◦ Carcinogenic effects
Propyzamide	Sanmarcro et al. (78)	Zebrafish (7 d.p.f.)	Immersed in TNBS-containing E3 ◦ Medium for 72 h.	Liquid surroundings	/	◦ Upregulate NF-κB-driven C/EBPβ proinflammatory gene expression ◦ Inhibit AHR signaling ◦ Boost intestinal inflammation	◦ Increase the risk of IBD and other gut diseases

"↑" means increased level or concentration; "↓" means decreased level or concentration.

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Herbicides, including glyphosate, 2,4-Dicholorophenoxy acetic acid (2,4-D), and dicamba, may damage the immune system and cause symptoms of IBD, like diarrhea, bowel inflammation, and maldigestion (77). Especially, people with weakened immune systems are more susceptible to chemical herbicide-related intestinal inflammation (82). Herbicides may disrupt the normal gut flora, and irritate the lining of the digestive tract, which can lead to gut inflammation and gastrointestinal diseases like IBD (83).

Glyphosate is an active ingredient in Roundup, the most widely-used herbicide (84, 85). Glyphosate exposure may be a critical environmental trigger in the etiology of diseases associated with gut microbiota dysbiosis, including IBD (73, 86, 87). Glyphosate can alter the structure of microbiota, interfere with the shikimate pathway in microbiomes, and hinder the production of aromatic amino acids (88, 89). And the dietary intake of aromatic amino acids may alleviate the antimicrobial effect of glyphosate (90). Glyphosate exposure induces inflammatory responses in the small intestine, and alters the gut microbial composition in rats, with the *Lactobacillus* significantly decreasing (73). It also destroys the intestinal mucosal barrier function, leading to dysbiosis and chronic inflammation.

Other essential elements in chemical herbicides include dicamba, 2,4-D, 2,4-Dinitrofluorobenzene, and 2,4,5-Trichlorophenoxyacetic acid. The sub-chronic low-dose 2,4-D exposure may influence gut microbiome homeostasis, significantly lower the acylcarnitine level, and decrease levels of plasma acylcarnitine (76). An IBD multi-omics research showed that the rising level of acylcarnitine is closely related to the development of IBD (91). But another explanation is that decreasing acylcarnitine levels can also produce toxicity, suggesting the perturbations in the fatty acid beta-oxidation pathway (92). Dicamba, 2,4-D, and glyphosate alone or in combination, account for genotoxicity in patients with gastrointestinal disorders, including DNA damage, oxidative stress, and unfolded protein response, which contributes to the development of IBD (77). Another herbicide, Propyzamide, can boost gut inflammation by upregulating NF-κB-driven C/EBPβ pro-inflammatory gene and inhibiting AHR signaling pathways, and further, inducing the development of IBD (78). Furthermore, an organic diet can reduce the human body's herbicide level, which is a possible solution to reduce herbicide residue and lower the risk of IBD.

Heavy metals

Heavy metals are naturally occurring elements with high atomic weight and density (93). They are also called trace elements, usually detected in trace concentrations (ppb range to <10 ppm) (94). The heavy metals we discuss are those accumulated in the food chain and are highly toxic to living organisms. Most of them come from natural resources and industry (95–98), including lead (Pb), manganese (Mn), arsenic (As), cadmium (Cd), mercury (Hg), and others. They are commonly used in people's daily life with widespread pollution (99, 100).

Human activities may increase the number of heavy metals residing in the environment, including metal processing, and the production of medical waste, plastic products, and electric wastes (101–104). Dietary is the main source of human exposure, with its detrimental effects including cardiovascular, neurological, reproductive, and intestinal disorders (105–108). Here we focus on five heavy metals: Pb, Mn, As, Cd, and Hg. They are not only ubiquitous in the environment, but also associated with gut microbiota dysbiosis and the severity of IBD (Table 4).

Lead

Most Pb emissions in life come from gasoline and enter the environment through burning exhausts. Pb enters the body mainly through dietary exposure, including food (65%) and water (20%) (117). For the Pb in the food, adults can absorb 10–15% of the ingested Pb, while children can absorb up to 50% through the gastrointestinal tract, which indicates children are more susceptible to Pb exposure.

Most Pb ingested accumulates in the kidneys, followed by the liver and other soft organs, like the heart and brain (94). When its dose is over 70 μg/dL, severe consequence happens (118). Pb can cause various disorders by inducing oxidative stress and breaking membrane integrity (119, 120). It also impairs gastrointestinal function and contributes to IBD pathogenies.

Epidemiological findings show that the level of Pb in IBD patients rises significantly (121). In CD patients, Pb in the scalp hair were significantly lower than that in healthy individuals, and its concentration in the serum is lower, which is consistent with the rising level in the tissue (122). In animals exposed to Pb, the amount of intestinal mucus increases, and the diversity and abundance of gut microbiota also change significantly (123, 124). Many metabolites related to glucolipid metabolism, amino acid metabolism, and nucleotide metabolism have changed after Pb exposure (109). Besides, Pb is highly toxic to Escherichia coli and Lactic acid bacteria, and long-term Pb exposure will induce chronic toxicity in a dose-dependent manner (11).

Developed countries have higher centration of Pb emission (125), which corresponds to the fact that IBD in developed countries shows a higher prevalence. Therefore, it is necessary for people in developed countries to take more precautions.

Manganese

Manganese (Mn) is the 12th most abundant element on the Earth. It exists mainly in the chemical oxidation state (126), and it is necessary for normal body functioning (94). It can activate various enzymes in the body and is indispensable for the development of intestinal immune functions (126).

The content of Mn in vegetables is higher than that of animal food (127). Seafood, chocolate, nuts, fruits, rice, and spices are also essential sources of Mn (127). The average concentration of Mn in human tissue is 1 mg/kg (126). Excessive dietary intake may lead to impaired intestinal immune function and over-activate oxidative stress, which is closely associated with the inflammation process in IBD patients.

The serum Mn concentration is different between healthy individuals and IBD patients, with the extent much greater in IBD patients (121, 128, 129). The concentration of Mn in blood is markedly higher in CD patients (121). But animal

TABLE 4 Adverse health effect of heavy metals in inflammatory bowel disease.

Contaminant	References	Experiment model/human group	Exposure time/dose	Route of exposure	Source of dietary intake	Impact on the gut	Other health risks
Lead	Yu et al. (11)	C57BL/6 mouse models	0.1, 0.5, and 1.0 g/L for 8 weeks	Dietary exposure	Drinking water	◦ ↓ Expression of tight junction proteins ◦ ↑ Abundance of Marvinbryantia and Ruminococcus ◦ ↓Abundance of Lactobacillus and Roseburia ◦ Induce gut dysbiosis	◦ Influence the metabolism of macronutrients, trace elements ◦ Neurodegenerative injury ◦ Inhibit CAT activity in kidney and GSH level in liver
	Xia et al. (109)	Male adult wide type AB strain zebrafish (*Danio rerio*)	Respective 10 and 30 μg/L for 7 days	/	/	◦ ↑ Gut mucus volume ◦ ↓The abundance of α-Proteobacteria ◦ ↑ The abundance of Firmicutes	◦ Induce hepatic metabolic disorder
Manganese	Choi et al. (110)	Wild type C57BL/6 mice aged 3–4 weeks	Mn: 0–0.5 ppm (deficient), 35–35.5 ppm (adequate), and 300-301 ppm (supplemented).	Dietary exposure	Diets	◦ Maintain the intestinal barrier ◦ ↑ Morbidity, weight loss, and colon damage ◦ ↑ Levels of inflammatory cytokines	/
	Mitchell et al. (111)	Male C57BL/6 mice	$MnCl_2$ (66 mg/kg)	i.p. injection	/	◦ Reduce chronic colitis	/
Arsenic	Zhong et al. (112)	1-day-old ducks	control group; low ATO group 4 mg/kg; high ATO group 8 mg/kg.	Oral administration and intubation	Drinking water	◦ Intestinal injury ◦ ↓ α diversity of intestinal flora ◦ Change bacterial composition ◦ ↓ Expression of intestinal barrier related proteins	◦ Liver inflammatory cell infiltration ◦ Vesicle steatosis ◦ ↑ Pro-inflammatory CKs (IFN-γ TNF-α IL-18 and IL-1β) in the liver
Cadmium	Breton et al. (113)	12-week-old female BALB/c mice	$CdCl_2$ (2.5 and 12.5 mg/kg) for 1, 4, or 6 weeks	Dietary exposure	Drinking water	◦ ↓ Epithelial permeability ◦ ↑ Oxidative defense mechanism ◦ ↓ Nf-κB and pro-inflammatory cytokine pathways ◦ Stimulate anti-oxidant pathways	/
Mercury	Zhao et al. (114)	Female Kunming mice	80 mg/L $HgCl_2$ for 90 days	Dietary exposure	Drinking water	↑ Faecalis, Helicobacter ◦ ↓ Halococcus and Bacillus ◦ intestinal injury	↑ Pro-apoptotic gene expression
	Zhao et al. (115)	Eight-week-old female mice	$HgCl_2$ (160 mg/L) for 3 days	Dietary intake	Drinking water	↓ Growth performance ◦ Induce oxidative stress ◦ ↑ Clostridium, Lactobacillus	/
	Seki et al. (116)	Female C57BL/6 mice	MeHg (5 mg/kg) for 14 days	Oral intubation	/	◦ Inhibit the growth of lactobacillus	◦ ↓ Gut bacteria after exposure to methylmercury ◦ Accelerated accumulation in the cerebellum, liver, and lungs

"↑" means increased level or concentration; "↓" means decreased level or concentration.

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experiments showed some contradictions. In Mn-deficient mice treated with DSS, the incidence of IBD increases, along with higher inflammatory cytokine levels, oxidative damage, and DNA damage, which indicates the level of Mn may be inversely correlated to the incidence of IBD.

Another two studies also indicate Mn's protective role in gut homeostasis. One shows that a decrease in absorption and accumulation of Mn will trigger the release of proinflammatory factors and exacerbate the severity of inflammation (111). Another study implies Mn can boost the immune system by enhancing the function of intestinal CD8+ T cells (130). Furthermore, some studies have indicated the protective role of Mn for IBD. A study on manganese metal-organic framework (Mn-MOF), is a practical application in the treatment of spontaneous IBD by scavenging ROS to relieve oxidative stress, and protect the intestinal barrier (131). The study on hollow MnO_2 ($hMnO_2$) carried out to achieve synergistic IBD therapy, is based on MnO_2, which has highlighted SOD-like and CAT-like activities (132, 133). According to the aforementioned viewpoints, the role of Mn for IBD is contradictory. For IBD patients, higher serum Mn concentration may be a protective reaction to avoid producing rapid and excessive ROS. The assumption can be consistent with the fact that IBD is a chronic disease. Experimenting to test the changes in serum Mn concentration with time is valuable. These exposure may increase the risk of IBD through oxidative stress and other mechanism in the gut. It is necessary to come up with the hypotheses to sort out the contradiction.

Arsenic

Arsenic (As) is an essential and poisonous substance commonly found in contaminated soils and water (134). It is also rich in fish and marine mollusks (135). The roots of crops and vegetables also contain high-concentration As (118).

According to World Health Organization, the permissible limit for As in drinking water is 10 μg/L (136). And if the exposure dose is over 50 μg/L, it can lead to gastrointestinal tract dysfunction and multiple organ disorder (136–139). Long-term exposure or high ingestion doses may increase the accumulated As in the gut (140).

Mounting evidence has revealed some intrinsic connections between As exposure and IBD. It enters the body through dietary intake, and metabolites to arsenic trioxide (ATO) in the gut, which is toxic to the gut microenvironment (112). It not only induces intestinal damage and liver inflammatory cell infiltration, but also reduces gut microbiota diversity (112, 141). In ATO exposure, the expression of intestinal barrier-related proteins, such as Claudin-1, MUC2, ZO-1, and occludin, significantly decreases, resulting in increased intestinal permeability (142, 143). However, with exposure to inorganic As, the expression of Claudin-1 reduces, resulting in increased permeability and intestinal barrier disruption. ATO can also activate inflammasome NLRP3, and induce a cascade effect of the LPS/TLR4/NF-κB signaling pathway, which exacerbates the inflammatory severity (112). However, ATO can inhibit NF-κB expression, increase procaspase-3, and induce caspase-3 activation leading to apoptosis to eliminate inflamed cells, which indicates the anti-inflammatory effect of ATO. Another study shows that ATO exposure can alleviate the inflammatory extent in DSS-induced IBD mice by increasing catalase and GSH levels to enhance antioxidants (144). In the epidemiological study, the level of serum As concentration is higher in CD patients compared to healthy adults (121). The causes for the distinct results may include the various metabolism of As in different species, exposure to the diverse form of As (inorganic As or ATO), the various regulation approaches, and the dose of As.

Cadmium

Cadmium (Cd) is mainly used as an anticorrosion agent, and it naturally occurs in ores (93). Cd can enter the human body through contaminated food and water *via* the gastrointestinal tract, inhalation, and dermal tissues (145). Food is the most important source of Cd exposure in the general non-smoking population, which indicates the risk of dietary exposure. Recent studies have found that Cd is highly enriched in some aquatic animals like zebrafish and crabs. Ingestion of Cd is highly related to gastrointestinal disturbances such as diarrhea, nausea, and abdominal pain. Meanwhile, its chronic exposure can increase risks concerning multiple organ dysfunction, bone deformation, and contribute to cancer cell progression (146).

Cd exposure can also significantly affect the gut microenvironment. It can perturb the diversity and abundance of gut microflora, especially decreasing the number of Firmicutes and γ-proteobacteria (145). In addition, Cd exposure also elevates the level of TNF-α, IFN-γ, and IL-17 in the colon (147). Additionally, Cd exposure can increase intestinal permeability through decreasing mRNA expressions of ZO-1, ZO-2, occludin, and claudin-1 in the jejunum and colon, accompanied by intestinal histological changes (148).

However, research also indicates the potential protective role of Cd. Cd may interfere with LPS signaling, particularly disrupting macrophage inflammation by inhibiting the NF-κB pathway in the gut, inhibiting the pro-inflammatory effect of M1 macrophage (149). Short-term exposure to Cd exacerbates the symptoms of acute DSS- and TNBS-induced colitis, while sub-chronic exposure to Cd significantly alleviates some symptoms in DSS-induced colitis and reduces the severity of colitis in a dose-dependent manner. Its potential mechanisms include reversible reduction in epithelial permeability, stimulation of anti-oxidant pathways, upregulation of oxidative defense mechanism, and downregulation of Nf-κB and pro-inflammatory cytokine pathways (113). Moreover, the study also implies that the outcomes of Cd exposure may vary as a function of dose and exposure time. Along with other common heavy metals like Mn, As, and Pb, the Cd concentration is markedly higher in CD patients (121). Thus, further studies concerning Cd exposure relationship with IBD are needed to clear out the Cd dose, exposure time, and the synthetic effect of Cd regulating in different pathways.

Mercury

Mercury (Hg) is a well-known component in medical apparatus like thermometers and other medical instruments

(93). The absorptivity of Hg is 8–15% in the gastrointestinal tract (104). Human's primary exposure to Hg is dietary intake (125, 150–152).

Hg can accumulate in untreated wastewater from factory and agricultural runoff, which directly contaminates the crops and fish. Correspondingly, methyl mercury, a chemical substance converted from Hg, is discovered to be highly enriched in vegetables and fish, which implies the ability of Hg to accumulate in the food chain.

The toxicity of Hg can induce multiple organ failures when the dose of Hg exceeds 10 μg/L in blood or 20 μg/L in urine, such as lung injury, intestinal damage, proteinuria, allergies, and chronic poisoning.

Exposure to Hg is closely associated with IBD. Methyl mercury can accumulate in organs, and change the composition of gut microbes (116). Moreover, dietary exposure to Hg affects the growth of mice, partly due to changed gut microbiota (114). Mice exposed to Hg have a decreased abundance of Bacteroidetes and Proteobacteria, and an increased abundance of Clostridium, Lactobacillus, Treponema, and Helicobacter in the gut (115). The toxicity of Hg may also contribute to the development of IBD (115). It can directly break the calcium homeostasis and activate multiple enzymes by affecting the electron transport chains in mitochondria, producing superfluous reactive oxygen species (ROS) (115, 153, 154). Besides, ROS promotes mitosis, polyploid aberration, and susceptibility to DNA damage in the gut (155–157). Epidemiological studies have also shown altered enzyme activity in people exposed to Hg (29).

In conclusion, diet exposure is the common exposure route for heavy metals. Along with the food chain, heavy metals enter the human body, generating adverse health effects by various mechanisms. Gut injury caused by heavy metals is highly associated with the occurrence and development of IBD. Heavy metals can alter the gut microbiota by increasing some flora and decreasing other flora, then causing gut dysbiosis. In addition, some uncommon impacts include damaged intestinal barrier function, increased levels of inflammation cytokines, oxidative stress, etc. Meanwhile, other organ dysfunction can occur due to heavy metals exposure and accumulation. As heavy metals tend to accumulate in fish (107), people with a disease or hypo-immunity should reduce their eating frequency. And IBD patients should avoid dietary exposure to heavy metals. For relevant authorities, they should supervise factories' proper treatment of sewage and sludge to reduce heavy metals accumulation in crops (158–160). Bioremediation can also work by changing pollutants into food and energy (161, 162). Some novel ways also focus on dealing with heavy metal contamination, such as Particle Capture Systems, soil displacement/isolation, and Soil-flow-electrode capacitive deionization.

Persistent organic pollutants

Persistent organic pollutants (POPs) are chemicals of global concern with the potential to persist in the environment. They can bio-accumulate and bio-magnify in ecosystems and threaten human health (71). POPs mainly include new pesticides, chemicals, and by-products of industrial production, which may lead to multiple effects on immune response and alter gut function (Table 5).

New pesticide

New pesticide is widely used to wipe out indoor and outdoor pests, such as imidacloprid, pyrethroids, and β-ketonitrile derivatives (169). It harms humans by taking the contaminated food and water (170), and causing intoxication through its accumulation in the food chain (171). Its exposure mainly includes the intake of vegetables, fruits, and grains. Among these, pesticide can residue more easily in grains. A food survey of Swedish adolescents showed that secondary school students who consumed grains had a higher exposure to pesticides than those who consumed vegetables and fruits, lending support to the findings (172).

Emerging links between pesticide residue and changes in the gut have emerged in recent years (173). Exposure to low-dose pesticide in diet seldom causes immediate health effect. Long-term exposure to chemicals in the pesticide can induce gut microbiota dysbiosis, alter the immune response in the gut, and contribute to the development of IBD (174, 175). Research on dietary exposure to chlorpyrifos, a widely used pesticide, suggests that dietary exposure can affect the population of immune-cell, induce inflammatory responses, and lead to severe tissue injury in DSS-induced colitis mice.

Another popular pesticide, imidacloprid (IMI), also adversely affects the gut microbiota (164, 165). IMI exposure can induce intestinal injury and oxidative stress in the gut of zebrafish (164). Additionally, it also results in a higher intestinal LPS level and the overexpression of inflammatory factors in the gut, as well as a rising level of the biochemical responses, transcriptome, and gut microbiota in the Pacific white shrimp. Human exposure to IMI is also observed in recent years. Currently, the maximum estimated daily intake of IMI [34.8 μg/kg bw/d] was lower than the chronic reference dose of IMI (57 μg/kg-bw/d) recommended by the United States Environmental Protection Agency (176). But IMI is already reported to have adverse effects on human semen quality parameters and the activation of macrophages in the body, which may increase the permeability of the intestine and impair the immune system (177, 178).

Chemicals and by-products of industrial production

Another type of POPs is chemicals and by-products derivate from industrial production (179). These pollutants include Polychlorinated biphenyls (PCBs), Polybrominated dibenzo-p-dioxins and furans (PBDD/Fs), 2,3,7,8-Tetrachlorodibenzo-p-dioxin (TCDD), etc. (167). They can accumulate in the environment and exert a long-term adverse effect on human health (180). The food chain and the food web are the primary pathways of human exposure (180). Human exposure to them in diets mainly includes ingesting contaminated food, like fruits, vegetables, and

TABLE 5 Adverse health effects of POPs in inflammatory bowel disease.

Study	References	Experiment model/human group	Exposure time/dose	Route of exposure	Source of dietary intake	Impact on the gut	Other health risks
New pesticide							
Chlorpyrifos (CPF)	Huang et al. (163)	Eight-week-old DSS-induced male C57BL/6 mice	AIN-93 diet at doses of 1, 2.5, or 5 mg/kg/day CPF,6 days	Diet exposure	Artificial feeding	◦ Affect immune-cell populations ◦ ↑Inflammatory responses	◦ Lead to severe tissue injury ◦ Exert adverse effect on the gut microenvironment
Imidacloprid (IMI)	Luo et al. (164)	Adult male zebrafish	IMI (100 and 1,000 μg/L) in water solution, 3 weeks	Water surroundings	Not mentioned	◦ ↑Superoxide dismutase and catalase (CAT) levels ◦ ↑LPS levels and inflammatory factors	◦ Cause intestinal barrier injury, oxidative stress, inflammatory response and gut microbiota dysbiosis
	Fu et al. (165)	The Pacific white shrimp *L. vannamei*	IMI (50, 100, 200, 400, and 800 μg/L) in water solution, 28 days	Water surroundings	Not mentioned	◦ Reshape the structure and interaction of gut microbiota ◦ ↑Gut pathogenic microbiota abundance ◦ Function disorders	◦ ↓Growth performance ◦ Gause tissue damage in shrimp ◦ Cause disorder of differential gene expression
Chemicals and by-products of industrial production							
TCDD, TCDF, and PCBs	Tian et al. (166)	Cecal contents isolated from 7-week-old C57BL/6 J male wild type mice	TCDD (0.6, 0.06 μM), TCDF and PCBs (6, 0.6 μM), 37°C for 4 h	Direct contact: incubation	/	◦ ↓Metabolic activity (dose-dependent) ◦ ↑Low nucleic acid (LNA) bacteria ◦ ↓High nucleic acid (HNA) bacteria	◦ Alter transcriptional and metabolic pathways in cecal bacterial mixtures ◦ Affect the physiological metabolism of individual bacteria
PBDD/**F**	Fernandes and Falandysz (167)	/	Some groups, particularly young children, may exceed the tolerable limit (2 pg TEQ/kg bw/week)	Present not only in soil, but also in plant foliage; Accumulate in the food chain	Mainly from dietary intakes: plant-based foods contain more PBDD/Fs	/	◦ Bind to the AhR, having carcinogenesis, immunotoxicity, enzyme induction and reproductive effects
TCDD	Li et al. (168)	C57BL/6 mice, 8–10 weeks old	0.1 and 10 μg/kg bw TCDD on embryonic day 0.5, ED 12.5, and post-natal 7 days	Oral gavage	Dissolved in dimethyl sulfoxide (DMSO) and diluted in olive oil	◦ Affect the structure and composition of the colonic microbiota ◦ Do not change the community diversity and richness ◦ Change the functional pathways of the colonic microbiota	/

"↑" means increased level or concentration; "↓" means decreased level or concentration.

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grains, and eating polluted meat, milk, eggs, and fish, which is closely associated with IBD.

Polychlorinated biphenyls

Polychlorinated biphenyls (PCBs) are synthetic organochlorine chemicals, which are mixtures of 209 different components (181). And PCBs are among the 12 initial POPs listed under the Stockholm Convention.

PCBs are mainly formed as by-products in manufacturing industries (182). They can reserve in soil and transfer to water surroundings, increasing the risk of human exposure *via* food chains (183, 184). Since PCBs are lipid-soluble, people who frequently eat animal fats can easily access PCBs (185). Contaminated meat and milk also show high concentrations of PCBs (186), and aquatic product consumption also increases the risk of PCBs exposure (181).

PCBs play a pro-inflammatory role in various diseases, which adversely affect IBD. They can induce oxidative stress by uncoupling CYP1A1 dose-dependently, and disrupt the normal endothelial barrier function (110, 187). Meanwhile, PCBs induce proinflammatory factors like IL-6 and vascular adhesion molecules such as VCAM-1, and then activate the NF-κB pathway (187–189). The expression of these molecules facilitates the recognition and migration of leukocytes, which are critical events of inflammatory responses (187). When exposed to PCBs, hosts can show disorders of gut microbiota, with reduced gut microbial diversity and variety (190, 191). In mice exposed to PCBs, the amount of Bacteroidales, Erysipelotrichales, Lactobacillales, Bifidobacteriales, Phyla Proteobacteria, Actinobacteria, Saccharibacteria, Deferribacteres, Firmicutes, and Verrucomicrobia increases significantly, while the level of Bacteroidetes decreases. Research in humans also shows that exposure to PCBs may interfere with the DNA hypomethylation of peripheral blood monocytes, inducing chronic inflammation (192).

PBDD/Fs and TCDD

Apart from PCBs, other POPs such as PBDD/Fs and TCDD are also related to IBD (193). These organics are by-products of industry, which are classified into unintentional POPs (194). Their primary exposure pathway is dietary intake.

Plant-based food is reported to show higher PBDD/F, and the overly dietary intakes of PBDD/F suggest some population groups, particularly young children, will exceed the tolerable weekly intake (2 pg TEQ/kg bw/week) (167). The metabolic mechanism of PBDD includes causing oxidative stress, apoptosis, and cell damage. It can induce gut inflammation and dysbiosis of the gut microenvironment, leading to IBD development.

TCDD is the most potent chemical carcinogen evaluated by the US Environmental Protection Agency (195). It has a long half-life of 5–10 years in humans, due to its high lipophilicity and low metabolism (195). Most TCDD released into the atmosphere eventually settles onto the plant, soil, and water surfaces. After being taken, it accumulates in blood serum and adipose tissue, which leads to further damage in the body (195). An animal experiment showed that maternal exposure to TCDD suppresses the differentiation of Type 3 innate lymphoid cells (ILC3s) in the offspring, and distinctly affects colonic ILC3 function (196). Since ILC3s play a significant role in the mucosal immune response in the pathogenesis of IBD, there is a close connection between TCDD exposure and the occurrence and development of IBD (197). Moreover, TCDD can impact the gut microbiota and metabolic pathways, such as upregulating harmful bacteria and downregulating beneficial bacteria (168).

In sum, people are exposed to POPs primarily through dietary intake. POPs can alter transcriptional and metabolic pathways in cecal bacterial mixtures, modify gut microbiota-host homeostasis, and affect the metabolism of individual bacteria *in vitro* (166). POPs can also alter the microbial community structure and metabolic activities, leading to host disorders (198). Some POPs can increase the amount of Proteus and proportion of Firmicutes/Bacteroidetes, and increase the synthesis of short-chain fatty acid, which is related to the inflammatory changes of IBD (191). Epidemiological studies also present the adverse health effects of POPs. The interplays between POPs and gut microbiota lead to intestinal inflammatory changes and resultant toxicity (198). That is to say, the variations in the microbial communities partially indicate the body's exposure to these pollutants. Therefore, it is essential to formulate some daily dietary interventions such as prebiotics, probiotics, or symbiotics, which could impede or alleviate detrimental impacts induced by exposure to POPs.

Conclusion

Mounting evidence of the underlying hazards of emerging contaminant exposures in IBD arouses increasing attention. However, the correlation of timing and frequency of such contaminant exposures to IBD incidence and disease phenotype was not specified. Environmental exposure may contribute to the pathogenesis of IBD through diet intake and the metabolic mechanism in the body. The currently accepted pathogenesis of IBD includes the interplay between genetic susceptibility and environmental factors, as well as the gut microbiome and the immune system.

The further relationship between these emerging contaminants and the risk of IBD deserves everyone's attention. Currently, these contaminants mainly exert long-term adverse effects by accumulating in the body and inducing chronic inflammation. Research in this field should focus more on the direct relationship and mechanism of these contaminants and the health of the human gut. And more epidemiological research about new emerging contaminants and their adverse health effects is needed.

In this review, we have summarized the standard ways of exposure and inclusion of emerging contaminants, and their adverse effects on IBD patients through various underlying mechanisms (Figure 2). Exposure to these pollutants will increase the risk of IBD in healthy individuals. And people with IBD should pay special attention to preventing daily exposure to these contaminants, as they may cause adverse health effects regardless of age and exposure time. Exposure to these new emerging contaminants may increase the risk of IBD and accelerate the process of IBD. Therefore, understanding the role of these contaminants, including how they enter the body,

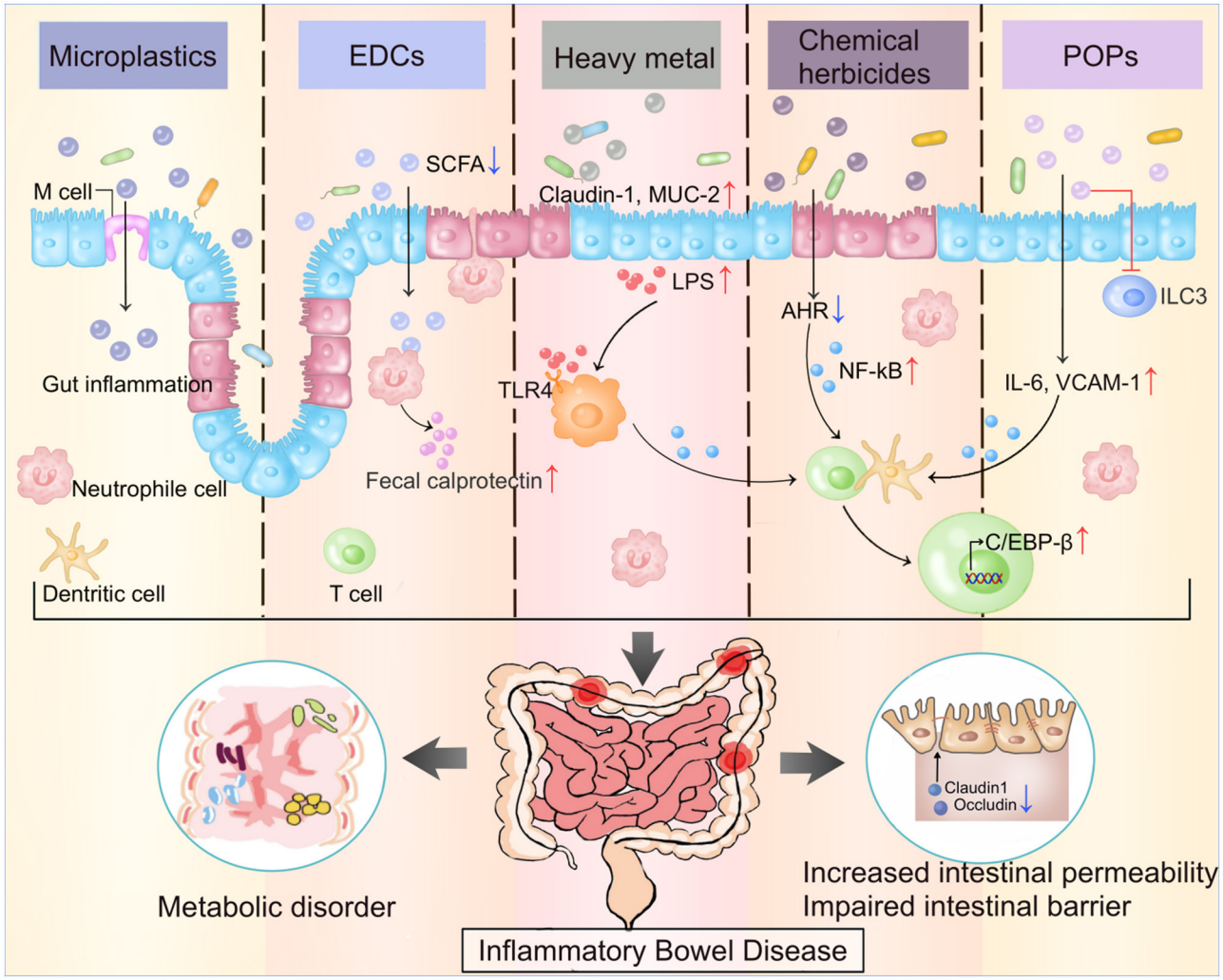

FIGURE 2
The main ECs and their impacts on gut microenvironment. ECs damage the intestine by regulating the intensity of immune response, releasing proinflammatory factors, impairing the intestinal barrier, and increasing the intestinal permeability through various mechanism. The figure illustrates the potential mechanism of five ECs mentioned in the review. ECs, Emerging contaminants; EDCs, Endocrine-disrupting chemicals; POPs, Persistent organic pollutants; SCFA, Short-chain fatty acid; TLR4, Toll-like receptors 4; ILC-3, Innate lymphoid cells; VCAM 1, Vascular cell adhesion molecule 1.

how they induce immune-related reactions, and how they affect certain inflammatory diseases like IBD, will enable the more comprehensive formation of policies concerning the prevention and control, and will reduce medical expenses and burdens on the families and countries.

Author contributions

XC: conceptualization, design, draft writing, and writing—review and editing. SW: design, draft writing, review, and writing—review and editing. XM: draft writing, review, and visualization. XX, AZ, YM, XY, and SP: methodology and review. SY: review and visualization. JC: methodology and writing—review and editing. XW: conceptualization, funding acquisition, and writing—review and editing. MD: conceptualization, design, methodology, and writing—review and editing. All authors contributed to the article and approved the submitted version.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Latency period of aristolochic acid-induced upper urinary tract urothelial carcinoma

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Purpose: Aristolochic acid (AA) is a carcinogen in upper urinary tract urothelial carcinoma (UTUC). This study investigated the latency period between AA exposure and UTUC development.

Materials and methods: This population-based cohort study was designed using record linkage of the National Health Insurance Research Database (NHIRD), Taiwan Cancer Registry Dataset, and cause-of-death data in Taiwan. Those aged 40–79 years were enrolled in this study. Patients who died or had renal insufficiency or UTUC before 2005 were excluded. The doses of AA exposure and rates of comorbidities between 2000 and 2005 were obtained. The Cox proportion hazard model was used to estimate the risk of UTUC between 2005 and 2016. In addition, the Cox model with time-varying coefficient of AA was used to measure the latency period of UTUC.

Results: Of the 752,232 participants enrolled from the NHIRD, 520,871 (68.29%), 210,447 (27.59%), and 31,415 (4.12%) were exposed to cumulative AA doses of 0–1 mg, 1–150 mg, and >150 mg, respectively. A total of 1,147 (0.15%) patients were diagnosed with UTUC between 2005 and 2016. The latency periods of UTUC in middle-aged (40–59 years old) men with cumulative AA doses of 1–150 mg and middle-aged women with cumulative AA doses of 1–150 mg and >150 mg were 8, 9, and 7 years, respectively. Among the aged (60–79 years) individuals, no time-varying effect was observed, and the latency period could not be measured.

Conclusion: A decreased risk of UTUC was observed after the ban on AA in Taiwan, especially in middle-aged women with moderate to high doses of AA exposure and men with moderate doses of AA exposure. The latency period of UTUC varies with age, the dose of AA exposure, and sex.

KEYWORDS

aristolochic acid, upper urinary tract urothelial carcinoma (UTUC), latency period, time-varying effect, slope-change model

1. Introduction

Aristolochic acid (AA) was identified as a nephrotoxin based on the unusual finding that a cluster of young Belgian women presented with rapidly progressive renal failure after consuming a slimming herbal remedy containing AA (1, 2). Furthermore, Nortier et al. (3) reported that 95% of these women were observed to have either urothelial carcinoma (UC) or urothelial dysplasia in their native kidneys. Therefore, AA was not a nephrotoxin but also a carcinogen in this Belgium cohort. In some villages in Balkan, the population

has consumed bread made from flour contaminated with seeds of *Aristolochia clematitis* for decades (4). Based on clinical and molecular evidence, AA was also demonstrated to be responsible for endemic nephropathy and upper urinary tract urothelial carcinoma (UTUC) in Balkan patients. In addition, nationwide exposure to AA from Chinese herbal remedies was observed in Taiwan. Between 1997 and 2003, at least 39% of Taiwanese people ingested AA-containing herbal products (AA-CHP) (5). AA-induced UTUC was confirmed in a considerable portion of UTUC patients according to the identification of both aristolactam-DNA adducts in the renal cortex and the signature mutation pattern in UTUC tumors in Taiwan (6). Using the nationwide health insurance database, those who took more AA-CHP were at higher risk of both UC (7) and end-stage renal disease (8).

Since the association between AA, renal failure, and UTUC has been confirmed, several countries have banned the prescription of AA-CHP, including Guangfangji (*Radix Aristolochiae Fangchi*), Guanmutong (*Caulis aristolochiae Manshuriensis*), Madouling (*Fructus Aristolochia*), Qingmuxiang (*Radix Aristolochiae*), Tianxianteng (*Herba Aristolochia*), and Zhushalian (*Radix Aristolochiae Tuberosa*) (9–11). This action limited the exposure of AA from major sources and might reduce the development of either renal insufficiency or UTUC in the population. Although the health authority in Taiwan banned the use of the abovementioned major types of AA-CHP in 2003, the incidence of UTUC continued to increase until 2010 (12). It reflects the carcinogenic effects of AA may persist long after the discontinuation of AA usage. However, there are no studies that represent the latency period of AA in the development of UTUC.

Wang et al. identified that the prescription frequency of AA-CHP in Taiwan for patients with end-stage renal disease decreased to nearly zero after 2005 (13). The combination of nationwide exposure to AA and the clear stop date of AA use makes Taiwan an ideal model to investigate the latency period. Therefore, this study was designed to explore the latency period in populations exposed to varied doses of AA using a nationwide registry database.

2. Materials and methods

This study was reviewed and approved by the Research Ethics Committee at National Taiwan University Hospital (201912201W). A population-based retrospective cohort study was designed using record linkage of the National Health Insurance Research Database (NHIRD) (14), the Taiwan Cancer Registry Dataset (TCRD) (15, 16), and cause-of-death data. The datasets used were retrieved from the Health and Welfare Data Science Center of the Ministry of Health and Welfare in Taiwan. A flowchart for the recruitment of study participants is shown in Figure 1. The study period was from January 2000 to December 2016. We conducted stratified random sampling according to sex, age, and administration divisions. Sex was divided into men and women. Age was divided into 20 groups at 5-year intervals from age 5 to 85. The region is classified into 6 categories based on the separate Divisions of the National Health Insurance Administration. A total of 240 subgroups ($2 \times 20 \times 6$) were created, and then the number of samples was drawn from each subgroup based on random sampling. A total of 2,000,120 participants were sampled from the 2005 registry for 20,926,602 beneficiaries of the NHIRD in Taiwan. In addition, we collected data including the status of AA exposure and comorbidities between 2000 and 2005, and enrolled study subjects aged between 40 and 79 years in 2005 (when the follow-up started). Subjects aged 80 years or older were not enrolled because of the conflict of competing risks of death. Participants who died or had been diagnosed with UTUC before 2005 were excluded from the study because of the unclear time-sequence between AA and UTUC.

Since 2003, the Ministry of Health and Welfare in Taiwan has banned the following herbs containing AA: (7, 12) Madouling (*Fructus Aristolochiae*), Tianxianteng (*Caulis Aristolochiae*), Guanmutong (*Aristolochia Manshuriensis*), Guangfangji (*Aristolochia Fangchi*), and Qingmuxiang (*Radix Aristolochiae*). A total of 923 Chinese herbal remedies containing these herbs were evaluated in this study. The study participants who used these AA-CHPs were identified from the NHIRD. The original amount of herbs and the total dose of AA for each AA-CHPs were determined using the detailed composition of AA-CHPs obtained from the Department of Chinese Medicine and Pharmacy, Ministry of Health and Welfare in Taiwan. The estimated average doses of AA per 1 g of Guanmutong, Guangfangji, Madouling, Qingmuxiang, and Tianxianteng are 2.59, 2.04, 0.63, 0.009, and 0.026, respectively (17–19). We calculated the estimated cumulative dose of AA exposure (CDAE) from 2000 to 2005 for each subject and categorized them into a reference group (0–1 mg) and two exposure groups (1–150 mg and >150 mg) for the comparability among the published studies (7).

Potential confounding factors, including demographic characteristics, living in arsenic-endemic areas or urbanized areas, and comorbidities, were considered and adjusted in the study. Study subjects were categorized into four age groups (40–49, 50–59, 60–69, and 70–79 in 2005 when the follow-up started). Arsenics was confirmed to be a carcinogen for urothelial carcinoma (20) and presented as a confounding factor in the identification of UTUC in Taiwan. A total of 21 townships in Taiwan (Supplementary Figure 1) where the concentration of arsenics in well water exceeded 0.35 ppm were defined as arsenic-endemic areas (21, 22). A total of 27 townships in Taiwan with a population size exceeding 20,000 and population density exceeding 300 per km^2 were defined as urbanized areas (Supplementary Figure 1). Comorbidities were collected from the NHIRD and defined by the following diagnoses: renal insufficiency (ICD-9-CM code: 585), hepatitis (ICD-9-CM code: 070), coronary artery disease (ICD-9-CM code: 414), chronic obstructive pulmonary disease (ICD-9-CM codes: 490–496), diabetes mellitus (ICD-9-CM codes: 249–250), and hyperlipidemia (ICD-9-CM code: 272). The UTUC cases (ICO-O-FT: 189.1 and 189.2; ICD-10-CM: C65.1–C65.9 and C66.1–C66.9) from 2005 to 2016 were collected from the TCRD.

Characteristics of study participants were presented as categorical variables and compared using the chi-square test and Cochran–Armitage test for trend. Multivariable analyses of UTUC risk between 2005 and 2016 were conducted using the Cox proportional hazards model. Because the hazard of UTUC might change after the discontinuation of AA, Cox models with a time-varying coefficient (23) of CDAE were constructed to measure

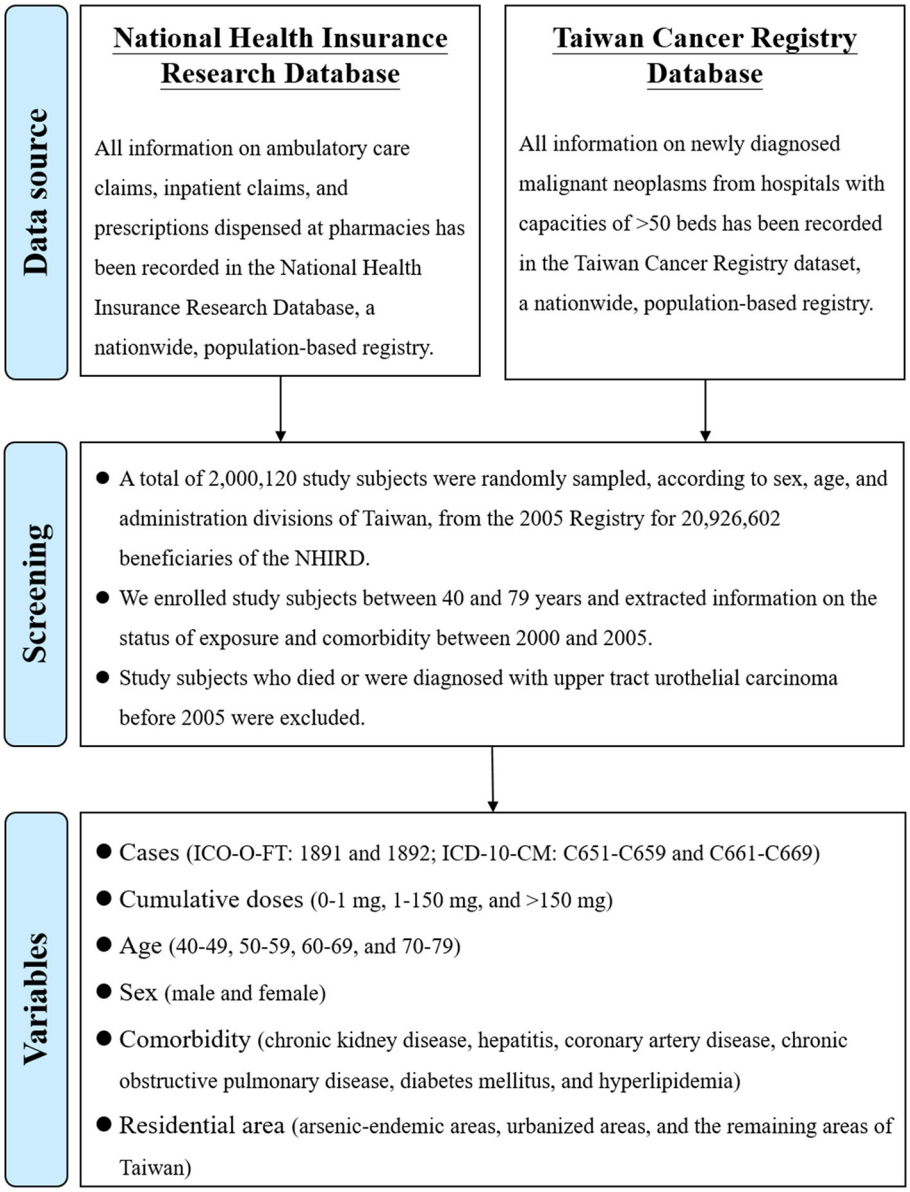

FIGURE 1
Flowchart for recruitment of study subjects.

the latency period. Specifically, we assumed a piecewise constant hazard function for the hazard ratios of CDAE, segmenting the calendar year into four consecutive periods (2005–2007, 2008–2010, 2011–2013, and 2014–2016) to depict the temporal change in adjusted hazard ratios (aHR). Besides, assuming that prohibiting the use of AA-CHPs would affect the trend in the UTUC occurrence after h years, we considered the slope-change model as follows:

$$\lambda\left(t|X,Z;\beta_0,\beta_1,\gamma\right) = \lambda_0\left(t\right) \times \exp\left(f\left(\beta_0,\beta_1,t\right)^T X + \gamma^T Z\right) \text{ and}$$
$$f\left(\beta_0,\beta_1,\ t\right) = \beta_0 + \beta_1 \times (t-h+1) \times I(t \geq h-\ 1),$$

where X is a vector of exposure groups, Z is a vector of confounding factors, and $I(.)$ is an indicator function. The model parameters include $\lambda_0\left(t\right)$ is the baseline hazard function, β_0 is the baseline effect of exposure, β_1 is the slope-change effect of exposure after h years, and γ is the effect of confounding factors. We regarded the "h" as the latency period. Subsequently, we performed model selection using the lowest Akaike information criterion (AIC) to determine the latency period. The latency period was defined as the duration between the exposure to AA and the identification of UTUC. The candidates included 11 slope-change models ($h = 1, 2, \ldots, 11$) and a proportional hazard model. Statistical interactions were examined using the likelihood ratio test. Statistical significance was set at p-value < 0.05. All analyses were performed using SAS version 9.4 and R version 3.5.2.

TABLE 1 Characteristics of study participants from the National Health Insurance Research Database registry between 2000 and 2005.

	Estimated cumulative doses of aristolochic acid from 2000 to 2005			
	0–1 mg	1–150 mg	>150 mg	
	N = 520,871	*N* = 210,447	*N* = 31,415	*P*-value
Sex, No. (%)				<0.001
Men	280,663 (73.48)	88,363 (23.14)	12,916 (3.38)	
Women	240,208 (63.08)	122,084 (32.06)	18,499 (4.86)	
Age, No. (%)				<0.001
40–49 years	219,718 (67.59)	91,263 (28.07)	14,113 (4.34)	
50–59 years	146,099 (67.67)	60,845 (28.18)	8,957 (4.15)	
60–69 years	89,431 (68.66)	35,664 (27.38)	5,165 (3.97)	
70–79 years	65,623 (71.74)	22,675 (24.79)	3,180 (3.48)	
Urbanized area, No. (%)				<0.001
No	468,301 (67.80)	193,489 (28.01)	28,969 (4.19)	
Yes	52,570 (73.04)	16,958 (23.56)	2,446 (3.40)	
Arsenic-endemic area, No. (%)				<0.001
No	511,537 (68.20)	207,501 (27.67)	31,001 (4.13)	
Yes	9,334 (73.53)	2,946 (23.21)	414 (3.26)	
Comorbidity, No. (%)				<0.001
No	499,312 (70.26)	187,040 (26.32)	24,332 (3.42)	
Chronic kidney disease	8,613 (69.26)	3,184 (25.61)	638 (5.13)	
Hepatitis	480 (24.01)	1,004 (50.23)	515 (25.76)	
Coronary artery disease	181 (31.87)	287 (50.53)	100 (17.61)	
Chronic obstructive pulmonary disease	8,259 (31.81)	13,555 (52.21)	4,149 (15.98)	
Hyperlipidemia	1,580 (32.57)	2,397 (49.41)	874 (18.02)	
Diabetes mellitus	2,446 (39.24)	2,980 (47.81)	807 (12.95)	

3. Results

3.1. Patient population

A total of 752,232 participants aged 40–79 years were enrolled in this study from the NHIRD. The demographics of the study participants stratified by the CDAE between 2000 and 2005 are shown in Table 1. In this cohort, 520,871 (68.29%), 210,447 (27.59%), and 31,415 (4.12%) were exposed to CDAE of 0–1 mg, 1–150 mg, and >150 mg, respectively. More women (36.92%) had a CDAE of >1 mg than men (26.52%, $p < 0.001$). With increasing age, the proportion of participants with CDAE >1 mg reduced gradually (40–49 years: 32.41%; 50–59 years: 32.33%; 60–69 years: 31.35%; 70–79 years: 28.27%; p for trend < 0.01). Fewer study subjects had CDAE of >1 mg in the urbanized areas (26.96%) than in the non-urbanized areas (32.20%, $p < 0.001$). Fewer study participants had a CDAE of >1 mg in arsenic-endemic areas (26.47%) than in non-arsenic-endemic areas (31.80%; $p < 0.001$). Subjects with comorbidities had a significantly higher CDAE than those without comorbidities ($p < 0.001$). Among people with comorbidities, 58.58 and 13.61% took CDAE of >1 and >150 mg, respectively, during the study period.

3.2. Univariable and multivariable analysis of UTUC risk

Between 2005 and 2016, 1,147 UTUC patients (0.15%) were diagnosed in this study cohort. Multivariable analysis was conducted based on the Cox proportional hazards model and presented in Table 2. The aHRs for all exposure groups (CDAE: 1–150 mg: aHR = 1.50, 95% CI 1.32–1.70; >150 mg: aHR = 2.21, 95% CI 1.77–2.77) were significantly higher than that in the reference group (0–1 mg). In addition, an apparent dose-response relationship (p for trend < 0.01) was observed between the dose of exposure and the risk of UTUC. There was no significant difference in the UTUC risk between men and women (women: aHR = 1.09, 95% CI 0.97–1.22) and between living in the urbanized areas and non-urbanized areas (urbanized areas: aHR = 1.06, 95% CI 0.87–1.28). Older participants had a higher risk of UTUC than younger participants (reference: 40–49 years; 50–59 years: aHR = 3.07, 95% CI 2.49–3.77; 60–69 years: aHR = 7.91, 95% CI 6.49–9.62; 70–79 years: aHR = 10.24, 95% CI 8.34–12.57). The participants living in arsenic-endemic areas were at a higher risk (aHR = 1.53, 95% CI 1.07–2.17) of UTUC

TABLE 2 Adjusted hazard ratios for the occurrence of upper tract urothelial carcinoma between 2005 and 2016, assuming proportional hazard and time varying effect of cumulative doses of aristolochic acid.

Model type	No. of subjects	UTUC cases	Proportional hazard model		Slope-change model	
			Adjusted HR	95% CI	Adjusted HR	95% CI
Cumulative doses of aristolochic acid						
Baseline						
0–1 mg	520,871	660	1.00	–	1.00	–
1–150 mg	210,447	398	1.50	(1.32–1.70)	1.48	(1.26–1.73)
>150 mg	31,415	89	2.21	(1.77–2.77)	2.52	(1.92–3.31)
Slope change in 2012						
0–1 mg	–	–	–	–	1.00	–
1–150 mg	–	–	–	–	1.00	(0.94–1.09)
>150 mg	–	–	–	–	0.89	(0.77–0.99)
Sex						
Men	381,942	514	1.00	–	1.00	–
Women	380,791	633	1.09	(0.97–1.22)	1.09	(0.97–1.23)
Age						
40–49 years	325,094	132	1.00	–	1.00	–
50–59 years	215,901	276	3.07	(2.49–3.77)	3.06	(2.49–3.77)
60–69 years	130,260	424	7.91	(6.49–9.62)	7.83	(6.34–9.53)
70–79 years	91,478	315	10.24	(8.34–12.57)	9.96	(8.12–12.22)
Urbanized area						
No	690,759	1,033	1.00	–	1.00	–
Yes	71,974	114	1.06	(0.87–1.28)	1.06	(0.87–1.29)
Arsenic-endemic area						
No	750,039	1,115	1.00		1.00	
Yes	12,694	32	1.53	(1.07–2.17)	1.52	(1.07–2.17)
Comorbidity						
No	710,684	896	1.00	–	1.00	–
Chronic kidney disease	12,435	174	10.18	(8.63–12.00)	9.85	(8.36–11.61)
Other comorbidities	39,614	77	1.09	(0.86–1.37)	1.09	(0.86–1.39)

compared to those in non-arsenic-endemic areas. The participants with chronic kidney disease were at higher risk (aHR = 10.18, 95% CI 8.63–12.00) of UTUC compared to those without chronic kidney disease. There was no difference in UTUC risk between the participants with (aHR = 1.09, 95% CI 0.86–1.37) and without other comorbidities.

3.3. Latency period in the whole population

To evaluate the latency period between AA exposure and the identification of UTUC, the slope-change model and the piecewise constant hazard model were constructed and presented in Tables 2, 3, respectively. Among subjects who took CDAE of 1–150 mg, no significant trend was observed in aHRs between 2005 and 2016 (Figure 2A). In contrast, among subjects with CDAE of >150 mg, the aHR increased from 1.96 (95% CI 1.22–3.16) in 2005–2007 to 3.02 (95% CI 2.01–4.53) in 2008–2010, and then gradually decreased to 1.76 (95% CI 1.09–2.83) in 2014–2016. The slope-change model showed that the aHR kept at 2.52 (95% CI 1.92–3.31) and decreased in 2011 (the 7th year after the ban on AA-CHPs; Figure 2B). The aHR will reach 1.00 in 2018–2019 based on the speed of 11% (95% CI 1–23%) per year.

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TABLE 3 Adjusted hazard ratios for the occurrence of upper tract urothelial carcinoma between 2005 and 2016, based on the piecewise constant hazard model.

	1–150 mg of aristolochic acid		>150 mg of aristolochic acid	
	Adjusted HR	95% CI	Adjusted HR	95% CI
The whole population				
2005–2007	1.43	(1.10–1.85)	1.96	(1.22–3.16)
2008–2010	1.45	(1.11–1.89)	3.02	(2.01–4.53)
2011–2013	1.70	(1.35–2.16)	2.20	(1.42–3.41)
2014–2016	1.41	(1.11–1.80)	1.76	(1.09–2.83)
Men aged 40–59 years				
2005–2007	0.91	(0.41–2.01)	1.51	(0.36–6.39)
2008–2010	2.59	(1.55–4.33)	2.57	(0.90–7.31)
2011–2013	1.48	(0.81–2.69)	1.25	(0.30–5.25)
2014–2016	1.06	(0.58–1.95)	1.30	(0.48–2.49)
Women aged 40–59 years				
2005–2007	3.30	(1.31–8.34)	4.66	(1.61–13.46)
2008–2010	1.54	(0.63–3.79)	3.73	(1.65–8.41)
2011–2013	1.90	(1.00–3.60)	1.84	(0.71–4.78)
2014–2016	0.77	(0.36–1.68)	2.09	(0.92–4.73)
Men aged 60–79 years				
2005–2007	1.34	(0.82–2.21)	1.51	(0.55–4.19)
2008–2010	1.13	(0.66–1.93)	2.71	(1.23–5.99)
2011–2013	1.98	(1.26–3.12)	3.24	(1.53–6.88)
2014–2016	1.69	(0.99–2.91)	1.58	(0.49–5.12)
Women aged 60–79 years				
2005–2007	1.38	(0.94–2.02)	1.84	(0.88–3.84)
2008–2010	1.19	(0.73–1.94)	3.12	(1.52–6.39)
2011–2013	1.70	(1.16–2.48)	2.11	(1.00–4.42)
2014–2016	1.46	(0.99–2.15)	2.25	(1.11–4.53)

3.4. Latency period in the middle-aged population

The latency period varied with sex, age, and CDAE (p for interaction = 0.04). Among middle-aged men (40–59 years) who took CDAE of 1–150 mg, the aHR increased from 0.91 (95% CI 0.41–2.01) in 2005–2007 to 2.59 (95% CI 1.55–4.33) in 2008–2010, and then gradually decreased to 1.06 (95% CI 0.58–1.95) in 2014–2016. The slope-change model showed that the aHR kept at 1.77 (95% CI 1.08–11.25), started to decrease in 2012 (the 8th year after the ban on AA-CHPs), and reached 1.00 in 2014–2015 based on the speed of 19% (95% CI −2–36%) per year (Figure 3A). In contrast, middle-aged men with a CDAE of more than 150 mg, the aHR increased from 1.51 (95% CI 0.36–6.39) in 2005–2007 to 2.57 (95% CI 0.90–7.31) in 2008–2010 and then declined to 1.30 (95% CI 0.48–2.49) in 2014–2016. The slope-change model showed that the aHR kept at 2.10 (95% CI 0.97–3.24) and decreased in 2009 (the 5th year after the ban on AA-CHPs; Figure 3B). The aHR decreased by 6% (95% CI −7–14%) per year and will reach 1.00 in 2020–2021.

In middle-aged women (40–59 years) with a CDAE of 1–150 mg, the aHRs substantially decreased from 3.30 (95% CI 1.31–8.34) in 2005–2007 to 0.77 (95% CI 0.36–1.68) in 2014–2016. The slope-change model showed that the aHR kept at 2.15 (95% CI 1.32–3.50), started to decrease in 2013 (the 9th year after the ban on AA-CHPs), and reached 1.00 in 2015–2016 based on the speed of 28% (95% CI −2–48%) per year (Figure 3C). Among middle-aged women having CDAE of more than 150 mg, the aHRs significantly decreased from 4.66 (95% CI 1.61–13.46) in 2005–2007 to 2.09 (95% CI 0.92–4.73) in 2014–2016. The slope-change model revealed that the aHR kept at 3.92 (95% CI 1.55–9.87), started to decrease in 2011 (the 7th year after the ban on AA-CHPs), and reached 1.00 in 2016 based on the speed of 22% (95% CI 1–38%) per year (Figure 3D).

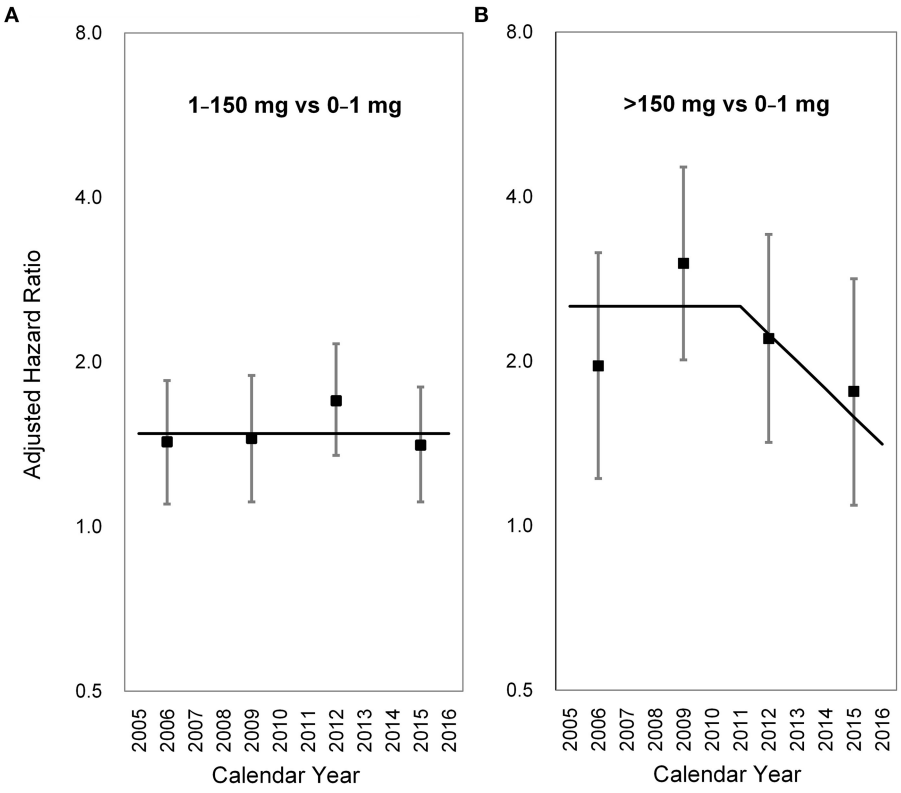

FIGURE 2
Long-term trends in the adjusted hazard ratio for upper tract urothelial carcinoma occurrence. **(A)** 1–150 mg vs. 0–1 mg, **(B)** >150 mg vs. 0–1 mg. Square points: adjusted hazard ratios from the piecewise constant hazard model. Vertical lines: 95% confidence intervals of the adjusted hazard ratios from the piecewise constant hazard mode. Solid lines: adjusted hazard ratios from the slope-change model.

3.5. Latency period in the older population

In the aged population (60 and 79 years), the aHRs among different time periods were not obvious in the subgroups of participants stratified by either sex or CDAE (Table 3, Figure 3). The selected proportional hazard models showed that the aHRs between 2005 and 2016 were 1.52 (95% CI 1.18–1.95; Figure 3E), 2.29 (95% CI 1.46–3.59; Figure 3F), 1.45 (95% CI 1.18–1.77; Figure 3G), and 2.26 (95% CI 1.57–3.26; Figure 3H).

4. Discussion

By investigating a nationwide cohort, our study revealed that the risk of UTUC decreased in the 7th year after the ban on AA in Taiwan. The latency period between the latest AA exposure and the identification of UTUC varied with sex, dose of exposure, and age. The risk of UTUC decreased in the 8th year after the ban of AA in middle-aged men who had exposure doses of 1–150 mg. Likewise, the risk of UTUC started to decrease in the 9th and 7th years after the ban on AA in middle-aged women who had exposure doses of 1–150 mg and >150 mg, respectively. In addition, the risk of UTUC disappeared approximately 10 years after the ban on AA in these women. No significant change in risk was observed in people aged ≥60 years.

Several studies have investigated the carcinogenesis of UTUC by AA. When the cells metabolize AA, the main components of AA, AA I and II become a reactive intermediate which binds to DNA. Then, aristolactam-DNA adducts can be detected in the DNA of cells exposed to AA (24). Besides, aristolactam-DNA adducts have been also identified in renal tissues from the patients with Balkan endemic nephropathy (4), and Taiwanese UTUC patients (6). In a human *p53* knock-in mice embryonic fibroblast cell line (Hupki), the signature mutation pattern, A:T to T:A transversion, was observed after exposure to AA (25). This mutation pattern have been also discovered in the animal models (26, 27), and urothelial carcinoma patients who ever used AA (28). In addition, a high mutation burden was observed in AA-related UTUCs when compared with smoking-related UTUC (29). Although it was difficult to distinguish mutated driver or passenger genes among the average 524 genes in each AA-related UTUC, several driver genes were frequently identified, including *TP53* (58%), *NRAS* (15%), *FGFR3* (8%), and *HRAS* (4%) (29).

Although the health authorities banned on AA according to the molecular (4, 6, 30) and epidemiological evidence (7), the incidence of UTUC seemed not to decrease immediately in the areas with nationwide exposure to AA (31). This implied that AA might have a longer latency period for UTUC. Recently, Jhuang et al. reported that the number of UTUC cases started to decrease after 2010 in Taiwan (12). Accordingly, our Taiwanese cohort was the ideal

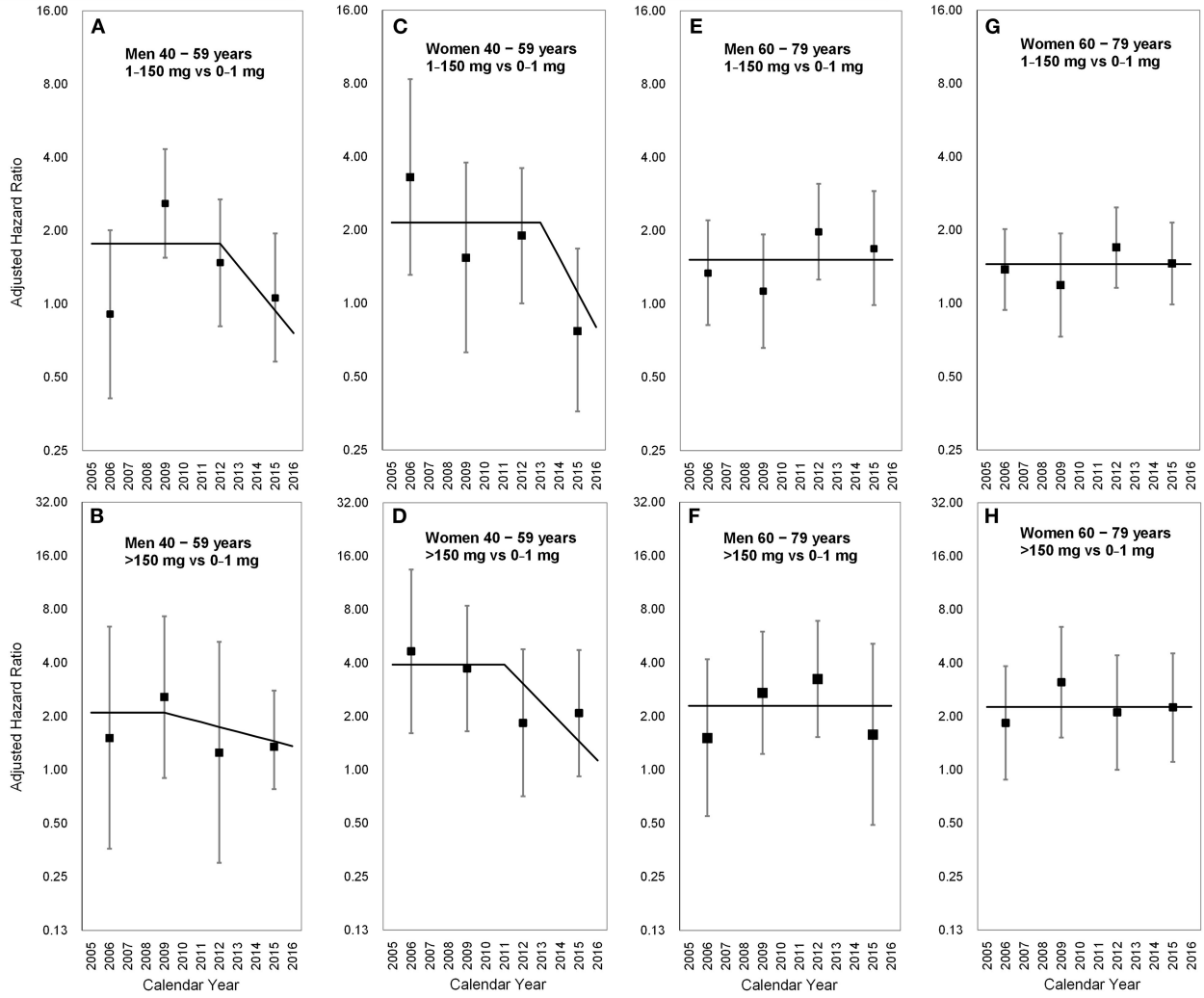

FIGURE 3
Long-term trends in the adjusted hazard ratio for upper tract urothelial carcinoma occurrence stratified by sex and age. **(A)** Men aged 40–59 years; 1–150 vs. 0–1 mg, **(B)** men aged 40–59 years; >150 vs. 0–1 mg, **(C)** women aged 40–59 years; 1–150 vs. 0–1 mg, **(D)** women aged 40–59 years; >150 vs. 0–1 mg, **(E)** men aged 60–79 years; 1–150 vs. 0–1 mg, **(F)** men aged 60–79 years; >150 vs. 0–1 mg, **(G)** women aged 60–79 years; 1–150 vs. 0–1 mg, **(H)** women aged 60–79 years; >150 vs. 0–1 mg. Square points: adjusted hazard ratios from the piecewise constant hazard model. Vertical lines: 95% confidence intervals of the adjusted hazard ratios from the piecewise constant hazard model. Solid lines: adjusted hazard ratios from the slope-change model.

model to investigate the latency period of AA-induced UTUC based on the below reasons, (1) nationwide exposure of AA, (2) the sharp interruption date of AA usage, (3) the nationwide health insurance which reimbursed AA-CHPs, and (4) the identification of the trend changes of UTUC incidence.

Previous population-based studies revealed that exposure to higher doses of AA resulted in a higher risk of UTUC (7, 20). Our analysis revealed the same finding and further identified the decreasing trend of UTUC risk with time after the ban of AA usage was obvious in the population who took CDAE of >150 mg of AA. This finding indicated that AA could be considered the major carcinogen in people exposed to a high dose of AA. When the responsible carcinogen, AA, was removed, the causal relationship was clearly confirmed by the decreasing risk of UTUC in Taiwan. Although there is no available literature reporting the risk change of UTUC in patients with different dosages of AA exposure, the evidence from studies considering the behavior of cigarette smoking cessation might support a similar phenomenon. The Korean National Health Insurance (NHI) Service database reported that a more reduced risk of all cancers was noted in the smoking reducers of heavy to light smokers than in moderate to light smokers (32).

Our results did not reveal a significant decreasing trend in UTUC risk in elderly people. The possible reasons include: (1) the competing carcinogens for the development of UTUC and (2) the difference in latency periods between long-term and short-term exposure to AA. Since several carcinogens, such as AA (3, 6, 7), smoking (33), and arsenic (20), may contribute to UTUC occurrence, the aged people were logically exposed to more kinds or higher cumulative dosages of carcinogens than the young or

middle-aged people. No literature has disclosed the effects of age on the trend of risk of AA-related disease until now, but other carcinogens, such as cigarette smoking, have addressed this issue (34). Using the US National Health Interview Survey for 1997 to 2014, which was linked to the National Death Index, Thomson et al. observed that smokers who quit smoking at an older age had a lower cancer mortality risk than those who quit smoking at a younger age (34).

Unlike in the United States (35), the UTUC risk was not significantly higher in men than in women in Taiwan (6, 31). Our studies revealed that Taiwanese women had taken more AA-CHPs than men. AA-induced UTUCs were more frequently observed in women in a previous cohort (36). The current investigation revealed a clear decreasing trend in UTUC risk after the ban of AA in middle-aged women regardless of AA dose. Although decreasing trends were also observed in middle-aged men regardless of AA dose, men may have multiple carcinogens. For example, cigarette smoking, a common risk factor for UC, was significantly higher in men (approximately 25–40%) than in women (4%) in Taiwan (37). Chen et al. also reported that male patients with UTUC smoked more than female patients in a Taiwanese cohort (36). Once AA was banned, a significant proportion of men were still exposed to carcinogens from cigarette smoking, which might mitigate the decreasing trend of UTUC.

This study has several limitations. First, only the herbal prescriptions reimbursed by the Taiwan NHI between 2000 and 2005 were used for the dosage calculation of AA exposure. We would underestimate the doses because the AA-CHPs prescribed before 2000 or not reimbursed by NHI were not included in this study. Second, some participants might be exposed to AA from the unidentified sources other than the AA-CHP reimbursed by NHI after 2005. Since our finding revealed the ban of AA reduced the incidence of UTUC, these unidentified sources of AA would be minor. Third, even Taiwan NHI covers more than 98% of the population and offers convenient medical access, it may not avoid underdiagnosed given their asymptomatic nature. Patients with either gross or microscopic hematuria can easily access medical resources. This mitigated the selection bias among populations with different doses of AA. Fourth, few UTUC cases in some subgroups might make the estimation of hazard ratio or hazard change imperfect. Even when we used the national database, the rare disease (cancer) still limited the total number of available cases for the analysis. Fifth, not all known carcinogens have been identified. Exposure to AA and arsenic, but not smoking behavior, was measured in this study. This was a natural limitation of the national database, which included only disease codes and prescriptions. The lack of information on smoking behavior made the analysis of this carcinogen impossible. In addition, some possible confounding factors, such as family history, occupation, and education level were not included in this study.

5. Conclusion

After the ban on AA was implemented, a decreasing trend in the incidence of UTUC was observed, especially in middle-aged women with AA exposure and in men with moderate doses of AA exposure. The latency period between AA exposure and UTUC development varied with age, dose of AA exposure, and sex.

Data availability statement

The data analyzed in this study is subject to the following licenses/restrictions: The data that support the findings of this study are available from the Health and Welfare Data Science Center but restrictions apply to the availability of these data, which were used under license for the current study, and so are not publicly available. Data are however available from the authors upon reasonable request and with permission of the Ministry of Health and Welfare, Executive Yuan, Taiwan. Requests to access these datasets should be directed to https://www.apre.mohw.gov.tw/.

Ethics statement

The studies involving human participants were reviewed and approved by National Taiwan University Hospital. Written informed consent for participation was not required for this study in accordance with the national legislation and the institutional requirements.

Author contributions

Conceptualization and writing: J-RJ and C-HC. Methodology and data interpretation: J-RJ, W-CL, and C-HC. Investigation: J-RJ, P-CC, and T-CH. Statistical analysis: J-RJ and W-CL. Writing review and editing: C-HC, Y-SP, and W-CL. All authors contributed to the article and approved the submitted version.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fpubh.2023.1072864/full#supplementary-material

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Surface ozone pollution in China: Trends, exposure risks, and drivers

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Introduction: Within the context of the yearly improvement of particulate matter (PM) pollution in Chinese cities, Surface ozone (O_3) concentrations are increasing instead of decreasing and are becoming the second most important air pollutant after PM. Long-term exposure to high concentrations of O_3 can have adverse effects on human health. In-depth investigation of the spatiotemporal patterns, exposure risks, and drivers of O_3 is relevant for assessing the future health burden of O_3 pollution and implementing air pollution control policies in China.

Methods: Based on high-resolution O_3 concentration reanalysis data, we investigated the spatial and temporal patterns, population exposure risks, and dominant drivers of O_3 pollution in China from 2013 to 2018 utilizing trend analysis methods, spatial clustering models, exposure-response functions, and multi-scale geographically weighted regression models (MGWR).

Results: The results show that the annual average O_3 concentration in China increased significantly at a rate of 1.84 $\mu g/m^3$/year from 2013 to 2018 (160 $\mu g/m^3$) in China increased from 1.2% in 2013 to 28.9% in 2018, and over 20,000 people suffered premature death from respiratory diseases attributed to O_3 exposure each year. Thus, the sustained increase in O_3 concentrations in China is an important factor contributing to the increasing threat to human health. Furthermore, the results of spatial regression models indicate that population, the share of secondary industry in GDP, NOx emissions, temperature, average wind speed, and relative humidity are important determinants of O_3 concentration variation and significant spatial differences are observed.

Discussion: The spatial differences of drivers result in the spatial heterogeneity of O_3 concentration and exposure risks in China. Therefore, the O_3 control policies adapted to various regions should be formulated in the future O_3 regulation process in China.

KEYWORDS

surface ozone, spatial-temporal pattern, exposure risks, health risks, dominant drivers

1. Introduction

Within the context of the yearly improvement of particulate matter (PM) pollution in Chinese cities, O_3 concentrations are increasing instead of decreasing and are becoming the second most important air pollutant after PM (1). According to the data published by the China General Environmental Monitoring Station, the daily maximum hourly average 90th percentile concentration of O_3 in 338 prefecture-level cities in China increased from 140.0 $\mu g/m^3$ in 2014 to 151.0 $\mu g/m^3$ in 2018, and the number of days exceeding the standard increased from 6.1% in 2014 to 8.4% in 2018, and the O_3 concentration in some regions has exceeded the secondary concentration limit (160 $\mu g/m^3$) for air quality in China (2). Long-term exposure to high O_3 concentrations not only affects urban air quality (3), damages human health (4), reduces food production (5), affects atmospheric radiation balance (6), and even influences global climate change (7). Due to its importance to the atmospheric

environment and climate change, O_3 has received continuous attention from the scientific community and relevant regulatory administrations in the past decades.

To deeply understand the O_3 pollution in China, a large number of researchers have conducted extensive investigations on O_3 pollution levels, spatial and temporal patterns, trends, exposure risks, and drivers in China from different spatial and temporal scales over the past decade (8–10). For example, Gong et al. (11) revealed the dominant meteorological controls on surface O_3 pollution in 16 Chinese cities from 2014 to 2016 using a generalized additive model (GAM); Cao et al. (12) studied the spatial and temporal patterns of O_3 pollution and ecological risks in the rainfed area of West China, Southwest China, based on ground-based data. Zhan et al. (2) estimated the health risk due to O_3 pollution in the Yangtze River Delta (YRD) region between 2015 and 2019 based on the exposure-response function, and their results showed that the population of premature respiratory deaths due to O_3 pollution was 5,889 cases per year from 2015 to 2019, and found that the population of premature deaths was extremely sensitive to O_3 pollution. In addition, Gao et al. (3), Maji et al. (13), and Lu et al. (14) also performed relevant studies on health risks due to O_3 pollution in China from different regions.

The numerous studies mentioned above are important references for a comprehensive assessment of the O_3 pollution in China, but these studies still have the following shortcomings. First, there is significant spatial heterogeneity in surface O_3 pollution, with a few individual cities or regions of O_3 pollution not being a substitute for the level of O_3 pollution in China. Second, there are potential spatial associations between exposure risk and health risk of populations to surface O_3 pollution, and unfortunately, previous studies have tended to ignore their interrelationships. Third, the effects of drivers on O_3 concentrations are spatially variable, and previous studies have tended to focus on the combined effects of drivers on O_3, neglecting the spatial and temporal differences in the effects of drivers on O_3 concentrations.

Therefore, the main objectives of this study include: (1) investigating the spatial and temporal patterns and trends of O_3 concentrations in China using trend analysis and spatial clustering based on a high spatial and temporal resolution O_3 concentration dataset; (2) examining the spatial and temporal associations of population exposure risk and health risk attributable to O_3 pollution using population exposure risk models and exposure-response functions; and (3) revealing the drivers of differences in O_3 distribution in China from a spatial perspective based on a multi-scale geographically weighted regression (MGWR) model. This study has important practical implications for assessing the future health burden caused by O_3 pollution and its resulting health costs in China; meanwhile, it has important implications for how to equitably allocate healthcare resources and environmental management costs in the future planning and construction of healthy cities and smart cities in China.

2. Materials and methods

2.1. Study area

This study focuses on China, including 31 provinces in the Chinese mainland, excluding Hongkong, Macau, Taiwan, and Hainan. Based on the social, natural, economic, and human environment, these 31 regions were further categorized into seven geo-administrative regions, including North China (Beijing, Tianjin, Hebei, Shanxi, Inner Mongolia), South China (Guangdong, Guangxi, Hainan), East China (Shanghai, Anhui, Fujian, Jiangsu, Jiangxi, Shandong, Zhejiang), Central China (Henan, Hunan, Hubei), Southwest China (Yunnan, Guizhou, Sichuan, Chongqing, Tibet), Northwest China (Shaanxi, Gansu, Ningxia, Qinghai, Xinjiang), and Northeast China (Heilongjiang, Jilin, Liaoning) (Figure 1).

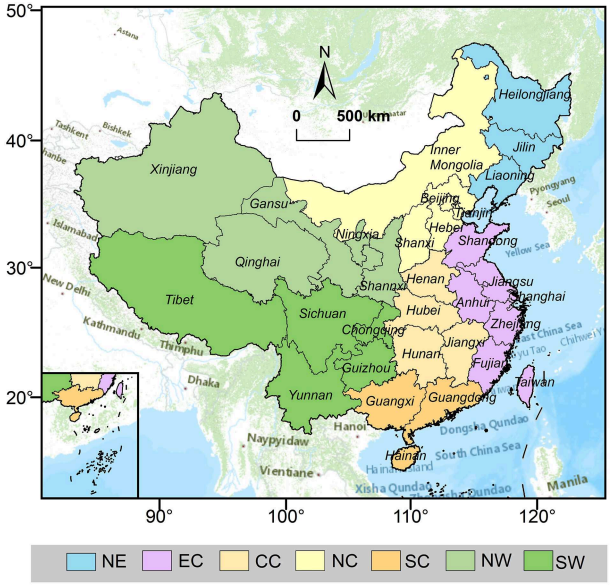

FIGURE 1
Study area spatial distribution map.

2.2. Data source

The daily maximum 8-h O_3 concentration (MDA8) reanalysis dataset of 10 × 10 km from January 1, 2013, to December 1, 2018, is from the tracking air pollution in China (http://tapdata.org/). The dataset is based on a machine learning algorithm and multi-data information fusion inversion. Its comprehensive construction combines ground monitoring data, satellite remote sensing data, high-resolution emission inventory data, air quality model simulation, and other multi-source data, which greatly improves the spatial and temporal accuracy of the data inversion results compared with the previous air quality reanalysis data (15). The daily O_3 concentrations in 360 prefecture-level cities in China during the study period were obtained from the China National Environmental Monitoring Center (http://www.cnemc.cn/sssj/). In order to reduce the error in the calculation of the health risk model, we calculated the 90th percentile concentration of the MDA8 O_3 concentration from the interannual scale based on the daily MDA8 O_3 concentration as the threshold.

The population size (Pop), the proportion of secondary industry to GDP (S_GDP), disposable income per capita (P_GDP), and soot emissions (Dust) for 360 prefecture-level cities in

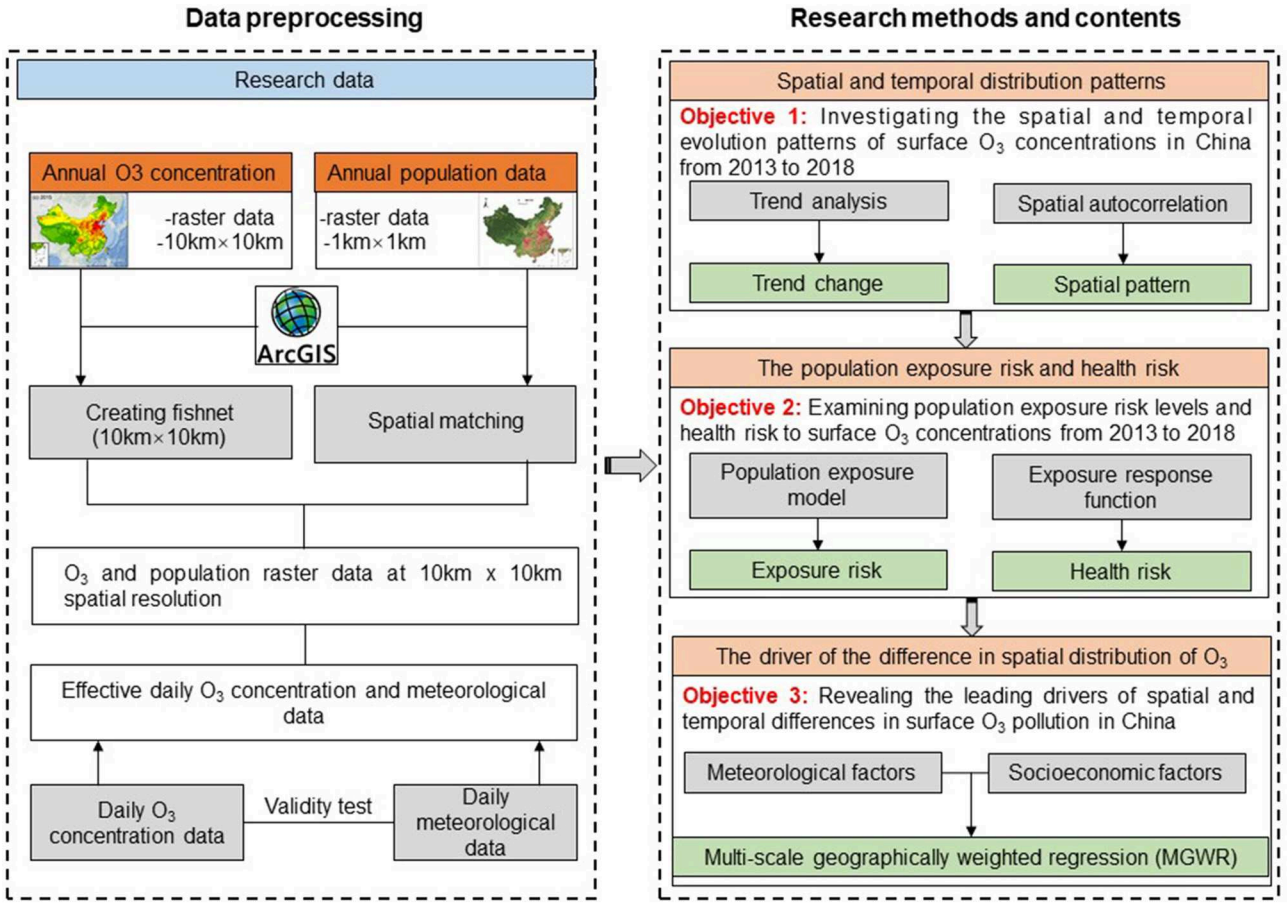

FIGURE 2
Research framework.

China during the study period were obtained from the China Statistical Yearbook (http://www.stats.gov.cn/tjsj/ndsj/#). The nitrogen oxide (NOx) and volatile organic compound (VOC) emissions were obtained from the China Multiscale Emissions Inventory Model (http://meicmodel.org/). The 1 × 1 km spatial resolution population data were obtained from the World pop dataset (https://www.worldpop.org/).

The daily meteorological data were obtained from the China Meteorological Data Network (http://data.cma.cn/) during the study period. The meteorological data obtained in this study mainly include air temperature (Tem, °C), sea level pressure (Pa, Pa), relative humidity (Hum, %), 2-m mean wind speed (WS, m/s), 1-h precipitation (Pre, mm), and 10-min mean visibility (Vis, m).

2.3. Trend analysis

Trend analysis is usually used for the analysis of temporal dynamics of air pollutants to explore the interannual rate of pollutant changes (16). In this paper, the rate of change of O_3 concentrations in China from 2013 to 2018 was analyzed based on the trend analysis method, which is calculated as Equation (1):

$$Trend = \frac{n \times \sum_{i=1}^{n} \left(i \times O_{3_i}\right) - \left(\sum_{i=1}^{n} i\right)\left(\sum_{i=1}^{n} O_{3_i}\right)}{n \times \sum_{i=1}^{n} i^2 - \left(\sum_{i=1}^{n} i\right)^2} \quad (1)$$

Where O_3 indicates the O_3 concentration of each cell; n indicates the time span, here the time span is 6; and i is the time unit.

2.4. Population exposure risk model

Previous studies have shown that significant heterogeneity in the spatial distribution of air quality concentrations and population density leads to major spatial differences in the exposure risks of populations to air quality (17). In addition, health risks due to exposure to pollutants are usually defined as a function of the multiplication of population density and pollutant concentration (18). Although the exposure risk intensity in the area can be quantified to some extent, it cannot distinguish the severity of the local area relative to the whole. To address this issue, we introduced a model for the relative exposure risk of the population attributable to O_3 exposure, as shown in Equation (2), which can evaluate the

exposure status in each pixel of the cells (19):

$$R_i = \frac{P_i \times C_i}{\sum_{i=1}^{n} P_i \times \frac{C_i}{n}} \quad (2)$$

where R_i indicates the risk of population exposure in grid cell i; P_i indicates the number of exposed populations in grid cell i; C_i indicates the O_3 concentration in grid cell i, and n indicates the total number of grid cells in the study area. To better reflect the spatial difference of relative population exposure risk, we categorized the population exposure risk as extremely low risk, low risk, lower risk, higher risk, high risk, and extremely high risk by using the reclassification method in ArcGIS10.8 software. The corresponding exposure risk values are $R_i = 0$, $0 < R_i \leq 1$, $1 < R_i \leq 2$, $2 < R_i \leq 3$, $3 < R_i \leq 5$ and $R_i > 5$, respectively. A higher value of R indicates a higher exposure risk.

2.5. Health risk model

In this study, a standard damage function was applied to estimate the population of premature deaths from respiratory diseases due to O_3 exposure. The specific equations are shown in Equations (3) and (4), and the relationships shown in the following equations have been extensively applied in previous studies (14, 20, 21).

$$RR = \begin{cases} e^{\beta(x-x_0)}, x > x_0 \\ 1, x \leq x_0 \end{cases} \quad (3)$$

$$\Delta M = y_0 \times Pop \times [(RR-1)/RR] \quad (4)$$

where RR is the relative risk; $(RR-1)/RR$ is the attributable fraction; x_i is the O_3 concentration in a city i or grid i; x_0 is the threshold concentration; β is the exposure-response coefficient, which represents the additional health risk associated with an increase in unit O_3 concentration (22, 23); ΔM is the number of premature deaths of respiratory diseases attributable to exposure to the O_3 environment; y_0 is the baseline mortality rate of respiratory diseases, and pop is the number of the exposed population. In this study, the mortality rate of respiratory diseases was obtained from the National Bureau of Statistics, where the crude mortality rate of respiratory diseases y_0 (1/100,000) in urban China from 2013 to 2018 was, 76.61, 74.17, 73.36, 69.03, 67.20 and 68.02, respectively. β values in this study were obtained from Shang et al. (24), per 10 $\mu g/m^3$ with a value of 0.48% (95% CL: 0.38%, 0.58%). Song et al. (25) concluded that the exposure-response coefficients obtained from a meta-analysis by Shang et al. (24) based on a 33–time series and case-crossover study conducted could to some extent reflect the health risks attributed to air pollution in China. Meanwhile, which has widely been used in several past studies for China (26, 27).

2.6. Multi-scale geographically weighted regression

Compared with the classical geographically weighted regression model (GWR), the MGWR model was a flexible regression model (28). Each regression coefficient was obtained based on local regression, and the bandwidth is specific. In addition, the GWR model uses weighted least squares in the fitting operation, while the MGWR model was equivalent to a generalized additive model (GAM), which could perform regression analysis on spatial variables with linear or non-linear relationships, and was also an effective tool for dealing with various complex non-linear relationships of spatial variables (29). Assuming that there are n observations, for observation $i \in \{1,2,3,\ldots, n\}$ at location (U_i, V_i), the MGWR were calculated as follows (30):

$$y_i = \beta_0(U_i, V_i) + \sum j\beta_{bwj}(U_i, V_i)X_{ij} + \varepsilon_i \quad (5)$$

where y_i is the response variable O_3 concentration, $\beta_0(U_i, V_i)$ is the intercept, X_{ij} is the j_{th} predictor variable i, $\beta_{bwj}(U_i, V_i)$ is the j_{th} coefficient, bwj in β_{bwj} indicates the bandwidth used for calibration of the jth conditional relationship, ε_i is the error term. In addition, the spatial kernel function type selected during the model operation is bisquare, the bandwidth search type is golden, and the model parameter initialization type takes GWR estimation as the initial estimation model.

2.7. Research framework

This study used the trend analysis method, spatial autocorrelation model, population exposure risk model, exposure-response function, and MGWR model to analyze the spatial-temporal pattern, exposure risk, health risk, and driving factors of O_3 concentration in China from 2013 to 2018. Firstly, we use the trend analysis method and spatial autocorrelation model to explore the changing trend and spatial-temporal distribution of O_3 concentration in China. Secondly, we selected the population exposure risk model and exposure-response function to investigate the population exposure risk and health risk attributed to O_3 pollution, and discussed their temporal and spatial correlation characteristics. Finally, we use the MGWR model to reveal the dominant factors of spatial distribution difference of O_3 concentration in China. Additionally, in this study we used O_3 concentration reanalysis data at 10 × 10 km resolution and population raster data at 1 × 1 km resolution to investigate the exposure risks and health risks attributed to O_3 pollution. To spatially match the 10 × 10 km O_3 concentration reanalysis data, we used the aggregation module of ArcGIS10.6 software to quantitatively change the spatial resolution of the 1 km×1 km population data. During the aggregation calculation, the output image element cell size was set to 10 × 10 km, i.e., 0.01°×0.01°, and the nearest neighbor assignment method was selected for the aggregation technique. Figure 2 shows the research framework of this paper.

3. Results

3.1. Spatial and temporal distribution patterns

Figure 3 shows the temporal and spatial distribution and changing trend of the annual average concentration of MDA8

(AMDA8, O_3) from 2013 to 2017 in China. From 2013 to 2018, the annual average O_3 concentrations in China were 110.75, 108.21, 111.13, 115.57, 120.49, and 115.95 $\mu g/m^3$, respectively, and changed at a rate of 1.84 $\mu g/m^3$/yr increase (Figure 3H). From a spatial and temporal perspective, the highest annual average O_3 concentrations were found in central China in 2013, 2015, and 2016, with annual average O_3 concentrations of 121.87, 118.84, and 122.78 $\mu g/m^3$, respectively. The highest annual average O_3 concentrations in 2014, 2017, and 2018 were all found in East China, with annual average O_3 concentrations of 116.98, 135.03, and 137.91 $\mu g/m^3$, respectively. In comparison, the lowest O_3 concentration in 2013 occurred in the Northeast region (98.33 $\mu g/m^3$), the lowest O_3 concentrations from 2014 to 2017 occurred in the Southwest region of China (90.86, 94.43, 99.20, and 104.27 $\mu g/m^3$), and the lowest O_3 concentration in 2018 occurred in the Northwest region (103.44 $\mu g/m^3$) (Figures 3A–G). Since 2013, 89.62% of China's territory has experienced a significant increase in annual average O_3 concentrations, with 2.73% of the regions experiencing an average rate of change in annual average O_3 concentrations exceeding 5.00 $\mu g/m^3$/yr. However, the rate of variation of O_3 concentration varies from region to region has strong spatial variability. The rate of change of O_3 concentration in the Central Plains urban agglomeration is the most variable in terms of the country, with its O_3 concentration change rate exceeding 4.0 $\mu g/m^3$/yr. In contrast, the rate of change of O_3 concentration in the Chengdu-Chongqing urban agglomeration (-0.3 ± 1.0 $\mu g/m^3$/yr), Southwest China (-0.5 ± 1.1 $\mu g/m^3$/yr) and South China (-1.0 ± 1.4 $\mu g/m^3$/yr) decreases significantly (Figure 3G).

Figure 4 represents the spatial clustering characteristics of the rate of variation of O_3 concentration at county-level units in China from 2013 to 2018. The results show that the global Moran's *I* index is significant at the 1% level, indicating a consistent and enhanced positive spatial autocorrelation in the rate of variation of O_3 concentration (Figure 4A). The results of the hot spot analysis show that there is a significant hot spot (HH) region for O_3 concentration growth rate, which is mainly contiguous and focused in Shaanxi, Shanxi, central Inner Mongolia, Beijing–Tianjin–Hebei (BTH), southwest Liaoning, central Henan, eastern Hubei, Anhui, Jiangsu, and Shandong in China, which are the regions with the strongest O_3 growth rate in China. In addition, we found a significant cold spot area (LL) covering a large part of China (about 90% of the territory). These regions are mainly located in northeastern, southern, southwestern, eastern, and northwestern China, where the growth rate of O_3 concentration is relatively low and even decreasing regions are observed (Figures 4B, C). The standard deviation ellipsometric analysis evaluated the overall variations in the spatial pattern of O_3 concentration growth rate from 2013 to 2018 in China (Figure 4D). It can be found that the regions with significantly increased O_3 concentration growth rates are mainly concentrated in BTH, Shanxi, Shandong, Jiangsu, Jiangxi, Anhui, Hubei, Henan, and Shaanxi in China. This result also indicates that the above-mentioned regions are the primary contributors of O_3 during the whole study period in China. Meanwhile, the center of the median growth rate of O_3 concentration is located north of the standard deviation ellipse arithmetic center, indicating that the growth rate of surface O_3 concentration is greater in northern China than in southern China.

3.2. The population exposure risk and health risk

Overall, the total population exposed to $O_3 > 160$ $\mu g/m^3$ increased from 1.2% in 2013 to 28.9% in 2018, compared to a decrease in the population exposed to $O_3 < 160$ $\mu g/m^3$ from 7.2% in 2013 to 3.6% in 2018 (Figure 5). Figures 6, 7 represents the spatial pattern of exposure risk levels attributed to O_3 pollution in 2013, 2015, and 2018. We found that most regions have remained at low (52.89–55.73%) or extremely low (19.48–20.48%) O_3 exposure risk levels over three time periods in China. From a temporal perspective, only 4.83% of the territory of the country was at high exposure to O_3 pollution in 2013, and this percentage increased to 6.45 and 7.19% in 2015 and 2018, respectively. Similarly, the area of the territory exposed to extremely high risk also exhibits a marked increasing trend, from 7.61% in 2013 to 9.62% in 2015 and further to 11.35% in 2018 (Figures 7A–C). Spatially, the distribution patterns of O_3 exposure risk levels were similar for the three time periods of 2013, 2015, and 2018 in China. With the rapid increase of O_3 concentration in the North China Plain, the high exposure risk level regions of BTH and YRD have been continuous, which constitute a high O_3 exposure risk level aggregation area including the Bohai Rim, YRD, Pearl River Delta (PRD), Shanxi and Guanzhong Plain urban clusters. Spatially, the distribution patterns of O_3 exposure risk levels were similar for the three time periods of 2013, 2015, and 2018 in China. In contrast, the extremely low risk and lower risk areas of O_3 pollution are widely distributed in China, which is mainly located in most regions of northwest, southwest, and northeast in China (Figures 7D–F).

Figure 8 indicates the spatial and temporal distribution of premature deaths from respiratory diseases attributable to O_3 exposure from 2013 to 2018. Overall, there was an average of over 24,000 premature deaths from respiratory diseases due to O_3 exposure per year in China from 2013 to 2018, and the growth rate fluctuated at 1,178 cases per year ($p < 0.05$). Specifically, the number of premature deaths attributable to O_3 exposure increased from 236,200 in 2013 to 272,300 in 2018, an increase of 36,100 cases compared to 2013. Spatially, the regions with <500 cases of premature death due to O_3 exposure are mainly located in Tibet, Qinghai, east of Xinjiang, west of Sichuan, west of Inner Mongolia, Liaoning, and Heilongjiang; the regions with more than 500 cases are mainly located in the region east of Hu line, mainly including most of eastern China and western Xinjiang, central Inner Mongolia, southern Gansu, most of southern China, most of northern China, and Liaoning in northeast China. The regions with more than 1,000 cases are mainly located in BTH, Sichuan–Chongqing region, Fenwei Plain, East China Plain, Jianghan Plain, Yangtze River Delta, and Pearl River Delta region. Meanwhile, the regions with more than 1,000 cases of premature death due to O_3 exposur e are further expanding over time.

3.3. The driver of the difference in the spatial distribution of O_3

Multicollinearity refers to the distortion of model estimates due to significant correlations between the independent variables in the

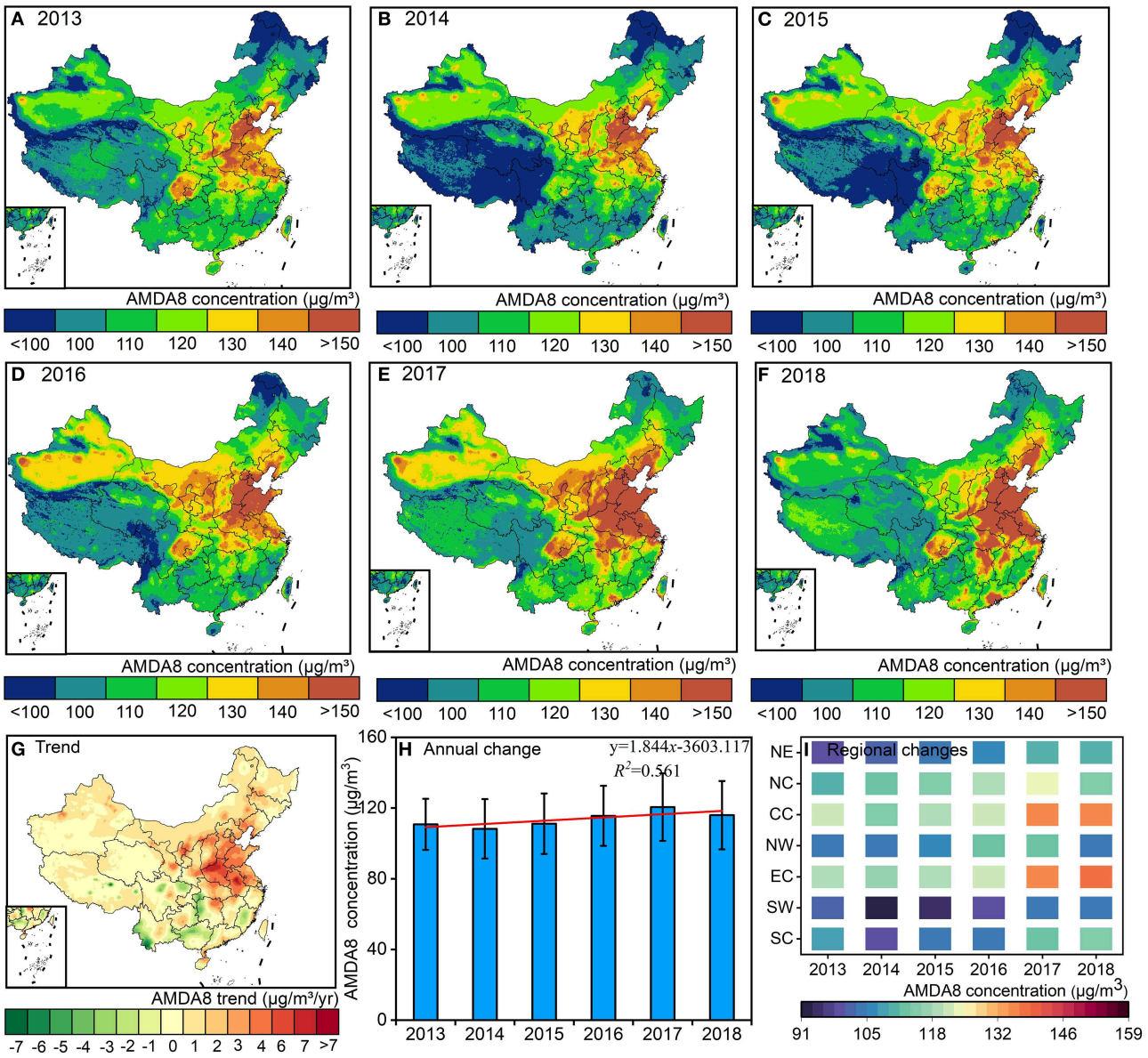

FIGURE 3
The spatial distribution and trend of O_3 concentration from 2013 to 2018. Where, **(A–F)** represents the spatial distribution of O_3 concentration; **(G)** represents the trend change of O_3 concentration; **(H)** represents the annual average O_3 concentration variation; **(I)** shows the annual average O_3 concentration change in different regions of China.

linear modeling regression process. Therefore, before conducting model regression analysis, to test whether there is multicollinearity between each explanatory variable, we use variance inflation factor (VIF) to test the multicollinearity problem between each explanatory variable, and previous studies have shown that when VIF $\geq$ 10, it indicates that there is a serious multicollinearity problem between the dependent variable and the independent variable. multicollinearity problem, which should be removed from the actual model operation. The collinearity test in this study was performed in SPSS 25.0 software and the results of the analysis showed that the range of VIF values for all explanatory variables was 1.000–9.765, which indicates that there was no cointegration between the dependent and independent variables. Table 1 indicates the diagnostic information of the MGWR model for the socioeconomic and meteorological factors. In terms of the number of valid parameters, the goodness–of–fit R^2 for the responses of socioeconomic and meteorological factors to O_3 concentrations are 0.861 and 0.799, respectively, and the residual sum of squares (RSS) is 136.297 and 136.51 $\mu g/m^3$, respectively, with the absolute values of the deficit information criterion (AIC) and the log-likelihood value (Log-likelihood) < 5,000. These regression results indicate that MGWR uses fewer parameters to obtain regression results that are closer to the true values and can be fully used to assess the relationship between O_3 pollution and socioeconomic and meteorological factors.

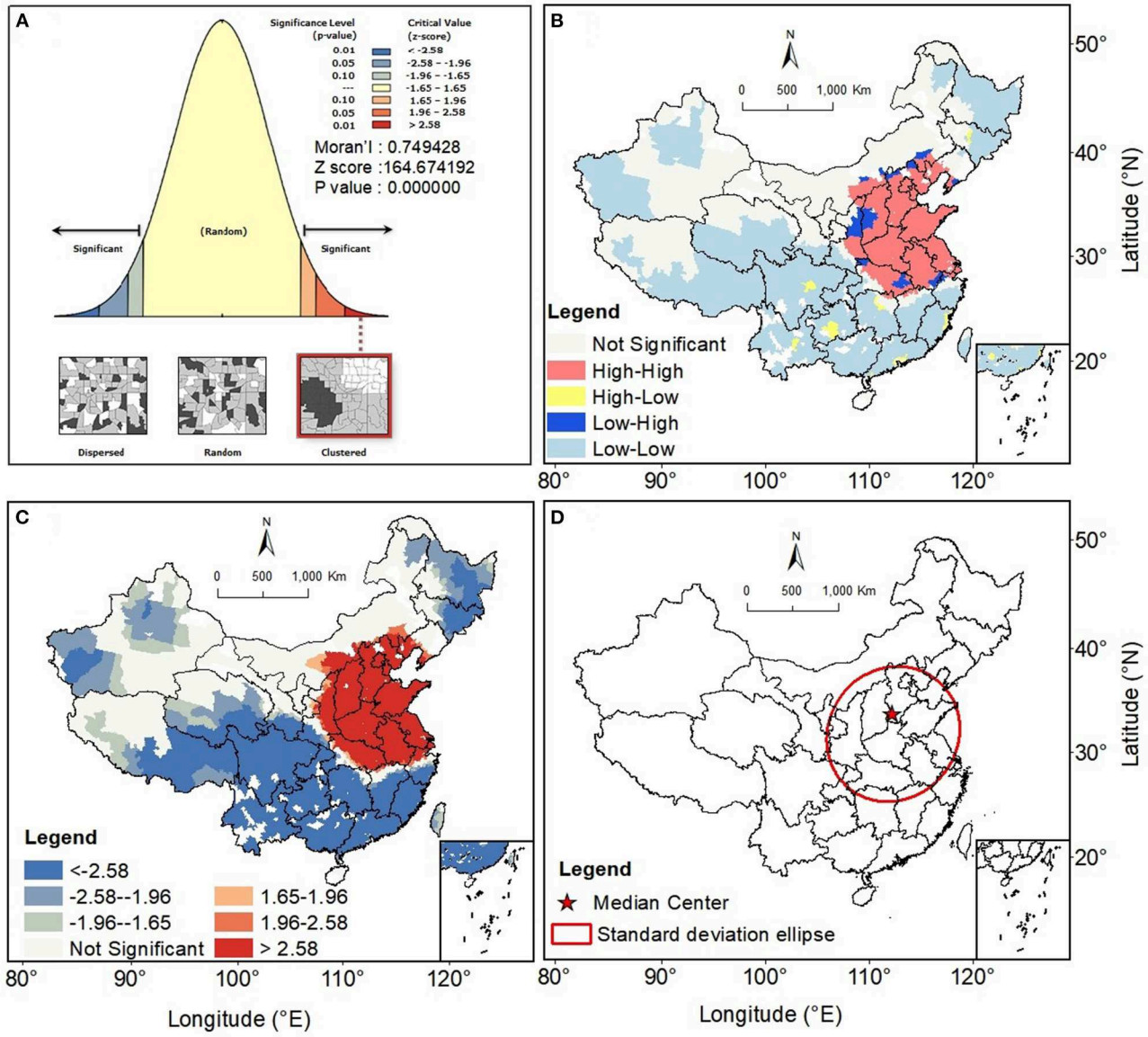

FIGURE 4
Spatial clustering characteristics of the rate of change of O_3 concentration in county-level units in China, 2013–2018. **(A)** Global spatial autocorrelation test results; **(B)** Spatial distribution of the spatial clustering of O_3 concentration variation rates; **(C)** Spatial distribution of cold and hot spots for the rate of change of O_3 concentration, in the cold and hot spot analysis we used Getis Ord Gi* analysis to calculate *Z* scores, where $|Z\text{scores}| > 1.65$ corresponds to $p < 0.10$, $|Z\text{scores}| > 1.96$ corresponds to $p < 0.05$, $|Z\text{scores}| > 2.58$ corresponds to $p < 0.01$. *Z* scores are negative indicating a cold spot, and a positive *Z* score indicates a hot spot; **(D)** Spatial distribution of the standard ellipse of the rate of variation of surface O_3 concentration and the center of change in China from 2013 to 2018.

Figure 9 indicates the spatial distribution of regression coefficients of socio-economic factors. The high values (>0.27) of regression coefficients for the total population are mainly located in North and East China, where the total population is significantly and positively correlated with its corresponding O_3 concentration. The influence of the share of secondary industry on surface O_3 in East and North China is significantly higher than that in other regions, and its regression coefficient exceeds 0.08. We also find that over 80% of the regional disposable income per capita is positively correlated with O_3, with regression coefficients ranging from 0.07 to 0.36. In contrast, Guangdong, Shandong, and Northeast provinces show a significant negative correlation between disposable income per capita and O_3, with regression coefficients, were below −0.02. The industrial dust emissions in Sichuan and Chongqing are significantly ($p < 0.001$) positively correlated with the corresponding O_3 concentration with a regression coefficient > 0.52, while industrial dust emissions in cities located in East China are significantly ($p < 0.01$) negatively correlated with the corresponding O_3 concentration with a regression coefficient ranging from −0.53 to −0.25, where O_3 concentrations in cities located in eastern Jiangsu and Anhui provinces are more affected by the negative correlation of

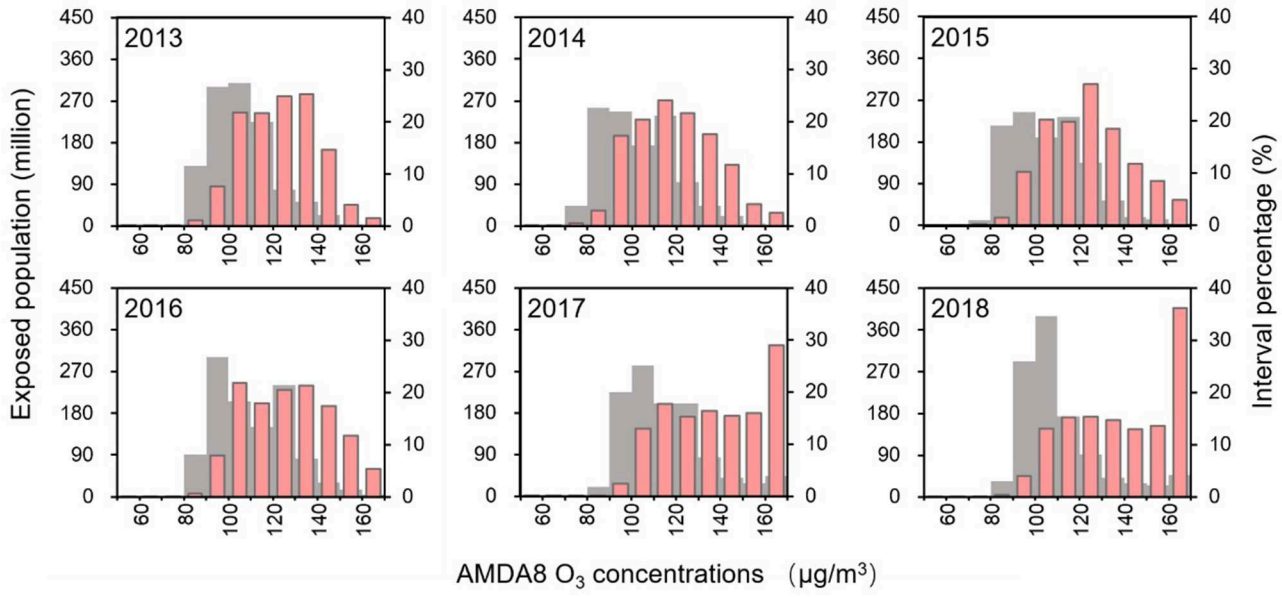

FIGURE 5
Probability distribution of the total population exposed to different O_3 concentrations from 2013 to 2018. The red and gray bars indicate the total population and the proportion of the population exposed to different O_3 concentrations, respectively.

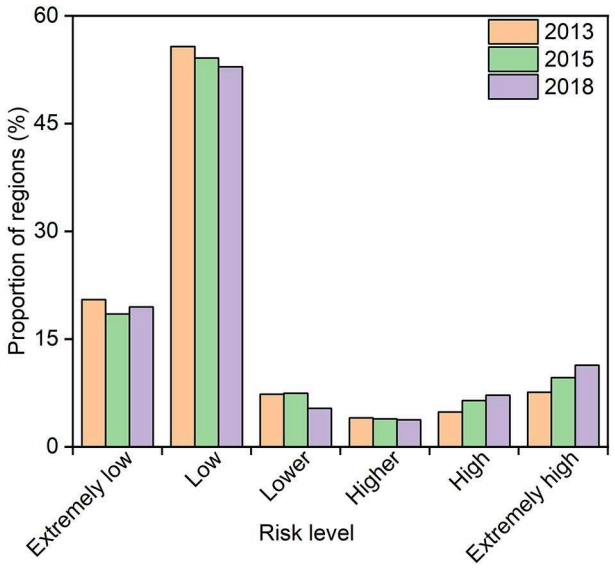

FIGURE 6
Proportion of regions with different O_3 exposure risk levels to the total land area (%).

industrial dust emissions. The NOx emissions were significantly and positively correlated with O_3 concentrations in Central China, East China, South China, Sichuan and Chongqing, and parts of Southwest and Northwest China ($p < 0.05$), with regression coefficients ranging from 0.60 to 1.26. There was a significant ($p < 0.01$) negative correlation between VOCs emissions and O_3 concentrations in Hubei, Jiangxi, Zhejiang, Anhui, Jiangsu, Shanghai, Guangdong, Fujian, and Guangxi cities with regression coefficients ranging from −0.53 to −0.35.

Figure 10 shows the spatial differences in the effects of various meteorological factors on O_3 concentration. It can be found that the temperature of cities in North, East, and Northeast China showed a significant ($p < 0.05$) positive correlation with O_3 concentration, with regression coefficients ranging from 0.23 to 0.49. The relative humidity was negatively correlated with O_3 concentration in all cities during the study period. Among them, cities in Heilongjiang, Jilin, Liaoning, Beijing, Tianjin, north-central Hebei, northwestern Shanxi, western Inner Mongolia, and northwestern Ningxia and northern Shaanxi showed a weak negative correlation between relative humidity and O_3 concentration with a non-significant ($p > 0.05$) regression coefficient of < -0.07. In contrast, cities in southern Zhejiang, southern Anhui, Jiangxi, central Hubei, Hunan, Chongqing, Guizhou, Yunnan, and cities in Fujian, Guangdong, and Guangxi regions showed a significant ($p < 0.01$) strong negative correlation between relative humidity (Hum) and its corresponding O_3 concentration with regression coefficients ranging from −0.18 to −0.15. Wind speed (WS) showed a significant ($p < 0.05$) negative correlation with O_3 concentrations in Heilongjiang, Jilin, Liaoning, Guangxi, southern Henan, Hubei, eastern Shandong, Jiangsu, Shanghai, Zhejiang, Sichuan and Chongqing regions, and northern Shanxi, with regression coefficients ranging from −0.02 to −0.06. It is particularly noteworthy that cities in BTH, southwestern Shanxi, northern Henan, central Shaanxi, Ningxia, southern Gansu, western Shandong, and Anhui have a significant positive correlation between their wind speed and O_3 concentration with regression coefficients >0.45. For air pressure, cities located in northern China showed a significant ($p < 0.05$) negative correlation between air pressure (Pa) and O_3

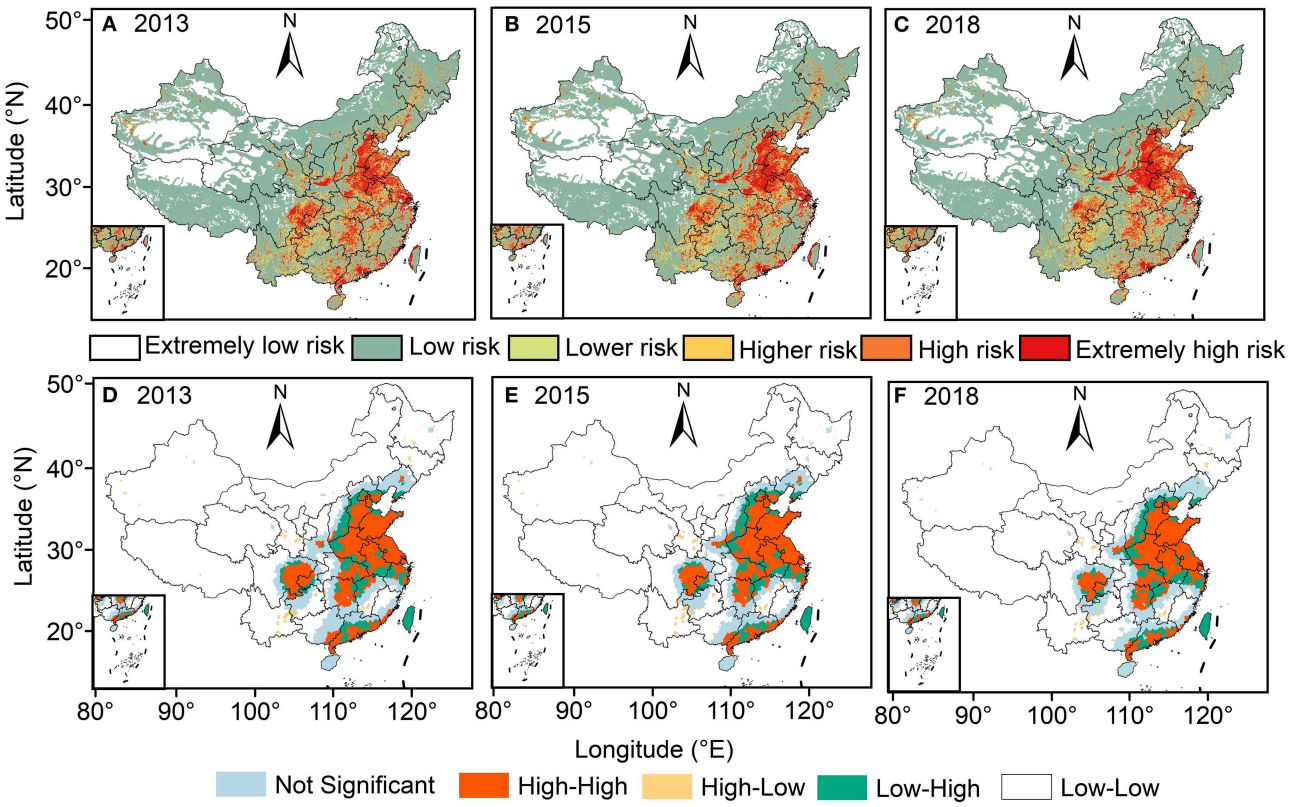

FIGURE 7
Spatial distribution pattern of population exposure risk levels attributed to O_3 pollution from 2013 to 2018. **(A–C)** Indicates the spatial distribution of population exposure risk levels in 2013, 2015, and 2018, respectively; **(D–F)** Indicates the spatial clustering of population exposure risk levels in 2013, 2015, and 2018, respectively.

concentration, with regression coefficients ranging from -3.6×10^{-3} to -1.3×10^{-3}. Precipitation showed a significant ($p < 0.05$) positive correlation with O_3 concentration in Heilongjiang, Jilin, South China, Guangxi, and Guangdong, with regression coefficients ranging from 3.93 to 19.21, while other regions showed negative correlations. Visibility was positively correlated with O_3 concentration in all cities.

4. Discussion

4.1. Spatial distribution difference of O_3 concentration

The results of the spatial and temporal pattern analysis of O_3 concentrations show that East, Central, and North China are the regions with the highest growth of O_3 concentrations in China from 2013 to 2018, which is mainly attributed to the huge amount of anthropogenic emissions. The areas of East, Central, and North China are one of the most densely populated and industrially developed regions in China, and the massive industrial activities, transportation, and human activities result in the emission of large amounts of O_3 precursors. In contrast, Southwest and South China are the regions with the largest decreases in O_3 concentrations in China. Previous studies have found that Southwest and Northwest China are located in high-latitude regions, and their corresponding atmospheric vertical exchange and photochemical reactions are stronger due to the special topography and intense solar radiation compared to inland regions, resulting in higher background values of O_3 concentrations in these regions (9, 31). However, the extent of the influence of solar radiation on O_3 in southwest and southern China is significantly weaker than the influence of anthropogenic emissions compared to the dramatic increase in O_3 concentrations due to strong anthropogenic emissions in East, Central, and North China (32).

4.2. Spatial heterogeneity of O_3 concentration drivers

There are strong spatial variations in the influence of different drivers on O_3. Relative to lower population density regions, a larger population size implies more energy consumption and pollution emissions, meanwhile, it also further compresses the green area of cities, leading to a significant reduction in the ability of cities to mitigate air pollution, which better explains why the positive correlation between population size and O_3 concentration is significantly higher in densely populated northern and eastern China than in other regions (21, 33). Previous studies have shown that industrial emissions are the predominant source of air pollution (34). Our study found that the share of secondary

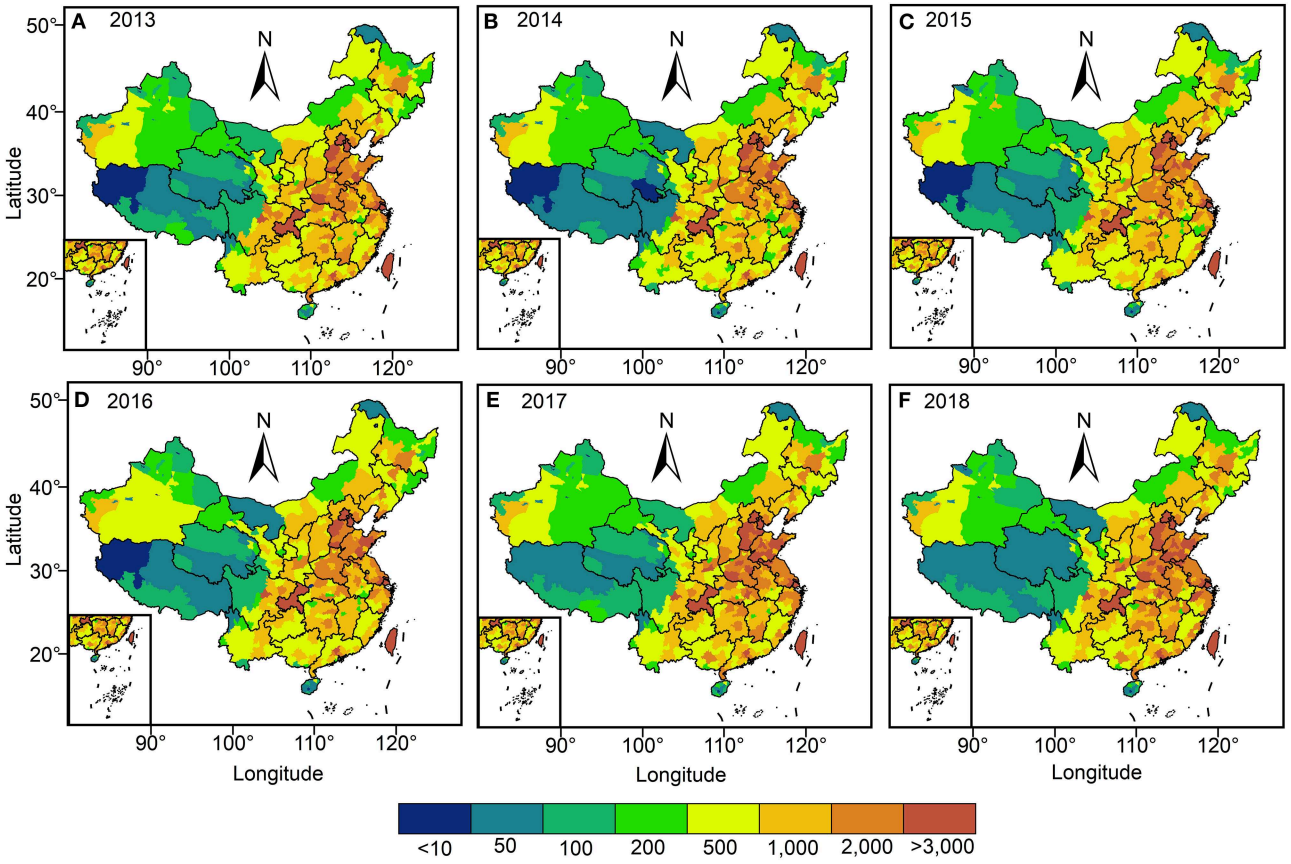

FIGURE 8
Spatial distribution of premature deaths attributable to O_3 exposure in China, 2013 to 2018. **(A–F)** indicates the spatial distribution of premature death population attributable to O_3 exposure at the prefecture-level city scale from 2013 to 2018 in China, respectively.

TABLE 1 Diagnostic information of MGWR model.

Evaluation indicators	Socio-economic factors	Meteorological factors
Residual sum of squares (RSS)	136.297	136.506
Log-likelihood	−423.861	−418.699
Degree of Dependency (DoD)	0.498	0.476
AIC	1,160.521	1,045.466
AICc	1,220.301	1,083.609
BIC	1,925.094	1,515.614
R^2	0.861	0.799
Adj. R^2	0.835	0.763

industry in GDP had a significant positive correlation with O_3 concentration, especially in central China, and eastern China, where industrial production is dominant, and the contribution of urban industrial production to O_3 concentration is stronger than in other regions. At the urban scale, the formation of O_3 concentrations depends on the VOCs–NOx ratio (35). In general, the higher the NOx emissions in cities, the lower the VOCs–NOx ratio. For example, the formation of O_3 in some cities located in Central and Northern China is often limited by VOCs (36, 37). In these cities, the reduction of VOCs emissions decreases the formation of O3, but the reduction of NOx emissions increases the formation of O_3. This chemical reaction tends to depend on the amount of VOCs and NOx emissions; the larger the emissions the more intense their reaction and the larger the O_3 emissions generated (38). In addition, industrial dust emissions indirectly affect solar radiation intensity by affecting atmospheric visibility, which further contributes to the O_3 photochemical reaction rate (39).

Temperature is an important ambient condition for photochemical reactions, and higher temperatures can promote the rapid production of O_3 concentration, therefore, temperature and O_3 concentration are mostly positively correlated, especially in cities in Northern, Eastern, and Northeastern China where the solar temperature is higher in the warm season (40). The wind speed has a diffusion and transport effect on pollutants in the atmosphere. For example, O_3 concentrations in cities in Northeast, South, Central, and East China, and Sichuan and Chongqing regions showed a significant ($p < 0.05$) negative correlation with wind speed. However, our results found a significant positive correlation between O_3 concentration and wind speed in most cities located in the North China Plain. Li et al. (41) attributed the significant positive correlation between O_3 concentration and wind speed in the North China Plain region to the influence of

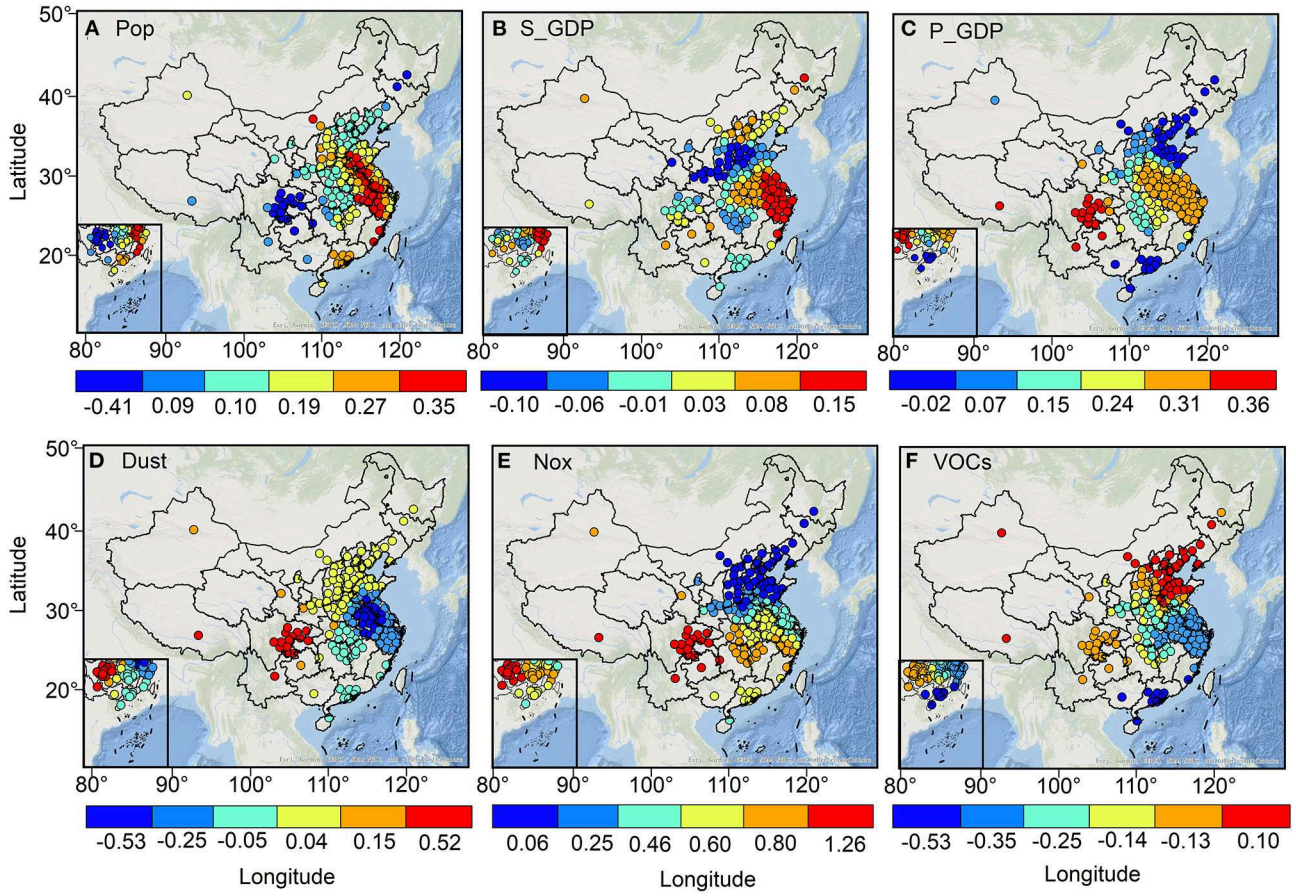

FIGURE 9
The spatial distribution of regression coefficients for the major socioeconomic factors of Pop **(A)**, S_GDP **(B)**, P_GDP **(C)**, Dust **(D)**, NOx **(E)**, and VOCs **(F)**.

warm-season burning winds, especially from June to August each year, when the burning winds blow from the mountains to the northern and western parts of the North China Plain, bringing dry the hot air further leads to a higher temperature in the region, which accelerates the photochemical reaction of O_3 production to some extent. Relative humidity has a negative correlation with O_3 concentration. Previous studies have shown that water vapor can not only absorb and release energy through changes in the aqueous phase but also undergo internal reactions, especially when controlling for other influencing factors, higher relative humidity leads to higher water vapor saturation, resulting in easy removal of O_3 and its precursors and lower O_3 concentrations (42). In addition, water vapor can reduce solar ultraviolet radiation through extinction mechanisms, thus affecting photochemical reactions and O_3 concentrations (43).

4.3. The O_3 control policy implications

In summary, O_3 pollution in China is gradually increasing, and more and more of China's population is exposed to high O_3 concentration pollution. Scientific and effective reduction of O_3 concentration exposure levels in China is crucial to reduce population exposure risks (44). Under these circumstances, this study proposes policy recommendations on how to reduce O_3 concentrations in Chinese cities from the perspective of the drivers affecting the spatial distribution of O_3 and epidemiology. For O_3 pollution areas dominated by O_3 precursors (e.g., NOx, VOCs, and CO), the authorities can ensure that their emissions comply with government regulations by optimizing the industrial structure and reducing the emissions of O_3 precursors. Meanwhile, the governmental department should focus on the synergistic management of $PM_{2.5}$ and O_3 compound pollution. Research shows that NOx is not only an important precursor for O_3 generation but also an important precursor for $PM_{2.5}$ (45). Therefore, strengthening the NOx deep regulation and emission reduction is a key step to promote synergistic control. Furthermore, the O_3 abatement measures in the future should pay attention to different seasonal O_3 control measures and strengthen regional cooperation for O_3 pollution prevention.

For O_3 pollution regions dominated by meteorological factors, the department should forecast the variation of O_3 concentration due to the change of meteorological factors promptly, meanwhile develop a detailed O_3 pollution early warning program to reduce the risk of public exposure and explore a sustainable development path for O_3 pollution management in China. From

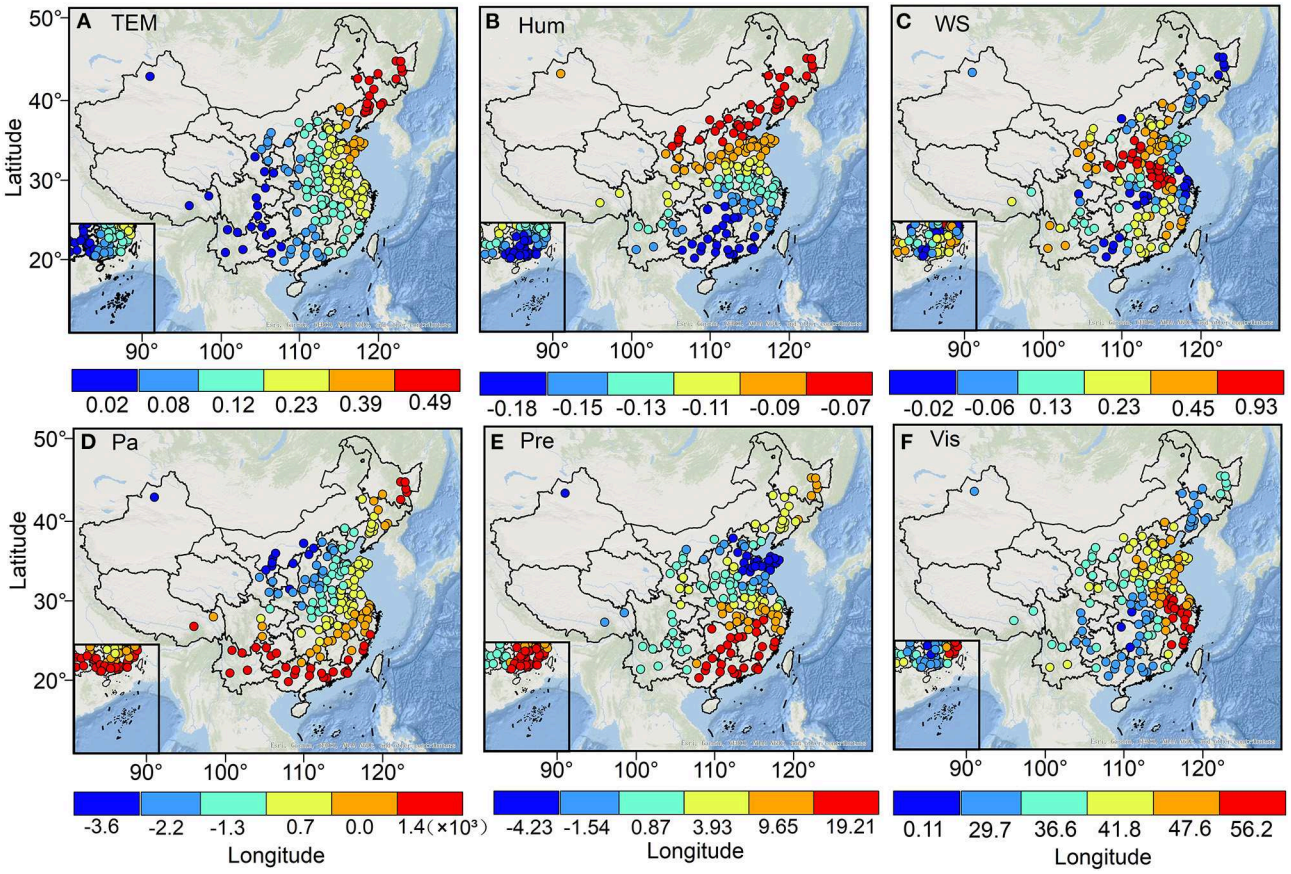

FIGURE 10
The spatial distribution of regression coefficients for the major meteorological factors of TEM **(A)**, Hum **(B)**, WS **(C)**, Pa **(D)**, Pre **(E)**, and Vis **(F)**.

an epidemiological perspective, to protect public health and improve the status of O_3 pollution, it is crucial to establish studies of health effects attributed to O_3 exposure from a national perspective. In addition, it is important for relevant government departments to establish a mechanism to revise the National Ambient Air Quality Standards (NAAQS) for regulatory assessment and health risk prediction of future O_3 air quality standards in China (46).

4.4. Research limitations and future prospects

Surface O_3 distribution has strong spatial and temporal heterogeneity, and there are significant differences in O_3 concentrations with time scales. This study only focused on the interannual spatial variability characteristics of O_3 concentrations, neglecting the seasonal variability of O_3 concentration changes. Furthermore, due to the lack of basic research data and inadequate research methods, this study only focused on the number of premature respiratory deaths attributed to O_3 pollution in the assessment of health risks attributed to O_3 pollution, neglecting the all-cause premature death group. Additionally, using the same exposure risk coefficient (β) may lead to spatial errors in the estimated health risks due to significant spatial differences in O_3 exposure levels. For example, Wang et al. (21) estimated the population of premature deaths from respiratory diseases caused by O_3 pollution between 2013 and 2017 in China using the method of Turner et al. (47), and their results found an average of 186,000 deaths from respiratory diseases due to O_3 pollution during the study period. This is slightly lower compared to our findings. A primary reason for this is that our study and Wang et al. (21) used different exposure response coefficients and critical thresholds. In addition, the interpolation of O_3 concentrations at large scales of pollution can also cause large errors in the assessment results. Therefore, in the future, we hope to conduct a detailed and comprehensive analysis of seasonal differences in O_3 pollution and all-cause health risks in China by utilizing more detailed surface O_3 monitoring data and meta-analysis methods. To provide a scientific basis for the improvement of O_3 pollution in China.

5. Conclusions

In this study, we quantitatively investigated the spatial and temporal patterns, trends, population exposure risks, health risks,

and drivers of surface ozone in China from 2013 to 2018. We observed the annual average O_3 concentration of China increased significantly at a rate of change of 1.84 $\mu g/m^3/yr$ from 2013 to 2018 ($p < 0.05$, $R^2 = 0.561$). The significant increase was mainly distributed in East China, Central China, and North China. Meanwhile, the growth rate of O_3 concentration has a consistent and enhanced positive spatial autocorrelation ($p < 0.05$), and there are significant hot and cold spots areas. During the research period, there was an average of over 24,000 premature deaths from respiratory diseases attributed to O_3 exposure in China from 2013 to 2018, and the growth rate fluctuated at 1,178 per year ($p < 0.05$). Spatially, there was a consistency in the spatial distribution of exposure risk and health risk of populations exposed to O_3. The results of the multi-scale geographically weighted regression model reveal spatial differences in the effect of various factors on O_3 concentration. The impact of the total population, disposable income, the share of secondary industry in GDP, and NOx emissions factors in eastern and northern regions are significantly greater than impacts in central and western regions. Meanwhile, we found that the effect of temperature on O_3 concentration in some cities in the north, east, and northeast is significantly higher than that in other regions, and relative humidity has a significant ($p < 0.01$) strong negative correlation with O_3 concentration in east, central, southwest and south China.

Data availability statement

The datasets presented in this study can be found in online repositories. The names of the repository/repositories and accession number(s) can be found in the article/supplementary material.

Author contributions

CH conceived the idea of the study and designed research, wrote the paper, discussed the results, and revised the manuscript. LZ conceived the idea of the study and design research, discussed the results, and revised the manuscript. XG involved in funding acquisition, resources, supervision, and analyzed the data. BL and JL discussed the results and revised the manuscript. QW analyzed the data, discussed the results, and revised the manuscript. All authors contributed to the article and approved the submitted version.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

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Endocrine disrupting chemicals: A promoter of non-alcoholic fatty liver disease

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Non-alcoholic fatty liver disease (NAFLD) is the most prevalent liver disorder. With the improvement in human living standards, the prevalence of NAFLD has been increasing in recent years. Endocrine-disrupting chemicals (EDCs) are a class of exogenous chemicals that simulate the effects of hormones in the body. There has been growing evidence regarding the potential effects of EDCs on liver health, especially in NAFLD. This paper aims to summarize the major EDCs that contribute to the growing burden of NAFLD and to raise public awareness regarding the hazards posed by EDCs with the objective of reducing the incidence of NAFLD.

KEYWORDS

non-alcoholic fatty liver disease, endocrine-disrupting chemicals, per-/polyfluorinated substance, bisphenol A, polychlorinated biphenyls, phthalates

1. Introduction

Non-alcoholic fatty liver disease (NAFLD) is an important public health issue that affects a large portion of the global population. Estimates indicate that the worldwide prevalence of NAFLD ranges from 13% in Africa to 42% in Southeast Asia (1). NAFLD encompasses a spectrum of liver conditions, including simple steatosis or non-alcoholic fatty liver (NAFL), which has a milder course, and non-alcoholic steatohepatitis (NASH), with potential progression to fibrosis or cirrhosis and hepatocellular carcinoma (HCC) (2, 3). The hallmark of NAFLD is the accumulation and deposition of excessive fat in liver cells, which may be related to genetic, dietary, and environmental factors (4). These factors promote the onset of insulin resistance (IR) in adipose tissue, leading to adipocyte dysfunction and increased influx of free fatty acids (FFAs) into the liver (5). These FFAs and their consequent lipotoxic intermediates have been shown to have adverse effects such as abnormal lipids metabolism, oxidative stress, and chronic liver inflammation, all of which contribute to the progression of NAFLD (6, 7).

Environmental endocrine disrupting chemicals (EDCs) are a class of exogenous chemicals that mimic the effects of hormones in the body, causing hormonal dysregulation and mediating various metabolic disorders. The liver, an organ crucial to metabolism and detoxification (8), has been shown to be impacted by EDCs, with studies suggesting exposure to these chemicals can lead to metabolic changes and liver disease (9–12). Because of the difficulty in getting biopsy-confirmed NAFLD histological specimens, the liver injury is typically assessed using serum biomarkers of hepatoxicity of NAFLD. Alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP) and γ-glutamyl transferase (GGT) are considered specific biomarkers of liver injury, they are widely used to evaluate the progression of NAFLD. Given the role of EDCs in the worldwide deterioration of metabolic health, it is imperative that the scientific community continues to study and understand their effects.

We aimed to provide an overview of EDCs and summarize the effects of EDC exposure on NAFLD.

1.1. Overview of EDCs

Environmental endocrine disruptors (EDCs) are a heterogenous group of chemicals that are widely distributed and easily enriched, present in many forms. In daily life, an increasing number of substances have been identified as EDCs; they enter the body through the digestive tract, respiratory tract, or skin, and produce adverse effects. EDCs can be classified as natural or synthetic, based on their origin. Natural EDC include phytoestrogens and mycotoxins, while synthetic EDCs include chemicals used as industrial solvents and their byproducts (polychlorinated biphenyls, polybrominated biphenyls, and dioxins), plastics (bisphenol A), plasticizers (phthalates), fungicides (vinclozolin), pesticides (methoxychlor and chlorpyrifos), heavy metals (mercury and lead), and pharmaceutical agents present in human and animal foods (11, 13–16).

Studies have shown that EDCs can interfere with various aspects of hormone regulation in the body, including production, release, transport, metabolism, binding, action, and elimination, leading to hormonal dysregulation and contributing to various metabolic disorders (17, 18). It's also been found that EDCs play a potential role in the regulation of genomic expression, promoting epigenetic modifications that result in the development of pathologies by mediating carcinogenic, neurotoxic, hepatotoxic, and immunotoxic effects (19–21). The challenge in understanding the impact of EDCs is compounded by the fact that humans are exposed, not to a single environmental pollution compound, but to a cocktail of EDCs, making it even more difficult to predict the net effect and evincing the association between a specific EDC and disease (13, 22). Moreover, exposure to persistent EDCs may initiate and promote the pathogenesis of NAFLD (23). EDCs affect the progression of NAFLD through the interaction of nuclear receptors. Activating transcription factors, triggering the imbalances between lipid flow/outflow in the liver, promoting mitochondrial dysfunction, and mediating the hepatic inflammatory are the possible mechanism of NAFLD (17).

2. Methods

In this study, we focused on several key EDCs that are closely related to human health, including per−/polyfluorinated substances, bisphenol A, polychlorinated biphenyls, and phthalates (as outlined in Table 1). Systematic search of PubMed and Embase databases was conducted from January 1, 2010, to December 28, 2022, to identify human studies investigating the relationship between non-alcoholic liver disease and these EDCs. Detailed search strategies are presented in Table 2. After screening the retrieved studies based on their titles and abstracts, we excluded studies without human data, case reports, non-original reports, studies without NAFLD outcomes, and pharmacological or ecological studies.

3. Relationship between NAFLD and EDCs

3.1. Per-/polyfluorinated substance

Per-/polyfluorinated substance (PFAS) is a series of organic compounds containing at least one perfluorinated carbon atom, it is lipophobic and hydrophobic that are useful for manufacturing wide ranges of consumer products (24, 25). PFASs have a stable chemical structure with a half-life of about 2–8 years, allowing them to persist and accumulate in the environment (10, 26). Because of these properties, PFASs are classified as "persistent organic pollutants" (POPs), and their delayed-elimination feature may cause long-term harmful health effects. PFASs have been detected in drinking water, various foods (meat, vegetables, milk, eggs), air, and early life placental or breast milk, and are able to accumulate in biological tissues and organs with high protein content (27–29). Nearly all adults in the U.S. have been found to have accumulated PFAS in their body tissues (10). Current studies have indicated that four congeners account for most human exposure: perfluorooctanesulfonic acid (PFOS), perfluorooctanoic acid (PFOA), perfluorohexanesulfonic acid (PFHxS), and perfluorononanoic acid (PFNA) (10, 30, 31). Compared to the general population, worker in certain occupations (e.g., professional ski-waxers, firefighters, fluorochemical plant workers) experience high PFAS serum concentrations based on their occupation (32). The median concentrations were 24-27 ng/ml (PFOS), 50-57 ng/ml (PFOA), 1.4–1.6 ng/ml (PFHxS) and 12-13 ng/ml (PFNA) in professional ski-waxers (33). And the median concentrations in the general population were 3.59–24.22 ng/mL (PFOS) (34, 35), 0.99–28.0 ng/mL (PFOA) (36, 37), 0.59–1.80 ng/mL (PFHxS) (38, 39), and 0.24–1.60 ng/mL (PFNA) (34, 37), respectively.

The liver is one of the main target organs of PFAS toxicity (40). However, the exact mechanism of PFAS hepatotoxicity remains unclear. PFAS are thought to act as ligands for peroxisome proliferator-activated receptors (PPARs), which promote liver inflammation and

TABLE 1 Characteristic of major endocrine-disrupting chemicals (EDCs).

Substance	Abbreviation	Source	Characteristic
Per-/polyfluorinated substance	PFAS	Contaminated drinking water, foods, air, etc	Environmental persistence, bioaccumulation, potential hazards
Polychlorinated Biphenyls	PCBs	Electrical equipment, soil, aquatic sediments, contaminated food, etc	Thermodynamically stable, degradation-resistant, bioaccumulation
Bisphenol A	BPA	Plastic containers and toys, food packaging materials, dental sealants, etc	Low lipophilicity, rapid degradation, short half-time
Phthalates	DEPH	Plasticizers in food wrapping and packaging, coatings, cosmetics, adhesives, medical tubes, ect	Rapid metabolism, strong adsorption

TABLE 2 Literature review search terms.

Substances	Database	Search terms
NAFLD	PubMed	NAFLD OR NASH OR "nonalcoholic fatty liver disease" OR "nonalcoholic steatohepatitis" OR "nonalcoholic fatty liver" OR "fatty liver" OR steatosis OR "liver enzymes" OR"liver damage" OR "liver injury" OR "liver fibrosis"
	Embase	nafld OR nash OR 'nonalcoholic fatty liver disease'/exp. OR 'nonalcoholic fatty liver disease' OR 'nonalcoholic steatohepatitis'/exp. OR 'nonalcoholic steatohepatitis' OR 'nonalcoholic fatty liver'/exp. OR 'nonalcoholic fatty liver' OR 'fatty liver'/exp. OR 'fatty liver' OR 'steatosis'/exp. OR steatosis OR 'liver enzymes'/exp. OR 'liver enzymes' OR 'liver damage'/exp. OR 'liver damage' OR 'liver injury'/exp. OR 'liver injury' OR 'liver fibrosis'/exp. OR 'liver fibrosis'
Per-/polyfluorinated substance	PubMed	Perfluoroalkyl OR Polyfluoroalkyl OR Perfluorinated OR polyfluorinated OR perfluoro* OR polyfluoro* OR PFAS OR PFAS* OR "Perfluorinated chemicals" OR Perfluorocarbons OR Polyfluorocarbons OR "Per- and Polyfluoroalkyl Substances"
	Embase	'perfluoroalkyl'/exp. OR perfluoroalkyl OR 'polyfluoroalkyl'/exp. OR polyfluoroalkyl OR perfluorinated OR polyfluorinated OR perfluoro* OR polyfluoro* OR pfas OR pfas* OR 'perfluorinated chemicals' OR perfluorocarbons OR polyfluorocarbons OR 'per- and polyfluoroalkyl substances'
Bisphenol A	PubMed	BPA OR "Bisphenol A" OR Bisphenol* OR "bisphenol A glycidyl methacrylate" OR "4,4-dihydroxy-2,2-diphenylpropane" OR "diphenylolpropane" OR "2,2-bis(4-hydroxyphenyl)propane" OR 'bisphenol A, sodium salt" OR "bisphenol A, disodium salt"
	Embase	bpa OR 'bisphenol a' OR bisphenol* OR 'bisphenol a glycidyl methacrylate' OR '4,4-dihydroxy-2,2-diphenylpropane' OR 'diphenylolpropane' OR '2,2-bis(4-hydroxyphenyl)propane' OR 'bisphenol a, sodium salt' OR 'bisphenol a, disodium salt'
Polychlorinated Biphenyls	PubMed	PCB OR PCB* OR "Polychlorinated Biphenyls" OR "Polychlorobiphenyl Compounds" OR "Polychlorinated Biphenyl" OR PBB OR PBB* or "Polybrominated biphenyls" OR "Polybromobiphenyl Compounds" OR "Polychlorinated terphenyls" OR PCN OR PCN* OR "Polychlorinated naphthalenes"
	Embase	'pcb'/exp. OR pcb OR pcb* OR 'polychlorinated biphenyls'/exp. OR 'polychlorinated biphenyls' OR 'polychlorobiphenyl compounds' OR 'polychlorinated biphenyl'/exp. OR 'polychlorinated biphenyl' OR pbb OR pbb* OR 'polybrominated biphenyls'/exp. OR 'polybrominated biphenyls' OR 'polybromobiphenyl compounds' OR 'polychlorinated terphenyls' OR pcn OR pcn* OR 'polychlorinated naphthalenes'
Phthalates	PubMed	"di-2-ethylhexyl phthalate" OR phthalate OR DEHP OR "Di(2-ethylhexyl) phthalate" OR "Phthalate" OR "Phthalates" OR "Dibutyl phthalate" OR "di-n-butyl phthalate" OR "di-isobutyl phthalate" OR DBP OR DiBP OR "mono (2-ethylhexyl) phthalate" OR "MEHP" OR "monomethyl phthalate" OR "mono (2-ethyl-5-carboxypentyl) phthalate" OR "MBP" OR "mono-(3-carboxypropyl) phthalate"
	Embase	'di-2-ethylhexyl phthalate'/exp. OR 'di-2-ethylhexyl phthalate' OR phthalate OR dehp OR 'di(2-ethylhexyl) phthalate'/exp. OR 'di(2-ethylhexyl) phthalate' OR 'phthalate'/exp. OR 'phthalate' OR 'phthalates' OR 'dibutyl phthalate'/exp. OR 'dibutyl phthalate' OR 'di-n-butyl phthalate'/exp. OR 'di-n-butyl phthalate' OR 'di-isobutyl phthalate' OR 'dbp'/exp. OR dbp OR dibp OR 'mono (2-ethylhexyl) phthalate'/exp. OR 'mono (2-ethylhexyl) phthalate' OR 'mehp' OR 'monomethyl phthalate'/exp. OR 'monomethyl phthalate' OR 'mono (2-ethyl-5-carboxypentyl) phthalate' OR 'mbp' OR 'mono-(3-carboxypropyl) phthalate'

triglyceride accumulation through the activation of PPARα and lead to liver injury or NAFLD (41–43). The complementary mechanism also includes activation of the constitutive androstane receptor (CAR) (42, 44), downregulation of nuclear factor erythroid 2-related factor 2 (NRF2) (45, 46), and upregulation of nuclear factor kappa-light-chain-enhancer of activated B cells nuclear factor-kappa B (NF-κ B) (47). Animal and epidemiological studies have shown that PFAS can cause an obviously increase in liver lipid volume, induce mitochondrial dysfunction and oxidative stress, and promote inflammatory responses in NAFLD progression (41, 48, 49).

A large number of nuclear receptors (NRs) were expressed in liver, making it a critical target for PFAS. In this review, we noticed that a few studies have evaluated the hepatic enzyme abnormalities associated with PFAS exposure. PFOA and PFNA exposure is usually positively associated with higher ALT and GGT levels, suggesting that changes in serum biomarkers are often accompanied by histopathological changes or liver disease (34, 39, 50–53). However, for AST, studies have reported different outcomes in different crowds. Khalil (37) found that there was no relationship between serum PFAS and AST levels in obese children, while PFOA and PFOS were positively correlated with AST in Japanese children (52). In addition, Attanasio et al. reported sex-specific histological effects of PFAS exposure and found positive associations between PFAS and ALT in female adolescents, but conversely in male adolescents, which could be mediated by sex hormones (54). Evidence for sex-specific differences was also found in rats, with ALT increasing more frequently in male rats (55–57). The authors suggest that PFAS exposure may play an important role in the development of NAFLD and carcinoma (Table 3). The PFAS was positively associated with lobular inflammation in adults undergoing bariatric surgery in Northern Europe, however, the reason for this association is unclear. It may be related to changes in lipid and bile acid metabolism (9, 59). In animal models, exposure to subchronic PFOS has been found to enhance hepatic stellate cell (HSC) activation and exacerbate carbon tetrachloride (CCl_4)-induced liver fibrosis (60), consistent with the outcomes of Sen's study, which showed that PFOS was positively associated with hepatic fibrosis in adults (9). In contrast, a study including adults from the C8 Health Project in the USA showed that cumulative PFOA exposure had no effect on all liver diseases, enlarged liver, or cirrhosis (58). Additionally, a study included 1,105 mother–child pairs from the European Human Early-Life Exposome (HELIX) cohort showed that higher exposure to PFAS during pregnancy was

TABLE 3 Epidemiologic studies on the relationship between PFAS and NAFLD or carcinoma.

Substance	Reference	Country	Population	Sample size	Biological materials	Measurement	Exposure assessment	Outcomes	Results	Adjustment factors
PFAS	Sen et al. (9)	Sweden	Adults undergoing laparoscopic bariatric surgery (18–75 years)	105	Serum	UPLC-QTOFMS	PFHxS, PFNA, PFOA, PFOS	NASH, hepatic fibrosis, macrosteatosis	PFOS, PFOA were positively associated with NASH (necroinflammatory grades), while PFOS was positively associated with hepatic fibrosis	NR
PFAS	Jin et al. (35)	USA 2007–2015	Children with NAFLD	74	Plasma	HRMS	ORs and 95%CI for liver histology in relation to PFOA, PFOS, PFHxS, PFAS score (per IQR increase)	Histologic severity of NAFLD	The odds of having NAFLD was significantly increased with each IQR increase of PFOS and PFHxS. Each IQR increase of PFHxS was associated with increased OR for liver fibrosis, lobular inflammation and higher NAFLD activity score.	NR
PFAS	Darrow et al. (58)	USA 2008–2011	C8 Health Project (age ≥ 20 years)	28,047	Serum	Exposure estimation	Median PFOA: 1.65 ng/mL	Validated liver disease, medically validated enlarged liver, fatty liver, cirrhosis	No evidence of an effect of cumulative PFOA exposure on all liver disease, nor on enlarged liver, fatty liver, and cirrhosis.	Age, sex, BMI, alcohol consumption, race, regular exercise, smoking status, education, household income, fasting status, worker at plant, insulin resistance
PFAS	Rantakokko et al. (59)	Finland 2005–2010	Kuopio Obesity Surgery Study	161	Serum	NR	Median(5th, 95th) ng/mL PFOA: 2.56(1.04, 4.66) PFNA: 0.83(0.30, 2.19) PFOS: 3.2(0.89–10.3) PFHxS: 1.18(0.54–2.90)	Steatosis, lobel inflammation, ballooning, fibrosis, liver phenotype	PFOA, PFNA, and PFHxS were inversely associated with lobular inflammation at baseline.	Age, fasting insulin, weight change

HRMS, high-resolution mass spectrometry; LC-HRMS, liquid chromatography with high-resolution mass spectrometry; UPLC-QTOFMS, ultra-high-performance liquid chromatography quadrupole time-of-flight mass spectrometry.

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associated with higher liver enzyme levels in children (38). PFAS can cross the placenta barrier efficiently and deposit in fetal tissues (61), they altered some amino acid (e.g., valine, leucine, phenylalanine) and lipid (e.g., glycerophospholipid) metabolism that related to NAFLD pathogenesis, which exerted adverse effects in liver (38).

3.2. Polychlorinated biphenyls

Polychlorinated biphenyls (PCBs) are also a type of POP that are manufactured and used commercially as dielectric fluids in transformers (62). They are thermodynamically stable polyhalogenated aromatic hydrocarbons consisting of up to ten chlorine substituents attached to a biphenyl ring (63). Based on their structures, PCB congeners have been subclassified into dioxin-like (DL) and nondioxin-like (NDL). DL PCBs have a coplanar structure, whereas NDL PCBs have a noncoplanar structure, which can be attributed to receptor-based modes of action. PCBs persist in the environment and accumulate in soil, aquatic sediments, and in species that consume these sources (fish, cows, dairy) (64). Despite a ban on their production and emission in 1979, human exposure to PCBs usually occurs through contaminated air, water, or food. As POPs, PCBs accumulate in adipose tissue and are gradually released into the bloodstream (65, 66).

The liver appears to be both a target and an effector organ for PCB-induced endocrine disruption. The occurrence of NAFLD is due to an imbalance between lipid production and elimination, which promotes excessive accumulation of hepatic lipids (17). PCBs have been documented to be related to this phenomenon, as they can induce pathological fat aggregation, both in the DL and NDL groups (67). DLPCBs activate the aryl hydrocarbon receptor (AhR) and peroxisome proliferator-activated receptors alpha and gamma (PPARα/γ) (63, 68), exerting a multimodal effect on lipid accumulation and causing steatosis by disrupting liver lipid metabolism. NDL PCBs, on the other hand, activate the constitutive androstane receptor (CAR) and pregnane X receptor (PXR) (69), which could reduce the protective response of the liver to promote diet induced NAFLD (67).

Studies have shown that PBC exposure can cause NAFLD related metabolic disorders, including insulin resistance, obesity, and lipid metabolic dysfunction. PCBs differentially regulate hepatic lipid metabolism and several related genes. PCB126 exposure increases hepatic lipids and causes toxicant-associated steatosis (mild small-droplet macro vesicular steatosis) (63). Mono-exposure to either PCB126 or Aroclor1260 increased hepatic lipid uptake while decreasing lipid biosynthesis, but these effects were abrogated by exposure to the NDL/DL PCB mixture (63). Pnpla3, a lipase implicated in NAFLD (70), was significantly upregulated by Aroclor1260 exposure, but was suppressed by either PCB126 or Aroclor1260/PCB126 exposure, potentially due to activation of different receptors among NDL or DL PCBs. PCBs have also been associated with the promotion of toxicant-associated steatohepatitis (TASH), which can ultimately lead to secondary liver necrosis, potentially due to a loss of protein phosphorylation and downregulation of the hepatic kinome (71). Positive associations were also observed between PCB concentrations and NAFLD-related biomarkers (Table 4), with most PCB congeners positively correlated with elevated alanine aminotransferase (ALT) levels. A study of 4,582 adults conducted by Cave et al. (76) showed that 10.6% of participants had unexplained ALT elevation, with older age significantly associated with total PCB levels in the highest quartile. Of those participants aged 70 years or older, 71.7% had high PCB levels compared to only 2.2% of those aged under 30 years. A study of 1,108 mother–child pairs from six countries by Midya (72) discovered that prenatal exposure to PCBs is a potential risk factor for pediatric NAFLD, and were further associated with increased CK-18 levels (a novel marker of hepatocyte apoptosis and NAFLD). Notably, researchers have identified potential therapeutic targets for improving PCB-induced NAFLD, including the anti-fibrotic compound recombinant FGF21, which reduced the overexpression of hepatic lipocalin-2 (LCN2), a group of transporters of small lipophilic molecules that are upregulated in several liver diseases, and attenuated NAFLD (62, 77).

3.3. Bisphenol A

Bisphenol A (BPA), which consists of two phenol rings attached by a methyl bridge with two methyl groups (78), is a plasticizer mainly used for polycarbonate plastics and epoxy resins in many consumer products (79). BPA exposure can occur *via* various sources such as plastic containers, toys, water bottles, food packaging materials, office supplies, and dental sealants (80, 81). BPA has low lipophilicity and degrades rapidly with a half-life of 4–5 h (82). Due to its broad application, BPA is detected in more than 90% of people, and the median urine BPA concentration in adults is 2.24–6.17 ng/mL (83, 84).

The liver is the main organ that metabolizes and transforms BPA into glucuronidation; therefore, it is more susceptible to BPA than other organs (85). BPA increases the risk of NAFLD owing to fat accumulation, obesity, and oxidative stress. Upregulation of lipogenic enzymes and transcription factors, such as sterol regulatory element binding protein-1c (*srebp-1c*), the carbohydrate responsive element binding protein (ChREBP), and liver X receptor (LXR) (86), promotes *de novo* lipogenesis (DNL) (87), increasing the risk of lipid accumulation and obesity. In addition, exposure to high doses of BPA decreased the activities of antioxidant indicators, such as superoxide dismutase (SOD) and glutathione (GSH), causing excessive accumulation of free radicals, such as reactive oxygen species (ROS), promoting liver damage and hepatotoxicity (88).

Aminotransferases are the most widely used biomarkers in experiments, and are released into the bloodstream following liver injury (89). Epidemiological studies have shown that BPA exposure could have negative effects on NAFLD-related biomarkers (Table 5). Higher urinary BPA levels usually led to the elevation of ALT (83, 96, 97). A study carried out by Lang et al. reported that higher BPA levels are linked to abnormal GGT (Odds ratio [OR]:1.29, 95% Confidence Interval [CI]:1.14–1.46) and ALP (OR:1.48, 95%CI:1.18–1.85) (98). In addition to liver biomarkers, urinary BPA levels were positively associated with the prevalence of NAFLD in adults and adolescents. In the Korean National Environmental Health Survey (83), which included 3,476 participants with a mean age of 52.96 ± 0.25 years old, the geometric mean concentration of BPA in the NAFLD group was significantly higher than in the non-NAFLD group (2.56 ug/L vs. 2.24 ug/L, $p = 0.001$). A study analyzing adolescents (12–19 years old) from NHANES in the USA also showed that the risk of suspected NAFLD (ALT≥30 U/L) was increased in participants in higher quartiles of BPA exposure (93). In terms of pathological findings, mice and rats treated with BPA showed liver tissue dilatation of sinusoids,

TABLE 4 Epidemiologic studies on the relationship between PCBs and NAFLD related biomarkers.

Substance	Reference	Country	Population	Sample size	Biological materials	Measurement	Exposure assessment	Outcomes	Results	Adjustment factors
PCB	Midya et al. (72)	France, Greece, Lithuania, Norway, Spain, UK 2021–2022	Mother–child pairs from the Human Early-Life Exposome project	1,108	Serum	GC-MS/MS	LOD used in NIPH PCB180: 0.91 pg./g PCB170: 0.61 pg./g PCB153: 0.61 pg./g PCB138: 0.61 pg./g PCB118: 0.31 pg./g	ALT, AST, GGT and CK-18 of children	A 1-quartile increase in prenatal exposure was associated with increased CK-18 for PCBs and constitute a potential risk factor for pediatric non-alcoholic fatty liver disease.	Subcohort, maternal age, maternal prepregnancy BMI, maternal educational level, parity, child age, child sex
PCB	Rantakokko et al. (59)	Finland 2005–2010	Kuopio Obesity Surgery Study	161	Serum	NR	Median ng/g lipid PCB-118: 15.2 (normal) 9.42(steatosis) 10.1(NASH)	Steatosis, lobel inflammation, ballooning, fibrosis, liver phenotype	PCB-118 was associated with NASH, lobular inflammation, few liver cell balloon, and S2-S3 steatosis grade at baseline.	Age, BMI, sex, fasting insulin
PCB	Clair et al. (73)	USA	ACHS adults	738	Serum	HRGC/HRMS	PCB congeners (28, 44, 49, 52, 66, 101, 105, 110, 128, 149, 151, 172, 178, 187, 195)	TASH, CK18	TASH was associated with increased exposures to specific PCB congeners.	Age, BMI, gender, race, diabetes status, alcohol use, total lipid levels
PCB	Kumar et al. (74)	Sweden	Prospective Investigation of the Vasculature in Uppsala Seniors (≥70 years)	992	Serum	HRGC–MS	PCB congeners (74, 99, 105, 118, 126, 138, 153, 156, 157, 169, 170, 180, 189, 194, 206, 209)	Bilirubin, ALP, ALT, GGT	PCBs was not associated with bilirubin, ALP, and GGT. PCB-74, 105, and 118 were found to be significant in positive direction with ALT.	Age, sex, kidney function, smoking BMI, education, physcial activity, waist circumference, fasting blood glucose, systolic blood pressure, use of cardiovascular medication
PCB	Serdar et al. (65)	USA 2003–2004	NHANES (>12 years)	1,935	Serum	HRGC/HRMS	PCB congeners	ALT, AST, GGT	Liver enzymes (AST, ALT, GGT) were significantly higher in the highest exposure groups of PCBs. ALP dropped as levels of PCBs increased.	Age, gender, relevant survey design, subsample, population weights
PCB	Christensen et al. (75)	USA 2003–2004	NHANES (>12 years)	1,345	Serum	HRGC/HRMS	PCB congeners (DL and NDL)	ALT	The DL PCB, the NDL PCB were significant associated with elevated ALT.	Age, sex, race/ethnicity, income, BMI
PCB	Cave et al. (76)	USA 2003–2004	NHANES adults	4,582	Serum	HRGC/HRMS	PCB congeners	ALT	9 of coplanar PCBs (66, 74, 105, 118, 126, 156, 157, 167, 169) were positively associated with elevated ALT. 11 of NDL PCBs (138 and 158, 146, 151, 153, 170, 172, 177, 178, 183, 187, 196 and 203,) were positively associated with ALT elevation.	Age, race/ethnicity, sex, BMI, poverty income ratio, insulin resistance.

GC-MS, gas chromatograph-mass spectrometry; GC-MS/MS, gas chromatography coupled to tandem mass spectrometry; HRGC/HRMS, high-resolution gas chromatography/isotope dilution high-resolution mass spectrometry; HRGC-MS, high-resolution gas chromatograph coupled to mass spectrometry.

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TABLE 5 Epidemiologic studies on the relationship between BPA and NAFLD related biomarkers.

Substance	Reference	Country	Population	Sample size	Biological materials	Measurement	Exposure assessment	Outcomes	Results	Adjustment factors
BPA	Fu et al. (90)	China 2017–2018	Children (5–14 years)	1,006	Serum	HPLC	Median BPA: 26.31 ng/mL	ALT, AST, TBIL	Exposure to BPA would have negative effects on hepatic function, and these effects showed differences in gender and geographical location.	Age, address, gender
BPA	An et al. (83)	Korea 2015–2017	KoNEHS (≥18 years)	3,476	Urine	UPLC	Geometric mean (SE) ug/L BPA: 2.24(0.08) non-NAFLD 2.56(0.15) NAFLD	NAFLD prevalence ALT, AST, GGT	The prevalence of NAFLD and abnormal ALT were increased in accordance with the increase of urinary BPA concentrations. There were no relationships between AST, GGT and BPA levels.	Age, sex, drinking and smoking status, physical activity, household income, education level, marriage, medication taking
BPA	Federico et al. (91)	Italy 2017	Male patients with NAFLD	32	Urine, plasma	HPLC LCMS/MS	mean ± SD ng/mL Plasm BPA: 6.45 ± 4.51 Urine free BPA: 2.73 ± 2.06 Urine total BPA: 5.84 ± 3.07	ALT, AST, GGT	NAFLD patients showed higher levels of ALT, plasmatic, free urine and total urine BPA.	NR
BPA	Kim et al. (92)	USA 2005–2014	NHANES adults	7,605 (HSI) 3,631 (USFLI)	Urine	SPE-HPLC	NAFLD and ALT according to BPA levels.	NAFLD defined by HIS or USFLI	The prevalence of NAFLD and abnormal ALT levels was correlated with urinary BPA levels.	Race/ethnicity, education, hypertension, diabetes, smoking status, alcohol consumption
BPA	Verstraete et al. (93)	Spain 2003–2010	NHANES adolescents (12–19 years)	944	Urine	HPLC-MS	NAFLD and ALT according to BPA levels. Median(IQR) BPA: 2.6(1.3–5.3) ng/mL NAFLD	NAFLD risk	Risk of suspected NAFLD was increased in the second quartile of BPA levels.	Age, gender, race/ethnicity, country of birth, poverty to income ratio, tobacco exposure, daily caloric intake
BPA	Lee et al. (94)	Korea 2005–2016	Children of Ewaha Birth and Growth Cohort Study	164	Urine	HPLC	Median(IQR) ug/L BPA: 0.61(0.35–1.09) 3–5 years old 0.60(0.34–1.15) 7–9 years old	AST, ALT, GGT	The urinary BPA concentrations at 7–9 years was associated with the serum levels of liver enzymes at 10–13 years of age, but 3–5 years not.	sex, age, BMI, monthly household income, maternal educational level, pubertal status, the frequencies of canned fish and soft drink consumption, exposure to secondhand smoke
BPA	Dallio et al. (84)	Italy	NAFLD patients with or without T2DM	60	Urine plasma	LC-MS/MS	Urine BPA: 6.17 ± 0.85 ng/ml NAFLD 0.80 ± 0.17 ng/mL control plasma BPA: 5.30 ± 0.78 ng/ml NAFLD 0.36 ± 0.06 ng/ml control	ALT, AST, GGT grade of NAFLD	BPA resulted to be significantly higher in NAFLD subjects compared to controls both in urine and plasma. BPA plasma levels in NASH patients was higher in NAFL patients.	NR
BPA	Albeldawi et al. (95)	USA 2005–2006	NHANES (18–74 years)	175	Urine	SPE-HPLC-MS/MS	OR (95%CI) Urinary BPA (1 ng/mL, increase): 0.92(0.83, 1.02)	ALT	BPA exposure was not associated with abnormal ALT levels and risk of liver disease.	Age, sex, race/ethnicity, education, smoking, BMI, waist circumference, urinary creatinine concentration

UPLC, ultra-high-performance liquid chromatography; HPLC, high-performance liquid chromatography; SPE, solid-phase extraction; HPLC-MS, high-performance liquid chromatography–tandem mass spectrometry; LC-MS, liquid chromatography-mass spectrometry; LC-MS/MS, liquid chromatography coupled to tandem-mass spectrometry.

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congestion, inflammation, and necrosis in a dose-dependent manner (99). Although BPA can be excreted quickly from the body, people are constantly exposed to it throughout their lives and BPA exposure is associated with metabolic health in offspring. Prenatal BPA exposure has been shown to alter gene expression profiles and result in peripheral insulin resistance and liver lipotoxicity (100, 101). Gestational BPA exposure can promote the development of NAFLD in rodent models through the perturbation of the nuclear transcription factor activity (102). Another study indicated that exposure to BPA may diminish the immune response following hepatitis B vaccination (79).

3.4. Di-(2-ethylhexyl) phthalate

Phthalates are a large group of ubiquitous industrial chemicals that are commonly used in a variety of products such as plasticizers in food wrapping and packaging, coatings, cosmetics, adhesives, medical tubes, and toys (103, 104). Phthalates may enter the human body through the skin, respiratory tract, digestive tract, or even intravenous injection, as they are prone to leaching and transfer to air, soil, or food (105, 106). These chemicals are usually rapidly metabolized and excreted within 24–48 h. Diester phthalates could hydrolyze into monoester phthalates, then excreted as glucuronide conjugates, in the urine (107).

The development of NAFLD may be related to the adverse effects of DEHP on lipid metabolism and oxidative stress. DEHP and its active metabolite mono-(2-ethylhexyl) phthalate (MEPH) can affect hepatic accumulation of TGs and exacerbate NAFLD in rodents (108). MEHP also can affect the lipid accumulation in BRL-3A hepatocytes through the inhibition of the Janus kinase 2/Signal transducer and activator of transcription 5 (JAK2/STAT5) pathway, suggesting that the regulation of STAT5 by MEPH plays a critical role in the activation of enzymes involved in fatty acid metabolism (109). Furthermore, DEHP also mediates the deterioration of antioxidant machinery and induces oxidative stress. Higher levels of ROS were observed in MEHP-treated cells, indicating the effect of ROS on pro-inflammatory cytokine production and apoptosis of hepatocytes by inducing NF-κB (110, 111). Other experimental studies in animals have shown that the toxicity of phthalates drives liver fibrosis by oxidative stress pathways (112, 113).

Phthalate exposure is strongly associated with the NAFLD prevalence (114) (Table 6). A study involving 5,800 Korean adults demonstrated that the prevalence of NAFLD defined by the hepatic steatosis index (HSI) was associated with high urinary levels of many types of phthalates, and higher quartiles of MEHHP revealed a significantly higher risk (OR 1.39, 95% CI:1.00–1.92) of NAFLD (119). In addition, NAFLD measured using vibration-controlled transient elastography (VCTE) was also found to be positively associated with MECPP and MEHHP exposure (116). Unlike in adults, DEHP exposure also affects the prevalence of NAFLD in adolescents. Berman et al. (115) studied 387 mother–child pairs in Australia and found that mid-level prenatal exposure to MnBP was associated with a greater incidence of NAFLD at 17 years old. Table 6 also lists epidemiologic studies on the relationship between NAFLD biomarkers. A study involving 102 males aimed to examine the influence of MEP and MEHP on liver function and found that phthalate exposure may be associated with a statistically significant increase in ALT and AST serum levels, while urinary phthalate levels may be correlated with increased serum TG and decreased HDL cholesterol levels (104). A transversal study also demonstrated that serum MEHP levels were correlated with GGT (122). Thus, DEHP may interfere with thyroid function and induce NAFLD. Yang et al. divided 2,308 adults with subclinical hypothyroidism (SCH) into NAFLD and non-NAFLD groups according to the HSI score and found that the levels of phthalate metabolites in urine are positively associated with NAFLD with SCH (118). DEHP possesses a thyroid receptor antagonistic function, while thyroid hormones can activate TH-Receptor β (a potential target in NAFLD therapy) and decrease hepatic steatosis, which may further induce NAFLD (123–125). However, not all studies have discovered an association between phthalates and thyroid hormones, and further studies ought to be conducted to investigate this association.

3.5. Sex differences of association between EDCs and NAFLD

Liver expresses androgen and estrogen receptors (126), thus research on sex-specific differences in EDCs has been a hot topic, but no consistent conclusion has been made so far. In the analyses of NHANES 2013–2016, it was observed that an opposite direction of the statistically significant association between PFAS and liver enzyme by sex, elevated AST is associated with increased PFOA in female adolescents, whereas there is an inverse association with increased PFOA, PFNA and PFHxS in males (54). However, Borghese et al. using the data from Canadian Health Measures Survey found that the association between PFOA and AST was twice as strong among men vs. women, this could be because menstruation, pregnancy, and breastfeeding are all prominent excretion pathways for PFAS in women (25). The sex difference was also reported in PCB, Li et al. (127) found that increased mortality from hepatic disease in PCB-exposed, it may be explained by sex-specific effects of estrogenic PCB congeners (73, 128). Relationships between BPA exposure and liver function at puberty were observed, serum AST levels were positively associated with BPA in boys, and the effect sizes were larger for all indicator in boys (94). Due to the differences in sex hormone associated BPA metabolism, women expressed higher levels of the UGT2B1 to catalyze BPA glucuronidation and accelerate the clearance of BPA (129, 130). Trasande et al. (131) identified a near-significant interaction of DEHP metabolites with sex, suggesting a possible role of reduced androgen activity. EDC's sex difference is complicated, further research is needed to be done.

4. Conclusion

Exposure to environmental chemicals is ubiquitous and poses a threat to human metabolic health. EDCs affect NAFLD by interacting with nuclear receptors (NRs) and activating transcriptional factors, which promote hepatic lipid accumulation, oxidative stress, and liver dysfunction. Data from epidemiological studies prove an interrelationship between EDCs exposure and NAFLD. However, several challenges remain. For example, EDC mixtures are a complicated issue, and it is difficult to predict the net effect of EDC mixtures at the individual level in humans because it is difficult to perform laboratory detection for individual isomer and every human has a unique exposome (22, 90). In addition, the interpretation of the results on the effects of EDCs has been complicated by using different

TABLE 6 Epidemiologic studies on the relationship between phthalates and NAFLD prevalence and biomarkers.

Substance	Reference	Country	Population	Sample size	Biological materials	Measurement	Exposure assessment	Outcomes	Results	Adjustment factors
DEHP	Berman et al. (115)	Australia 1989–1992 (prenatal)	Mother–child pairs from the Raine Study	387	Maternal serum	LCMS/MS	Phthalate diesters	NAFLD at 17 years old ALT, AST, GGT	Mid-levels of prenatal exposures to MnBP were associated with a greater incidence of NAFLD.	Age, household income at birth, maternal education level at birth, duration of breast feeding, BMI z-score, height
DEHP	Chen et al. (116)	USA 2017–2018	NHANES adults	1,450	Urine	HPLC-ESI-MS/MS	Mean ± SD MECPP: 1.89 ± 0.03 ug/g MEOHP: 0.99 ± 0.03 ug/g MEHHP: 1.44 ± 0.03 ug/g MCiNP: 0.16 ± 0.02 ug/g MCiOP: 1.51 ± 0.04 ug/g MCiNP: 0.19 ± 0.03 ug/g	NAFLD prevalence	Higher prevalence of NAFLD is correlated with MECPP and MEHHP. There is no significant relationship between phthalates and liver fibrosis.	Age, sex, smoking status, education, race/ethnicity, physical activity, diabetes, blood pressure, BMI, total cholesterol levels
DEHP	Fu et al. (90)	China 2018.7–8	Children (5–14 years)	1,006	Serum	HPLC	Median DMP: 31.62 ng/mL	ALT, AST, TBIL	Serum DMP concentration and TBIL level were significantly positively correlated.	Age, address, gender
DEHP	Li et al. (117)	USA 1999–2014	NHANES participants	17,878 (HIS-NAFLD) 8,487 (USFLI-NAFLD)	Urine	HPLC-ESI-MS/MS	13 phthalates OR (95%CI) Urinary phthalates: 1.18(1.09–1.4)	NAFLD prevalence	Urinary phthalates were positively associated with NAFLD development.	Age, sex, race, education, family income-to-poverty ratio, marital status, employment, insurance, self-reported comorbidities, alcohol consumption, cigarettes smoking, leisure time physical activity, diet quality
DEHP	Midya et al. (72)	France, Greece, Lithuania, Norway, Spain, UK 2021–2022	Mother–child pairs from the Human Early-Life Exposome project	1,108	Serum	GC-MS/MS	10 phthalates	ALT, AST, GGT and CK-18 of children	Decreased odds of liver injury were associated with high-molecular-weight phthalates.	Subcohort, maternal age, maternal prepregnancy BMI, maternal educational level, parity, child age, child sex

(Continued)

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TABLE 6 (Continued)

Substance	Reference	Country	Population	Sample size	Biological materials	Measurement	Exposure assessment	Outcomes	Results	Adjustment factors
DEHP	Yang et al. (118)	Korea 2012–2014	Adults with subclinical hypothyroidism from KoNEHS	2,308	Urine	UPLC-MS	Geometric mean(95%CI) ug/L MEHHP: 3.02(2.97–3.06) EH 3.10(2.98–3.23) SCH MEOHP: 2.66(2.61–2.71) EH 2.76(2.64–2.89) SCH MECPP: 3.15(3.10–3.19) EH 3.22(3.11–3.33) SCH MBzP: 1.13(1.05–1.21) EH 1.02(0.84–1.20) SCH MnBP: 3.32(3.26–3.39) EH 3.35(3.22–3.48) SCH	Risk of NAFLD	The levels of phthalate metabolites in urine are positively associated with NAFLD in adults with subclinical hypothyroidism (SCH).	Age, gender, drinking, smoking, physical activity, monthly household income, education, marital status, clinical variables
DEHP	Cai et al. (114)	USA 2003–2016	NHANES adults (>20 years)	4,206	Urine	HPLC-ESI-MS/MS	9 phthalates (MEOHP, MEP, MEHHP, MECPP, MnBP, MEHP, MiBP, MBzP, MCPP)	ALT, AST, GGT	Phthalates exposure was independently associated with NAFLD both in males and females.	Age, gender, education levels, race/ethnicity, marital status, family poverty income ratio, BMI, total cholesterol, survey circle, smoking status, physical activity, hypertension, alcohol consumption
DEHP	Yang et al. (119)	Korea 2012–2014	KoNEHS adults	5,800	Urine	UPLC-MS	GM ± SE MEHHP: 2.922 ± 0.011 ug/L MEOHP: 2.571 ± 0.011 ug/L MECPP: 3.059 ± 0.010 ug/L MnBP: 3.211 ± 0.012 ug/L MBzP: 1.047 ± 0.015 ug/L	NAFLD prevalence	The prevalence of NAFLD was associated with urinary levels of MEHHP, MEOHP, MECPP, MBzP, MnBP compared to the reference group.	Age, gender, smoking, drinking, exercise level, marital status, education level, socioeconomic status.

(Continued)

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TABLE 6 (Continued)

Substance	Reference	Country	Population	Sample size	Biological materials	Measurement	Exposure assessment	Outcomes	Results	Adjustment factors
DEHP	Yu et al. (120)	USA 2007–2016	NHANES adults (≥20 years)	6,046	Urine	HPLC-ESI-MS/MS	15 phthalate metabolites Median ΣDEHP: 3.1 ug/mmol	ALT, AST, ALP, TBIL	Positive dose–response relationships between urinary phthalate metabolites and ALT or AST, ΣDEHP and GGT were observed. Significant positive associations of ΣDEHP with TBIL were found after adjusting for potential confounders.	Age, sex, race/ethnicity, education level, the ratio of family income to poverty, physical activity, alcohol consumption, medications
DEHP	Milošević et al. (121)	Serbia	Adults (18–50 years)	305	Urine	GC-MS	10 phthalates metabolites mean ± SD all phthalates: 304.55 ug/g MEP: 132.2 ± 188.6 ug/g MEHP: 80.36 ± 96.27 ug/g	ALT, AST, GGT	Phthalates exposure was associated with elevated AST levels. ALT and AST values were increased in MEP exposed while GGT levels were enhanced in MEHP exposed.	Obesity, diabetes
DEHP	Milošević et al. (104)	Serbia 2015–2016	Male participants (18–55 years)	102	Urine	GC-MS	MEP, MEHP, MPP, MiAP, MnAP, MCHP, MBzP, MOP, MBP	ALT, AST, GGT	Significant increment in transaminase serum levels was observed in MEP-positive normal weight sub-group. The phthalates exposure may be related to statistically significant ALT and AST serum levels increment.	NR

UPLC, ultra-high-performance liquid chromatography; HPLC, high-performance liquid chromatography; HPLC-MS, high-performance liquid chromatography-tandem mass spectrometry; UPLC-MS, ultra-high-performance liquid chromatography mass spectrometry; LC-MS, liquid chromatography-mass spectrometry; LC-MS/MS, liquid chromatography coupled to tandem-mass spectrometry; HPLC-ESI-MS/MS, High-performance liquid chromatography-electrospray ionization-tandem mass spectrometry; GC-MS, gas chromatograph-mass spectrometry; GC-MS/MS, gas chromatography coupled to tandem mass spectrometry.

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routes of administration under many experimental conditions such as, difference of doses, absence of dose–response relationships, or small sample sizes (132). In the future, research with larger samples, longer follow-up periods, and a multidisciplinary approach to explore the effect of EDCs in the human body is required. Moreover, the scientific community should help draw public attention to the hazards of EDCs and promote more regulation of industrial pollution.

Author contributions

YC: design, information retrieval, draft writing, and article review and editing. YW: information retrieval, draft writing, and article review and editing. ZC: information retrieval, draft writing, and information visualization. WL: methodology, review, and information visualization. BL: methodology and review. QZ: article review and editing. XZ: article review and editing. JD: methodology, and article review and editing. JC: conceptualization, design, funding acquisition, and article review and editing. All authors contributed to the article and approved the submitted version.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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The relationship between urinary selenium levels and risk of gestational diabetes mellitus: A nested case–control study

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Background: Selenium (Se) is an essential trace element for the human body. Serum Se and urinary Se are also biomarkers to assess Se exposure status. However, studies focusing on the association between urinary Se and the risk of gestational diabetes mellitus (GDM) are rare.

Objective: To investigate the association between urinary Se and the risk of GDM.

Methods: A nested case–control study based on a prospective birth cohort in Wuhan, China, which focuses on the effects of prenatal environmental factors exposure on pregnant women and children's health was conducted. Two hundred and twenty-six cases and 452 controls were included. Maternal urine samples were collected before GDM diagnosis, and the urinary Se levels were determined. We assessed the association of urinary Se with GDM by conditional logistic regression with maternal urinary Se level as a categorical variable, and estimated the association between Se and glucose levels by multiple linear regression. The potential modifier roles of maternal age and fetal sex have also been assessed.

Results: Lower urinary level of Se was significantly associated with a higher risk of GDM (OR=2.35 for the tertile 1, 95% CI:1.36-4.06; adjusted OR=1.79 for the tertile 2, 95%CI:1.09-2.95; *p* for trend=0.01). Fetal sex had an interaction with Se in the association with GDM. The association was more pronounced among pregnant women with female fetuses than with male fetuses.

Discussion: Our study suggested a significant negative association between urinary Se and the risk of GDM, and this association may vary depending on the fetal sex.

KEYWORDS

urinary selenium, gestational diabetes mellitus (GDM), nested case–control study, maternal age, fetal sex

1. Introduction

Selenium (Se) is an essential trace element for humans and animals (1). As a component of the enzyme glutathione peroxidase, Se protects against oxidative stress damage (2) and also has antioxidant (3), anti-cancer, and anti-viral properties (4). The general population mainly consume Se from eating food, such as grains, meat, and dairy products (5, 6). Deficiency for Se

for human body can increase the risks of cardiovascular disease, miscarriage, cancer, and other diseases (7). On the other hand, over-exposure to Se can also be harmful to human health (8).

Gestational diabetes mellitus (GDM) is one of the most common medical complications of pregnancy. As of 2019, the global prevalence of GDM ranges from 7.5 to 27% (9). The prevalence of GDM in China was 14.8% (10), but due to the increasing age of pregnant women, rising obesity rate, unhealthy lifestyle, and more rigorous GDM diagnostic guidelines, it is expected that the prevalence rate of GDM will further increase (11). The International Diabetes Federation has reported that 1 in 6 live births is affected by hyperglycemia in pregnancy (12). GDM is associated with both short-term and long-term adverse health outcomes in mothers and children, such as pre-eclampsia, macrosomia, and increased risks of future type 2 diabetes mellitus (T2DM), obesity, and cardiovascular disease (13). Studies have shown that some factors are associated with the increased risk of GDM, such as age, obesity, metabolic dysregulation, and daily diet (14–17). As an essential trace element, the relationship between Se and the risk of GDM was found. However, their findings are inconsistent. Most recently, a meta-analysis, based on 12 studies, demonstrated that women with GDM had lower Se levels than women without GDM (18). Nevertheless, the difference reported by this meta-analysis was non-significant after correction of the reporting bias by means of the trim-and-fill method (18).

The nested case–control study design can reduce selection bias because both case and control subjects are sampled from the same population, and can also reduce cost and minimize effort because only a fraction of the parent cohort is included (19). Therefore, in the present study, we conducted a nested case–control study based on a large birth cohort study to investigate the relationship between the urinary concentrations of Se of pregnant women and the risk of GDM.

2. Materials and methods

2.1. The design and population of the study

The design of the present study is a nested case–control study. All GDM cases and controls are from a perspective birth cohort in Wuhan, China, which focuses on the effects of prenatal environmental factors exposure on pregnant women and children's health. The details of this cohort study have been reported previously (20).

At 24–28 weeks of gestation, pregnant women were given an oral glucose tolerance test (OGTT). Pregnant women need to collect fasting venous blood after 10–12 h of fasting overnight, and then dissolve 75 g of anhydrous glucose in 250-300 ml of water. After drinking for 3–5 min, venous blood will be collected 1 and 2 h after taking sugar. The blood glucose measurement sample shall be venous plasma or serum, and the blood glucose measurement method shall be the glucose oxidase method. Obstetricians of the study hospital would follow the diagnostic criteria of the International Association of Diabetes and Pregnancy Study Groups (IADPSG) (21) that if any of the 75 g OGTT glucose levels met or exceeded the following criteria: fasting: 92 mg/dl (5.1 mmol/l); 1 h after a meal: 180 mg/dl (10.0 mmol/l); and 2 h after meal:153 mg/dl (8.5 mmol/l). The controls were pregnant women who are negative in the OGTT result.

The inclusion criteria for our study are: (1) singleton; (2) live births; (3) voluntary participation in this study. The exclusion criteria for our study are: (1) missing information or urine samples not provided; (2) history of pre-pregnancy cardiovascular disease, diabetes mellitus, renal disease and hypertension; (3) multiple pregnancies, birth defect, and stillbirth. For each selected GDM case, two consecutive controls are randomly selected in the birth cohort and matched according to the infant gender and maternal age at conception (that is, the case was matched by two controls of the same age). Women with multiple pregnancies, birth defects, stillbirths, and women whose urine samples could not be analyzed was excluded. In the end, a total of 226 cases and 458 controls were included (Figure 1).

2.2. Ethics approval

The study protocol was approved by the ethical committee of the Tongji Medical College, Huazhong University of Science and Technology, and Women and Children Medical and Health care Center of Wuhan. After explaining the detailed description of the research process, each participant signed the informed consent document.

2.3. Data collection

Professionally trained nurses conducted face-to-face interviews with pregnant women before the OGTT test or after delivery in the hospital. Demographic and socioeconomic characteristics (e.g., maternal age, residence, employment, household income, education, and self-reported height and weight before pregnancy at the hospital), and lifestyle habits during pregnancy (e.g., exercise, smoking, and alcohol consumption) were collected through the interview. Information about maternal disease history, complications, and birth outcomes were retrieved from the hospital's medical record. Gestational age was estimated based on the date of the last menstrual period of the pregnant woman. The pre-pregnancy body mass index (BMI) was calculated based on the height and the weight of the pregnant woman before getting pregnant.

2.4. Urine sample collection and Se measurements

The maternal mid-stream urine sample was collected in the second trimester (within 3 days before the OGTT). All the mid-stream urine samples collected would be labeled and stored separately in polypropylene tubes and stored at −20°C until further analysis. Determination of Se was carried out by professional laboratory personnel, who could not identify the status of the case and control. Urine samples were thawed at room temperature before analysis, and 1 ml of urine from the supernatant was introduced in Kirgen polypropylene conical centrifuge tubes. Then, 3% HNO_3 was added to the final volume of 5 ml for overnight nitrification. The resulting sample was digested by ultrasound at 40°C for 1 h and then analyzed using inductively coupled plasma mass spectrometry (Agilent 7,700, Agilent Technologies, Santa Clara, CA, United States). The standard Reference Material Human Urine (SRM2670a, National Institute of Standards and Technology, Gaithersburg, MD, United States) was used

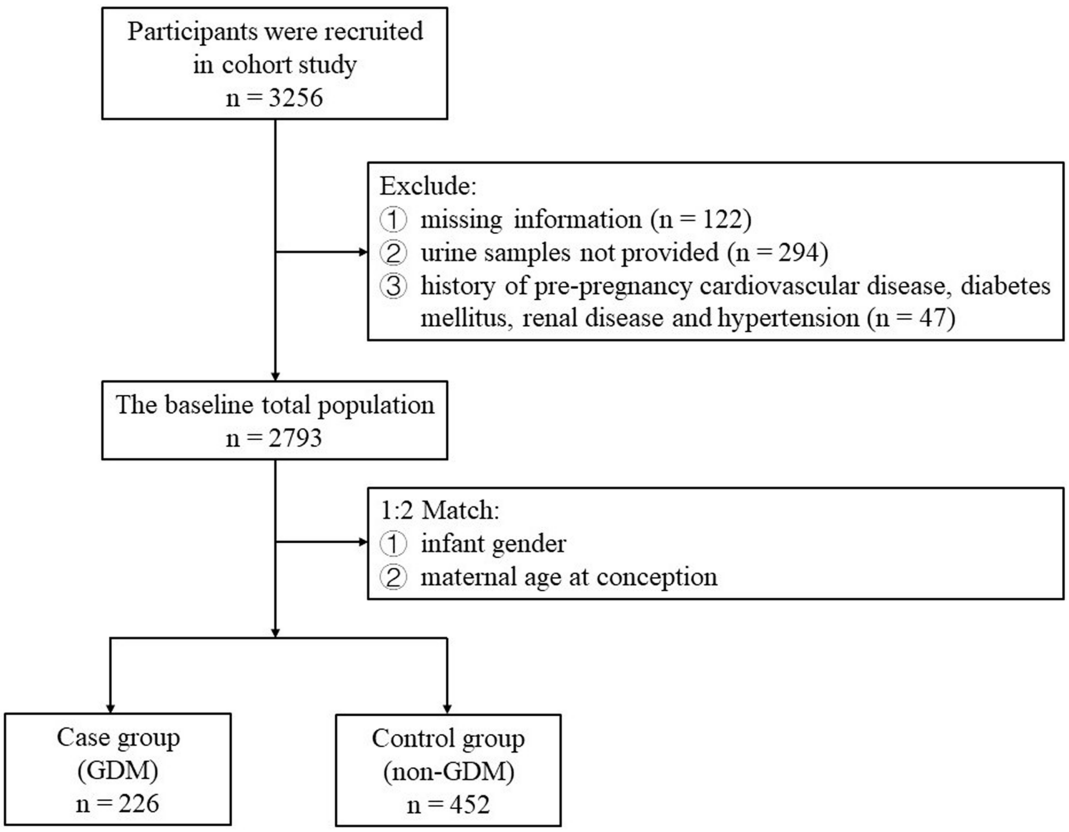

FIGURE 1
Flowchart of participant recruitment and case–control selection.

as an external quality control, and sample spike-recoveries were used to confirm analytical recovery, which was 95%. A 3% HNO_3 blank was processed in each batch of samples to control for possible contamination. The samples were analyzed with an external calibration method, using eight standard concentrations ranging from 0 to 500 μg/L. The limit of detection (LOD) for Se in urine was 0.08 μg/L. Field blanks were also included for quality control and the levels of Se in the field blanks were <LOD. The urinary Se concentrations below the LOD were given a value of one-half the LOD. Urinary creatinine concentrations which were determined by the Sarcosine Oxidase Method with Mindray BS-200 CREA Kit (Shenzhen Mindray Bio-medical Electronics Co., Ltd., Shenzhen, China), were used to correct for the effect of variation in urine dilution. Finally, the concentrations of urinary Se were reported as μg/g creatinine.

2.5. Statistical analyses

We used the Chi-square test to compare the differences of basic characteristics between GDM cases and controls. After Kolmogorov–Smirnov normality test, we found that Se concentrations in urine samples were skewed distribution. Therefore, we chose to use Wilcoxon rank-sum test to evaluate the difference in the distribution of urinary Se concentrations between cases and controls. Conditional logistic regression models were used to assess the association between maternal urinary Se concentration and the risk of GDM. In the conditional logistic regression models, we used maternal urinary Se levels as a categorical variable which were categorized into three levels. And the criteria for variable conversion is based on the tertile distribution [tertile 1/tertile 2/tertile 3 (T1/T2/T3)] of urinary Se concentrations in the control group. The T3 was assigned as the referent group. The median value with each tertile was used as the score variable in the regression model to test for a linear trend in the risk between GDM and urinary Se concentration.

Maternal education (less than high school, high school, more than high school), pre-pregnancy BMI (<18.5, 18.5–23.9, ≥24 kg/m^2), and gestational weight gain (<15, 15–20, ≥20 kg) were included in the adjusted models. Because gravid (1, ≥2) has a great impact on GDM, we also include it in the adjustment model (22–24). The association between confounders and GDM were analyzed. Besides, to test the robustness of the results, we conducted the sensitivity analyses by adjusting multivitamin supplement use during pregnancy, hypertension during pregnancy, occupation, and household income, sequentially. The risk estimates were further stratified by maternal age (<30, ≥30 years old) and infant gender. Statistical significance was defined as a two-sided p value <0.05. The statistical analyses were performed using the SAS (version 9.4; SAS Institute Inc., Cary, NC, United States).

3. Results

The basic characteristics of the cases and controls are shown in Table 1. Among the 678 participants, the mean age of all mothers at delivery was 30.65 ± 4.07 (mean ± SD) years old. Compared with

TABLE 1 Basic characteristics of GDM cases and controls.

Characteristics	Cases (n =226)	Controls (n =452)	p
	n (%)	n (%)	
Maternal age (years)			1.00
<25	8 (3.54)	16 (3.54)	
25–29	100 (44.25)	200 (44.25)	
30–34	73 (32.30)	146 (32.30)	
≥35	45 (19.91)	90 (19.91)	
Infant sex			1.00
Male	131 (57.96)	262 (57.96)	
Female	95 (42.04)	190 (42.04)	
Pre-pregnancy BMI (kg/m^2)			0.01
<18.5	20 (8.85)	64 (14.16)	
18.5–23.9	140 (61.95)	324 (71.68)	
≥24.0	66 (29.20)	64 (14.16)	
Education			0.01
Less than high school	12 (5.31)	21 (4.65)	
High school	46 (20.35)	15 (11.28)	
More than high school	168 (74.34)	380 (84.07)	
Household income (yuan per year)			0.60
<50,000	23 (10.18)	55 (12.17)	
≥50,000	202 (89.38)	393 (86.95)	
Missing	1 (0.44)	4 (0.88)	
Gravid			0.06
1	95 (42.04)	225 (49.78)	
≥2	131 (57.96)	227 (50.22)	
Pregnancy-induced hypertension			0.49
No	219 (96.90)	442 (97.79)	
Yes	7 (3.10)	10 (2.21)	
Occupation			0.60
Employed	194 (85.84)	392 (86.73)	
Unemployed	28 (12.39)	56 (12.39)	
Missing	4 (1.77)	4 (0.88)	
Gestational weight gain (kg)			0.01
<15	122 (53.98)	167 (36.95)	
15–20	70 (30.97)	190 (42.04)	
≥20	34 (15.04)	95 (21.02)	
Multivitamin supplement during pregnancy			0.26
No	32 (14.16)	79 (17.52)	
Yes	194 (85.84)	372 (82.48)	
Urinary Se (μg/g creatinine)[a]	19.13 (14.00, 24.94)	22.24 (15.97, 29.34)	

GDM, gestational diabetes mellitus; BMI, body mass index. [a]Values are presented as median (25–75th percentile).

TABLE 2 Association between maternal urinary Se levels and GDM (n =678).

Se (μg/g creatinine)	Cases	Controls	OR[a] (95% CI)	OR[b] (95% CI)
Tertile 1 (<18.10)	96	150	2.91 (1.71–4.95)	2.35 (1.36–4.06)
Tertile 2 (18.10–26.77)	82	151	2.12 (1.32–3.40)	1.79 (1.09–2.95)
Tertile 3 (≥26.77)	48	151	1.00	1.00
p for trend			0.01	0.01

OR, odds ratio; CI, confidential interval. [a]Crude model. [b]Adjusted for education, gravid, pre-pregnancy BMI, and gestational weight gain.

controls, the cases were more likely to be overweight (≥24.0 kg/m^2), and with lower educational level. The average gestational weight gain (kg) of women in the cases and controls were 14.47 ± 4.81 and 16.12 ± 4.87 (mean ± SD), respectively. The urinary Se levels of pregnant women in GDM cases were significantly lower than those of controls (median: 19.13 vs. 22.24 μg/g creatinine; $p < 0.05$).

Table 2 presents the association between maternal urinary Se levels and the odds of GDM. In the crude model, there was a significant increase in the risk of GDM with reduction of Se levels [T3: reference; T2: OR = 2.12 (95%CI: 1.32–3.40); T1: OR = 2.91 (95%CI: 1.71–4.95); p for trend <0.01]. After adjustment for potential confounding factors, the dose–response relationship between Se and GDM was still statistically significant [adjusted ORs = 1.79 (95%CI: 1.09–2.95) for the T2 and 2.35 (95%CI: 1.36–4.06) for the T1; p for trend <0.01]. The association between each confounder and GDM was provided in Supplementary Table S1. In addition, we conducted sensitivity analysis by adjusting the intake of multiple vitamin supplements during pregnancy, hypertension during pregnancy, occupation, and family income. There is no material change in the observed correlation (Supplementary Table S2).

We further performed analyses stratified by pregnant women's age and fetal sex (Table 3). In women with age ≥ 30 years old, lower Se concentration was significantly associated with the risk of GDM (T2 of adjusted OR = 1.84, 95%CI: 0.93–3.63; T1 of adjusted OR = 3.31, 95%CI: 1.52–7.23; p trend <0.01), but no significant interaction was found (p for heterogeneity = 0.14). As for fetal sex, the observed association was more pronounced in pregnant women with female fetuses (adjusted OR = 3.58, 95%CI: 1.30–9.58 for the T1 vs. T3) than those women with male fetuses (adjusted OR = 2.25, 95%CI: 1.16–4.38; p for heterogeneity = 0.03).

4. Discussion

In this nested case–control study, we found a significant association between decreased urinary Se concentration with the risk of GDM in pregnant women, and this association did not change after adjustment for a series of potential confounding factors. We further found that the association between lower urinary Se and risk of GDM was more pronounced in pregnant women who had female fetuses.

Previous studies have shown that Se in urine is a useful biomarker to assess Se exposure status (25, 26). A comparison of urine Se levels between pregnant women in this study and previously published data is presented in Table 4. Urinary Se levels of our study population

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(median: 11.9 μg/L and 21.12 μg/g creatinine; geometrical mean (GM): 11.38 μg/L and 20.71 μg/g creatinine) were comparable to those adults in the United Kingdom (median: 13.4 μg/L) (27), but were lower than the general population in Belgium (median: 25.1 μg/L and 21.6 μg/g creatinine) (28). Besides, our study population also had lower levels of urinary Se compared with pregnant women in developed countries, such as Australia (median: 19.1 μg/L and 25.6 μg/g creatinine) (29), Japan (GM: 37.6 μg/g creatinine) (30), the United States (GM: 35.4 μg/g creatinine) (31) and Canada (GM: 44 μg/L) (32). Compare with median urine Se of other countries in our study, the reference significance may be unclear. The possible reason was that considering the heterogeneity of the population, the distribution of urine Se in different populations was also different.

TABLE 3 The association between maternal urinary Se levels and gestational diabetes mellitus stratified by maternal age and fetal sex.

Se (μg/g creatinine)	Cases/ controls (*n*)	OR[a] (95% CI)	*p* for heterogeneity
Maternal age (years)			**0.14**
<30 (*n* = 324)			
Tertile 1 (<17.26)	35/72	1.36(0.60–3.07)	
Tertile 2 (17.26–27.38)	49/72	2.13(1.00–4.55)	
Tertile 3 (≥27.38)	24/72	1.00	
p for trend		0.43	
≥30 (*n* = 354)			
Tertile 1 (<18.51)	57/79	3.31(1.52–7.23)	
Tertile 2 (18.51–26.48)	38/79	1.84(0.93–3.63)	
Tertile 3 (≥26.48)	23/78	1.00	
p for trend		0.01	
Fetal sex			**0.03**
Male (*n* = 393)			
Tertile 1 (<19.08)	68/87	2.25 (1.16–4.38)	
Tertile 2 (19.08–27.10)	34/88	1.17 (0.63–2.19)	
Tertile 3 (≥27.10)	29/87	1.00	
p for trend		0.01	
Female (*n* = 285)			
Tertile 1 (<17.01)	35/63	3.58 (1.30–9.85)	
Tertile 2 (17.01–26.42)	45/64	4.31 (1.68–11.05)	
Tertile 3 (≥26.42)	15/63	1.00	
p for trend		0.03	

OR, odds ratio; CI, confidence interval. [a]Adjusted for education, gravid, pre-pregnancy BMI, gestational weight gain. The bold values "<30 (*n* = 324), ≥30(*n* = 354), Male(*n* = 393), Female (*n* = 285)" meant the stratification by maternal age and fetal sex. The bold values "0.14" and "0.03" meant *p* for heterogeneity.

Tan et al. (33) found that the serum Se levels of pregnant women with impaired glucose tolerance and GDM were significantly lower than those of normal pregnant women in a population from Shanghai, China. In a cross-sectional study in Turkey, Kilnc et al. (34) also reported that pregnant women with GDM and those with glucose intolerants had lower Se level than that of the normal pregnant women. Most recently, a meta-analysis, including 12 studies (940 pregnant women with GDM and 1749 controls), suggested that Se levels of women with GDM were lower than those of women without GDM (18). Similar to these above results, our study also found that the urine Se levels of pregnant women in GDM cases were significantly lower than those of controls, and observed a negative correlation between maternal urinary Se concentrations and the risk of GDM. However, there were some controversies about the relationship between Se and GDM in previous studies. Molnar et al. (35) conducted a cross-sectional study in Hungary and reported that the serum Se concentrations of GDM pregnant women were significantly higher than those of normal pregnant women. Liu et al. (36) found that Se levels in the first trimester were not related to GDM in a cohort study. One possible explanation is that this study collected blood samples in the first trimester of pregnancy, while other studies chose the second or third trimester. The Se levels may change in the second or third trimester of pregnancy (37).

We found that the association between the urinary Se levels and the risk of GDM is different in age. Women who were above 30 years old had relatively low urinary Se levels. Similar to our findings, in a cohort study of 506 adults in Attica Province, Greece, Letsiou et al. (38) found that in people aged 18–75, serum Se levels decreased with age. This may be related to the distribution and retention of Se in different tissues of the human body (38). In addition, animal experiments have shown that preserving Se in young rats was more effective than in adult rats (39). Moreover, we found that there was an interaction in the association between maternal urinary Se level and GDM risk by infant sex. There is no reasonable explanation to this finding in existed studies, and further research is needed.

TABLE 4 Comparison of Se levels in urine between the present study and previous studies.

References	Location	Population	*N*	Median	Geometric mean
Present study 2014	Hubei, China	Pregnant women	678	11.91 μg/L	11.38 μg/L
Morton et al. (27)	UK	Adult	1,001	21.12 μg/g creatinine	20.71 μg/g creatinine
Hoet et al. (28)	Belgium	Adult	132	25.1 μg/L	—
Callan et al. (29)	Australia	Pregnant women	173	19.1 μg/L 25.6 μg/g creatinine	—
Shirai et al. (30)	Japan	Pregnant women	78	—	37.6 μg/g creatinine
Kim et al. (31)	USA	Pregnant women	380	—	35.4 μg/g creatinine
Hu et al. (32)	Canada	Women	156	—	44 μg/g creatinine

We have discovered several possible mechanisms to explain the link between low Se levels and increased risk of GDM. Firstly, Se has the characteristics of insulin simulation (40), which can promote glucose transport, regulate cell glucose utilization, and ions, and reduce insulin resistance (41). Secondly, a possible mechanism is that Se improves the defense function of the antioxidant system, which may protect β cells to some extent and promote the increase of insulin secretion (42). Thirdly, Se participates in the production of the catalytic site of Se GSHp, which is an enzyme in the body (42). And the activity of enzyme Se GSHp is related to the activation of the nuclear factor-κB (NF-κB) (43). Researchers have proved that the activation of NF-κB is associated with macrovascular complications in late diabetic (44). However, by the role of Se in lipid peroxidation, Se could help to reduce the activity of NF-kB (45).

Nevertheless, there are some limitations in our study. First, the urine Se level of pregnant women was measured only at a certain time point, which may not accurately reflect the Se level of pregnant women during the whole pregnancy. Second, for the dietary information and dyslipidemia of pregnant women, our questionnaire was not comprehensive enough to exclude the possibility of residual confounding. Third, our study was aimed at the Han population in China. In the future, the daily dietary intake questionnaire should be added to comprehensively analyze the relationship between Se levels and GDM. Future researchers can conduct prospective cohort studies to investigate urinary Se status in populations of different ethnicities and countries.

In this nested case–control study, we found that a correlation between low urinary Se concentration in pregnant women and increased risk of GDM in pregnant women. This association suggests that low Se concentration during pregnancy may be one of the risk factors for GDM. In the future, more in-depth studies are needed to find out the possible mechanisms, and to provide the basis for guiding pregnant women to supplement Se reasonably.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Ethics statement

The study was conducted according to the guidelines of the Declaration of Helsinki, and approved by the ethics committee of Tongji Medical College, Huazhong University of Science and Technology (no. [2014] 14#), and the study hospital (no. 2010009). The patients/participants provided their written informed consent to participate in this study.

Author contributions

YLiu: conceptualization, methodology, software, formal analysis, and writing—original draft preparation. HC: investigation and validation. MZ: investigation. GZ: data curation. YY: data validation. YLi: project administration and funding acquisition. WL: resources, writing—review and editing, and supervision. HZ: conceptualization, writing—review and editing, and resources. All authors contributed to the article and approved the submitted version.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

Supplementary material

The Supplementary material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fpubh.2023.1145113/full#supplementary-material

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Time trends in cardiovascular disease mortality attributable to non-optimal temperatures in China: An age-period-cohort analysis using the Global Burden of Disease Study 2019

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Background: Associations between non-optimal temperatures and cardiovascular disease (CVD) mortality risk have been previously reported, yet the trends of CVD mortality attributable to non-optimal temperatures remain unclear in China. We analyzed trends in CVD mortality attributable to non-optimal temperatures and associations with age, period, and birth cohort.

Methods: Data were obtained from the Global Burden of Disease Study (GBD) 2019. Joinpoint regression analysis was used to calculate annual percent change (APC) and average annual percent change (AAPC) from 1990 to 2019. We used the age-period-cohort model to analyze age, period, and cohort effects in CVD mortality attributable to non-optimal temperatures between 1990 and 2019.

Results: The age-standardized mortality rate (ASMR) of CVD attributable to non-optimal temperature generally declined in China from 1990 to 2019, whereas ischemic heart disease (IHD) increased slightly. Low temperatures have a greater death burden than high temperatures, but the death burden from high temperatures showed steady increases. Joinpoint regression analysis showed that CVD mortality decreased in all age groups except for IHD, and the decreases were greater in females than in males. The mortality of CVD attributable to non-optimal temperatures of males was higher than females. The mortality rate showed an upwards trend with age across all CVD categories. Period risks were generally found in unfavorable trends. The cohort effects showed a progressive downward trend during the entire period.

Conclusion: Although there have been reductions in CVD mortality attributable to non-optimum temperatures, the mortality of IHD has increased and the burden from non-optimal temperatures remains high in China. In the context of global climate change, our results call for more attention and strategies to address climate change that protect human health from non-optimal temperatures.

KEYWORDS

cardiovascular diseases, myocardial ischemia, stroke, mortality, non-optimal temperatures, age-period-cohort model

1. Introduction

Cardiovascular disease (CVD), principally ischemic heart disease (IHD) and stroke, are the leading causes of death and disability-adjusted life years (DALYs) globally in 2019, especially in low-income and middle-income countries (1–3). As the Global Burden of Disease 2019 Study reported, there were ∼523 million cases and 18.6 million deaths worldwide caused by CVD in 2019 (2, 4). With the largest population in the world, China had always experienced a heavy health and economic burden of CVD due to population aging and the increasing prevalence of many risk factors (5, 6). In 1990, stroke and IHD were ranked as the 3rd and 7th leading causes of DALYs in China; in 2017, they rose higher to the 1st and 2nd leading cause, respectively (5). CVD is caused by various risk factors, mainly including metabolic factors (i.e., lipids, diabetes, obesity, and hypertension), behavioral factors (i.e., tobacco use, alcohol, diet quality, and physical activity), socioeconomic and psychosocial factors (i.e., education, depression), and environmental factors (i.e., air pollution, ambient temperatures) (7–9).

Climate change will become the biggest health threat of the 21st century and an urgent problem to be solved, the Lancet Countdown to climate change has warned (10). In recent years, there are more and more extreme heat and cold waves around the world due to climate change, and it affects human health directly by increasing exposure to extreme temperatures. According to the GBD 2019 study, non-optimal temperatures are among the top 10 causes of death globally (11). There were 1.69 million deaths would attributable to non-optimal temperature globally in 2019, and low temperature has a greater overall effect on mortality than does high temperature (12). The epidemiological evidence suggests a U- or J-shaped association between ambient temperatures and the risk of death from cardiovascular and cerebrovascular, which means both low and high temperatures may increase the risk of mortality (13–15). Recently, a study reported that 399.7 thousand deaths who were diagnosed with CVD were attributed to non-optimal temperatures across China in 2019, indicating a substantial burden of CVD due to non-optimum temperatures (16). However, there are only 3 years left to achieve the target of reducing CVD mortality in China by 15% by 2025 compared with 2015, which was adopted in the document Medium- to Long-Term Plan for the Prevention and Treatment of Chronic Diseases (2017–2025) (17). In this context, understanding the CVD death and trends attributable to non-optimum temperatures in China is key to guiding CVD prevention and control efforts under climate change.

To date, previous analyses have focused on the association between CVD and non-optimal temperatures in China (18–20). None of the existing studies explore the long-term trends of CVD mortality attributable to non-optimal temperatures between different age groups and gender, and there is a lack of comprehensive analyses of the possible causes underlying the long-term trends. Therefore, in this study, we examine the effects of age, period, and cohort on CVD mortality attributable to non-optimal temperatures and the temporal trends from 1990 to 2019 in China, using data from the GBD 2019.

2. Materials and methods

2.1. Data source

The attributable burden of CVD data was obtained from the GBD 2019 study, which was provided by the Institute for Health Metrics and Evaluation (IHME). The GBD 2019 study used DisMod-MR 2.1, a Bayesian meta-regression tool as the primary method to comprehensively estimate disease burden (e.g., incidence, prevalence, mortality, and DALYs) for 369 diseases and injuries and 87 risk factors in 204 countries and territories from 1990 to 2019 (2, 11). Details of the data, methodology to enhance data quality and comparability, and statistical modeling for the GBD 2019 have been explained previously. All anonymized data have been publicly available on the website of IHME and can be accessed online (http://ghdx.healthdata.org/gbd-results-tool). The University of Washington Institutional Review Board reviewed and approved the informed consent. Original data of CVD mortality in China were mainly from Disease Surveillance Points, Maternal and Child Surveillance System, Chinese Center for Disease Control and Prevention Cause of Death Reporting System (21). IHD and stroke cases were classified using the International Classification of Diseases and Injuries, 10th Revision.

In the GBD 2019 study, the daily averages of temperature for each location were obtained from the European Center for Medium-Range Weather Forecasts. The theoretical minimum risk exposure level for temperature (TMREL), which meant the temperature associated with the lowest mortality risk for all included causes combined, was estimated for a given location and year. Given varying TMREL for different regions (e.g., higher in warm regions than colder locations), years, and diseases, the GBD study 2019 employed both spatially and temporally varying to estimate TMREL and are not using a globally uniform TMREL (11, 13, 22). Exposure to non-optimal temperature is defined as the same-day exposure to ambient temperature that is either warmer or colder than the temperature associated with the minimum mortality risk (11). High-temperature exposure is defined as exposure to temperatures warmer than this TMREL and low-temperature is defined as temperatures colder than this TMREL. The population attributable fraction (PAF) is defined as the meaning that, if the exposure to a risk factor is reduced to the theoretical minimum exposure level, then the proportion of associated disease or death in the population would decrease (i.e., the proportion of cause-specific deaths attributable to high or low daily temperatures). The PAF associated with non-optimal temperature is an aggregate of high-temperature and low-temperature PAFs in each location and year. We computed PAF by age-sex-location-year using the following general formula for a continuous risk:

$$PAF = \frac{\Sigma_i^n P_i\,(RR_i - 1)}{\Sigma_i^n P_i\,(RR_i - 1) + 1} \quad (1)$$

where P_i is the percentage of the population exposed to level i of high or low temperature, n is the total number of exposure level. RR_i is the relative risk as a function of exposure level i of high or low temperature, and was estimated based on 81 published systematic reviews, whose specific methods have been outlined previously (11).

The number of attributable deaths (ADs) was calculated by multiplying the PAFs with the number of CVD death cases (N) (23). It can be expressed as follows:

$$AD = PAF{*}N \quad (2)$$

Age-standardized mortality rate (ASMR) and the 95% uncertainty intervals (UIs) was calculated using the GBD 2019 global standard population. The detailed methods were introduced in GBD 2019 report and the official website.

2.2. Statistical analysis

The rate of mortality with 95% UI of the CVD is reported according to age and gender. All the rates are reported per 100,000 population. Identifying changes in the secular trend is critical to analyzing disease mortality data. Joinpoint regression analysis was used to determine temporal trend changes of CVD mortality attributable to non-optimal temperature from 1990 to 2019. Annual percent change (APC) and 95% confidence interval (CI) were calculated for each mortality trend, and Average annual percent change (AAPC) and 95% CI were calculated for the full range of period analyzed. The APC and AAPC were used to describe the temporal trends of CVD mortality. Significant changes of the time points were tested using a Monte Carlo substitution method. The hypothesis test was whether AAPC/APC was significantly different compared to zero. An increasing trend was defined as APC/AAPC > 0, and a decreasing trend was defined as APC/AAPC < 0, vice versa. The analysis was carried out by the Joinpoint Regression Program software (version 4.9.0.1; Statistical Research and Applications Branch, National Cancer Institute).

The age-period-cohort model is a common statistical model to extract information hidden in mortality, including the risk of death experienced by the population in a given year and the accumulation of health risks since birth. This model allows the analysis of the independent effects of age, period, and cohort on temporal trends in the mortality of CVD. It has been used in the descriptive epidemiology of certain chronic diseases, including cardiovascular disease (24). The age effect represents the different risks in various age groups (25). Period effect reflects changes over time affecting non-optimal temperature-attributable CVD mortality in all age groups, presumably arising from changes in social, cultural, economic, or physical environments. Birth cohort effects reflect the characteristics of individuals with the same birth year and consider the risk factors and exposure to environmental factors present in early life. For age-period-cohort analyses, we arranged the mortality and population data into successive 5-year age groups from 25–29 years to 80–84 years, consecutive 5-year periods from 1990 to 2019, and correspondingly consecutive 5-year birth cohort groups starting from 1910–1914 to 1990–1994. The estimated coefficients of parameters (perfect collinearity of the age, period, and cohort variables) were obtained by the age-period-cohort analyses with intrinsic estimator method (26). These coefficients were converted to the exponential value [exp (coef.) $= e^{coef.}$], representing the RRs of CVD mortality for a given age, period, or birth cohort relative to the average level of all ages, periods, or birth cohorts combined. Age-period-cohort analysis was performed using STATA 15.0 software (StataCorp, College Station, TX, United States). The Wald's chi-square test was adopted to assess the significance of the estimable parameters and functions. All statistical tests were 2-sided and P-values <0.05 were considered statistically significant.

3. Results

3.1. Descriptive analysis

The mortality of CVD attributable to non-optimal temperature in China from 1990 to 2019 is shown in Figure 1. Deaths due to the non-optimal temperature were dominated by low temperature. Generally, the ASMR of CVD and stroke attributable to non-optimal temperature showed a downward trend in China from 1990 to 2019. However, slight increments were observed in IHD among both sexes. For CVD, stroke, and IHD, the annual ASMR in males were significantly higher than those in females during the observation period. The ASMR of CVD, stroke, and IHD attributable to non-optimal temperature by age group in China in 2019 were shown in Table 1. In 2019, the ASMR of CVD attributable to non-optimal temperature in China were 31.38 (95% UI 24.69 to 38.81) and 18.82 (95% UI 14.59 to 23.76) per 100,000 population of males and females, respectively. The ASMR of stroke attributable to non-optimal temperature was 16.80 (95% UI 12.44 to 21.94) in males and 9.66(95% UI 7.07 to 12.59) in females, and 12.23 (95% UI 8.63 to 16.29) in males and 7.53 (95% UI 5.18 to 10.11) in females for IHD, respectively, per 100,000 population.

Figures 2A–C showed the increased mortality of CVD, stroke, and IHD attributable to non-optimal temperature with age group, and the rate accelerated after the group aged 65–69 years. Meanwhile, a declining trend was observed in mortality between 1990 to 1994 and 2015 to 2019. As Figures 2D–F showed, the mortality of CVD and stroke attributable to non-optimal temperature showed a decreased trend across birth cohorts, whereas mortality of IHD first showed a decrease, then an increase, and finally decreased again across all age groups, suggesting a relatively lower risk of mortality in those born later. Because the cohort variation could be confounded by age and period, and unable to assess the net cohort effect. Therefore, age-period-cohort analyses were used to address this limitation.

3.2. Joinpoint regression analysis

The APC and AAPC by joinpoint regression analysis are listed in Table 1 and Figure 3. From 1990 to 2019, the ASMR of CVD attributable to non-optimal temperature in China decreased by 0.90% (95% CI 0.31–1.48, Figure 3A) in males and 1.77% (95% CI 0.91–2.62, Figure 3B) in females. The ASMR of stroke decreased by 1.53% (95% CI 0.81–2.25) in males (Figure 3C) and 2.54% (95% CI 1.94–3.13) in females (Figure 3D), whereas the ASMR of IHD rose by 0.69% (95% CI 0.30–1.07) in males (Figure 3E) and 0.13% (−0.80 to 1.07) in females (Figure 3F). Moreover, there were marked sex differences in the AAPC of CVD, stroke, and IHD across all age groups, with less improvement in mortality in males than in females.

3.3. Age-period-cohort analysis

The estimated RR of age, period, and cohort effects of CVD mortality attributable to non-optimal temperature for both sexes were shown in Table 2 and Figure 4. Age effects of CVD mortality showed an expected exponential distribution for both sexes in China. After adjustment for period and cohort deviations, the age effect on CVD mortality increased from 0.08 (95% CI 0.04–0.17) in the group aged 25–29 to 10.35 (95% CI 8.92–12.01) in the group aged 80–84 for males, and from 0.10 (95% CI 0.04–0.26) in the group aged 25–29 to 14.18 (95% CI 11.12–18.08) in the group aged 80–84 for females (Figure 4A). The period effects of the mortality risk showed a slight increase from 0.85 (95% CI 0.75–0.96) in 1990 to 1.24 (95% CI 1.10–1.39) in 2019 for males, whereas the period effects were flat

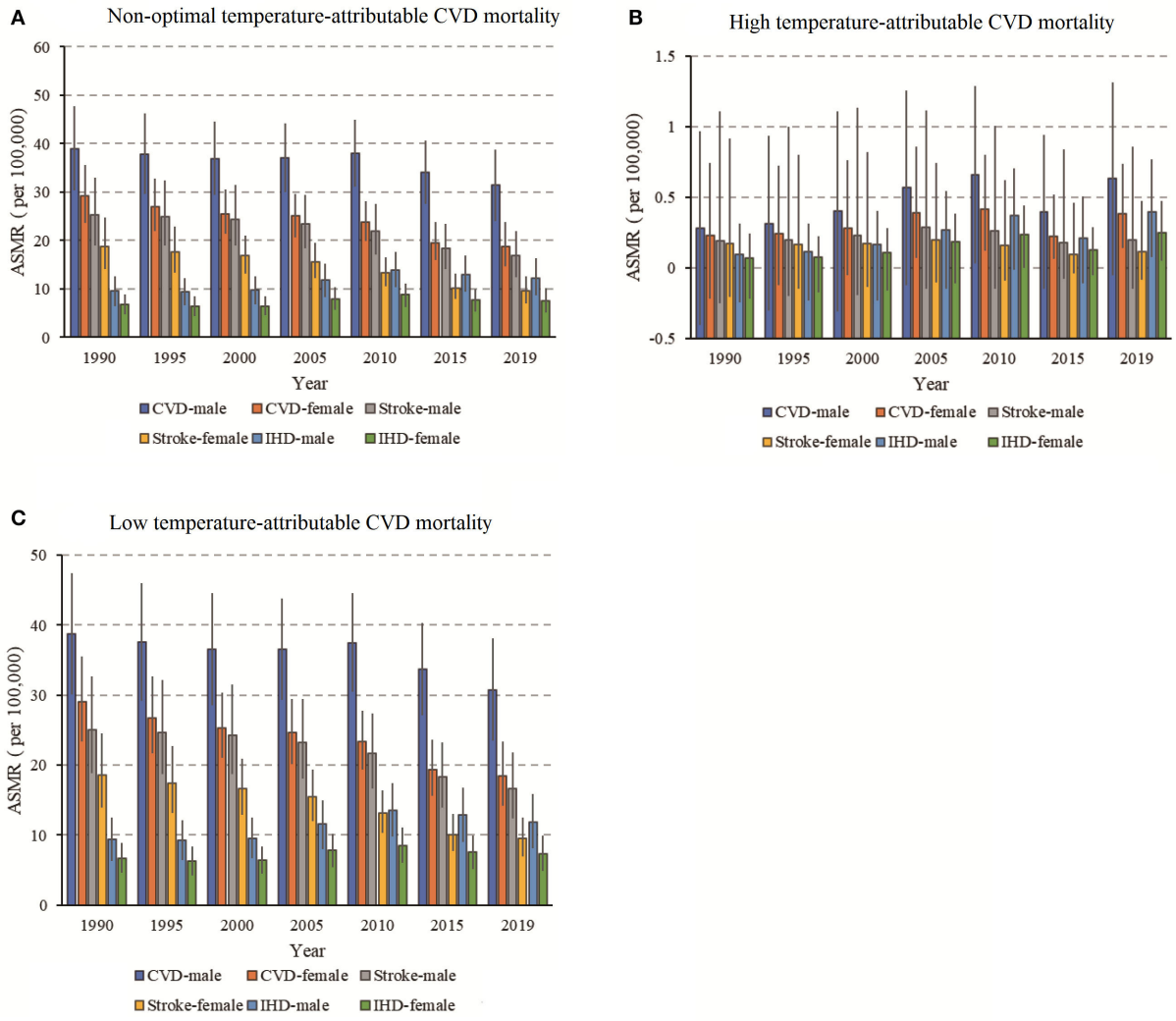

FIGURE 1
The trends of age-standardized mortality rate (ASMR) for CVD **(A)**, stroke **(B)**, and IHD **(C)** mortality attributable to non-optimal temperatures during 1990–2019 and the corresponding 95% CI.

for females, indicating no improvements for the whole population during the study period (Figure 4B). The cohort effects for both sexes continuously decreased, which means the later birth cohorts experienced a relatively lower mortality risk compared to the earlier cohorts (Figure 4C).

The estimated RR of age, period, and cohort effects of stroke mortality attributable to non-optimal temperature for both sexes were shown in Supplementary Table 1. With regard to stroke, the mortality risk also increased markedly with age, regardless of sex (Figure 4D). Periods have a non-significant effect on stroke mortality attributable to non-optimal temperature. Estimated period effects showed an upward trend in males during the entire period. In contrast, the females have seen a slight improvement in stroke mortality (Figure 4E). The cohort effects for both sexes also showed downward trends, which were similar to CVD (Figure 4F).

The estimated RR of age, period, and cohort effects of IHD mortality attributable to non-optimal temperature for both sexes were shown in Supplementary Table 2. The mortality risk caused by IHD attributable to non-optimal temperature increased markedly with advancing age for both sexes (Figure 4G). Period effects were greater for IHD mortality than on stroke across the study period (Figures 4E, H). The period effects of the mortality risk caused by IHD for both sexes showed an upward trend between 1990 and 2019. It increased from 0.77 (95% CI 0.62–0.94) in 1990 to 1.44 (95% CI 1.19–1.75) in 2019 for males, and from 0.99 (95% CI 0.74–1.32) in 1990 to 1.20 (95% CI 0.91–1.59) in 2019 for females. For cohort effects, it showed downward trends for both sexes, which were similar to CVD and stroke (Figure 4I).

4. Discussion

This study comprehensively estimated on temporal trends in CVD deaths attributable to non-optimal temperature from 1990 to 2019 in China. Although a downward trend of the ASMR was observed for CVD mortality attributable to non-optimal temperature among males and females, the death burden remains substantial, with stroke being the main burden of temperature-related deaths from

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TABLE 1 Sex- and age-specific mortality rates of CVD, stroke, and IHD attributable to non-optimal temperature in China in 1990 and 2019 and their average annual percentage changes (AAPC) from 1990 to 2019.

Categories	Males		Females	
	Rates in 2019, 95% UI (per 100,000 population)	AAPC, 95% CI (%,1990–2019)	Rates in 2019, 95% UI (per 100,000 population)	AAPC, 95% CI (%, 1990–2019)
CVD				
ASMR	31.38 (24.69 to 38.81)	−0.90 (−1.48 to −0.31)*	18.82 (14.59 to 23.76)	−1.77 (−2.62 to −0.91)*
25–29 years	0.88 (0.66 to 1.11)	−0.82 (−1.65 to 0.02)	0.30 (0.21 to 0.40)	−3.12 (−4.82 to −1.39)*
30–34 years	1.81 (1.36 to 2.31)	−0.51 (−1.35 to 0.35)	0.53 (0.38 to 0.72)	−2.85 (−4.18 to −1.51)*
35–39 years	3.40 (2.52 to 4.42)	−0.48 (−0.81 to −0.14)*	0.96 (0.69 to1.30)	−3.47 (−3.82 to −3.12)*
40–44 years	6.11 (4.41 to 8.11)	−0.74 (−1.49 to 0.01)	1.96 (1.41 to 2.64)	−3.47 (−3.81 to −3.14)*
45–49 years	8.69 (6.12 to 11.71)	−1.42 (−2.01 to −0.82)*	3.31 (2.42 to 4.49)	−3.45 (−4.69 to −2.20)*
50–54 years	15.02 (10.82 to 20.15)	−1.88 (−2.21 to −1.55)*	6.56 (4.73 to 8.91)	−3.70 (−4.11 to −3.28)*
55–59 years	24.40 (17.39 to 32.70)	−1.98 (−2.38 to −1.59)*	11.64 (8.46 to 15.71)	−3.37 (−4.20 to −2.54)*
60–64 years	42.05 (30.79 to 55.68)	−1.78 (−2.28 to −1.27)*	22.25 (16.64 to 29.83)	−3.00 (−3.64 to −2.35)*
65–69 years	72.60 (55.42 to 94.96)	−1.74 (−2.40 to −1.07)*	43.01 (32.71 to 55.77)	−2.59 (−3.56 to −1.62)*
70–74 years	143 (108.07 to 182.22)	−1.43 (−2.15 to −0.70)*	89.82 (68.97 to 116.23)	−2.16 (−2.66 to −1.65)*
75–79 years	256.45 (196.65 to 327.39)	−1.10 (−1.74 to −0.46)*	168.25 (130.76 to 214.87)	−1.80 (−2.31 to −1.30)*
80–84 years	495.54 (389.24 to 610.42)	−0.74 (−1.50 to 0.02)	338.79 (262.76 to 426.06)	−1.34 (−2.31 to −0.37)*
Stroke				
ASMR	16.80 (12.44 to 21.94)	−1.53 (−2.25 to −0.81)*	9.66 (7.07 to 12.59)	−2.54 (−3.13 to −1.94)*
25–29 years	0.43 (0.29 to 0.59)	−1.27 (−2.05 to −0.49)*	0.15 (0.10 to 0.21)	−3.39 (−5.11 to −1.63)*
30–34 years	0.88 (0.60 to 1.19)	−0.97 (−1.78 to −0.15)*	0.26 (0.18 to 0.37)	−3.26 (−4.58 to −1.91)*
35–39 years	1.70 (1.15 to 2.34)	−0.88 (−1.63 to −0.12)*	0.52 (0.35 to 0.74)	−3.81 (−4.13 to −3.49)*
40–44 years	3.17 (2.10 to 4.38)	−1.33 (−1.70 to −0.95)*	1.13 (0.78 to 1.59)	−3.97 (−4.27 to −3.66)*
45–49 years	4.69 (3.16 to 6.59)	−1.97 (−2.56 to −1.38)*	1.98 (1.35 to 2.81)	−3.92 (−5.16 to −2.67)*
50–54 years	8.38 (5.67 to 11.72)	−2.56 (−2.90 to −2.21)*	4.09 (2.79 to 5.72)	−4.14 (−4.54 to −3.74)*
55–59 years	14.01 (9.43 to 19.53)	−2.51 (−2.86 to −2.15)*	7.17 (4.89 to 10.00)	−3.94 (−4.60 to −3.27)*
60–64 years	24.99 (17.14 to 35.14)	−2.21 (−3.03 to −1.38)*	13.40 (9.29 to 18.77)	−3.56 (−4.06 to −3.06)*
65–69 years	44.10 (31.30 to 60.14)	−2.05 (−2.83 to −1.26)*	25.71 (18.52 to 35.10)	−2.96 (−3.87 to −2.05)*
70–74 years	86.96 (62.40 to 116.97)	−1.80 (−2.40 to −1.19)*	52.50 (38.03 to 70.44)	−2.65 (−3.18 to −2.12)*
75–79 years	152.44 (110.73 to 203.34)	−1.61 (−2.28 to −0.95)*	94.66 (69.16 to 126.01)	−2.44 (−2.93 to −1.95)*
80–84 years	268.48 (200.09 to 348.96)	−1.24 (−2.23 to −0.25)*	174.61 (128.69 to 227.14)	−2.03 (−2.64 to −1.41)*
IHD				
ASMR	12.23 (8.63 to 16.29)	0.69 (0.30 to 1.07)*	7.53 (5.18 to 10.11)	0.13 (−0.80 to 1.07)
25–29 years	0.42 (0.28 to 0.57)	−0.02 (−0.95 to 0.92)	0.14 (0.08 to 0.20)	−2.53 (−4.16 to −0.87)*
30–34 years	0.87 (0.60 to 1.20)	0.26 (−0.68 to 1.20)	0.24 (0.15 to 0.35)	−2.30 (−4.78 to 0.25)
35–39 years	1.59 (1.06 to 2.23)	0.35 (−0.01 to 0.71)	0.40 (0.25 to 0.58)	−2.53 (−2.95 to −2.11)*
40–44 years	2.73 (1.79 to 3.86)	0.08 (−0.17 to 0.34)	0.74 (0.46 to 1.09)	−2.31(−2.42 to −2.20)*
45–49 years	3.68 (2.32 to 5.23)	−0.30 (−0.99 to 0.41)	1.18 (0.74 to 1.71)	−2.20 (−3.46 to −0.92)*
50–54 years	6.02 (3.85 to 8.57)	−0.47 (−0.80 to −0.15)*	2.14 (1.34 to 3.10)	−2.24 (−2.68 to −1.80)*
55–59 years	9.35 (5.93 to 13.26)	−0.67 (−1.53 to −0.20)	3.86 (2.41 to 5.53)	−1.87 (−2.81 to −0.81)*
60–64 years	15.07 (9.75 to 21.42)	−0.50 (−0.98 to −0.01)*	7.62 (4.80 to 10.91)	−1.53 (−2.16 to −0.90)*
65–69 years	24.62 (16.23 to 34.36)	−0.44 (−1.26 to 0.37)	14.63 (9.47 to 20.34)	−1.01 (−2.16 to 0.15)
70–74 years	47.16 (31.69 to 65.39)	0.13 (−0.42 to 0.68)	30.97 (20.73 to 42.41)	−0.16 (−0.64 to 0.33)
75–79 years	86.43 (59.68 to 117.20)	0.54 (−0.17 to 1.26)	60.19 (40.56 to 82.20)	0.02 (−0.96 to 1.01)
80–84 years	186.98 (130.56 to 248.56)	1.05 (0.24 to 1.86)*	133.90 (92.25 to 179.91)	0.78 (−0.18 to 1.75)

*Indicated the AAPC was significantly different from zero at the $\alpha = 0.05$ level.

UI, uncertainty interval; CI, confidence interval; ASMR, age–standardized mortality rates; AAPC, average annual percent change.

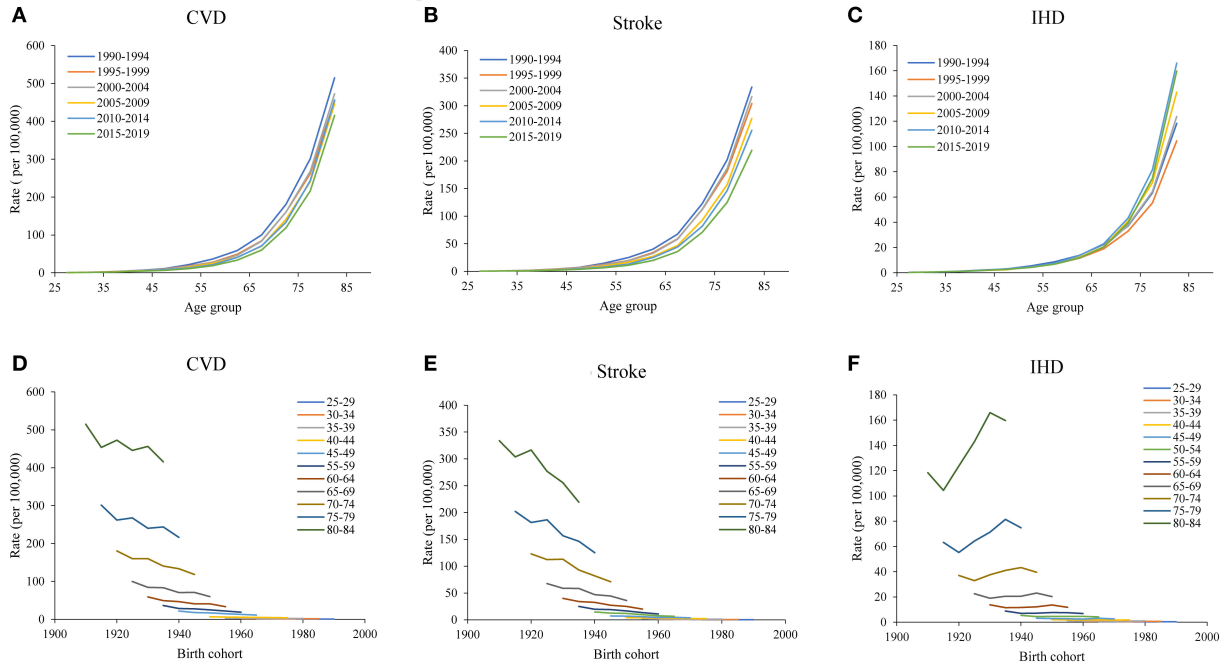

FIGURE 2
Age-specific mortality of CVD attributable to non-optimal temperatures from 1990 to 2019 and cohort-specific mortality of CVD attributable to non-optimal temperatures from 1990 to 2019. **(A–C)** Survey years were arranged into consecutive 6-year periods from 1990 to 1994 (median, 1992), 1995 to 1999 (median, 1997), 2000 to 2004 (median, 2002), 2005 to 2009 (median, 2007), and 2010 to 2014 (median, 2012), and the CVD, stroke, and IHD mortality attributable to non-optimal temperatures increased with age group. **(D–F)**, the data of CVD, stroke, and IHD mortality attributable to non-optimal temperatures were arranged into 17 consecutive birth cohorts, including those born from 1910 to 1914 (median, 1912) to 1990 to 1994 (median, 1992), and successive 5-year age intervals from 25 to 29 years (median, 27 years) to 80 to 84 (median, 82 years) years of age.

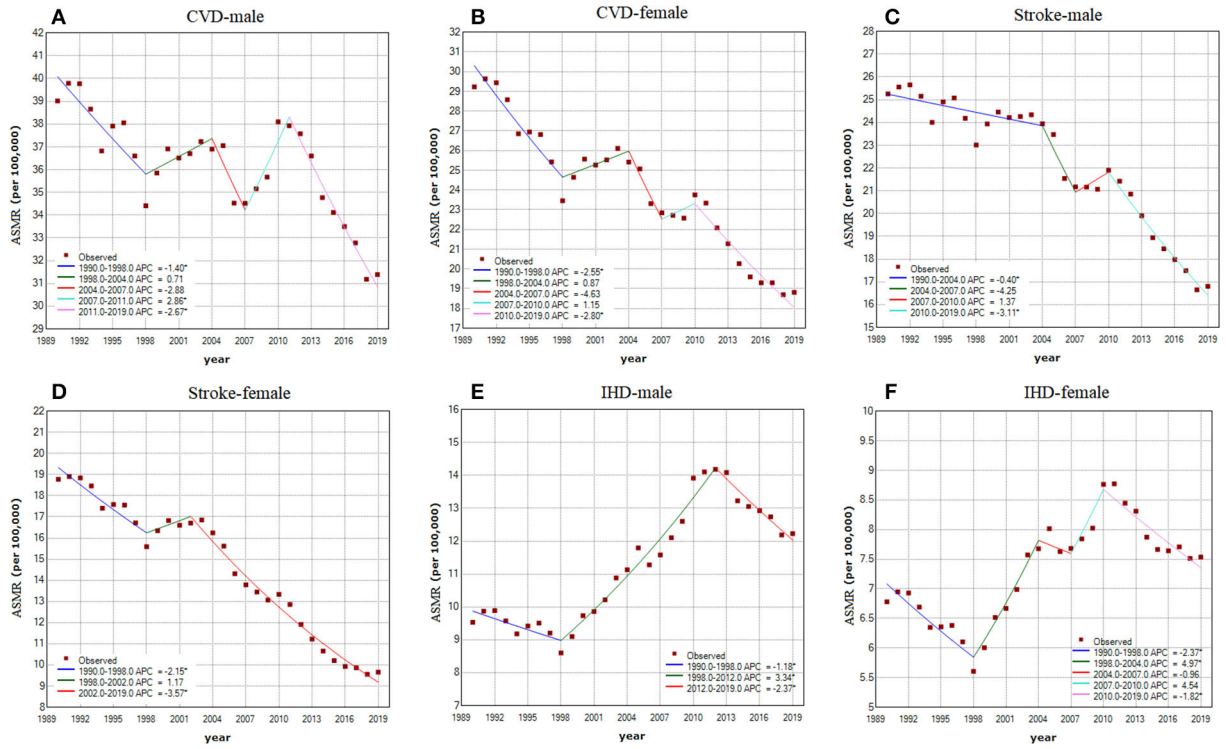

FIGURE 3
Joinpoint regression analysis in sex-specific age-standardized mortality rate (ASMR) of CVD, stroke, and IHD attributable to non-optimal temperatures from 1990 to 2019. **(A)** CVD in males; **(B)** CVD in females; **(C)** stroke in males; **(D)** stroke in females; **(E)** IHD in males; **(F)** IHD in females. Notes: an asterisk indicates that the annual percent change is statistically significantly different from zero at the $\alpha = 0.05$ level.

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TABLE 2 Sex-specific relative risks of CVD death attributable to non-optimal temperatures in China due to age, period, and cohort effects.

Factor	Mortality in males		Mortality in females	
	RR (95% CI)	*P*-value	RR (95% CI)	*P*-value
Age				
25–29	0.08 (0.04–0.17)	<0.001	0.10 (0.04–0.26)	<0.001
30–34	0.14 (0.08–0.24)	<0.001	0.14 (0.07–0.29)	<0.001
35–39	0.24 (0.16–0.37)	<0.001	0.20 (0.11–0.36)	<0.001
40–44	0.42 (0.30–0.59)	<0.001	0.33 (0.21–0.53)	<0.001
45–49	0.58 (0.43–0.77)	<0.001	0.50 (0.34–0.72)	<0.001
50–54	0.89 (0.71–1.12)	0.310	0.80 (0.60–1.06)	0.120
55–59	1.28 (1.06–1.53)	0.009	1.10 (0.87–1.38)	0.440
60–64	1.81 (1.57–2.09)	<0.001	1.69 (1.41–2.03)	<0.001
65–69	2.66 (2.37–2.99)	<0.001	2.71 (2.31–3.17)	<0.001
70–74	4.31 (3.87–4.80)	<0.001	4.93 (4.19–5.79)	<0.001
75–79	6.50 (5.76–7.33)	<0.001	7.99 (6.57–9.72)	<0.001
80–84	10.35 (8.92–12.01)	<0.001	14.18 (11.12–18.08)	<0.001
Period				
1990–1994	0.85 (0.75–0.96)	0.011	1.10 (0.92–1.30)	0.292
1995–1999	0.85 (0.78–0.93)	<0.001	0.93 (0.83–1.05)	0.242
2000–2004	0.96 (0.90–1.02)	0.174	1.00 (0.93–1.08)	0.980
2005–2009	0.99 (0.93–1.05)	0.741	0.96 (0.89–1.03)	0.270
2010–2014	1.18 (1.08–1.28)	<0.001	1.01 (0.90–1.14)	0.813
2015–2019	1.24 (1.10–1.39)	0.001	1.00 (0.85–1.19)	0.961
Cohort				
1910–1914	2.75 (2.18–3.47)	<0.001	2.27 (1.63–3.17)	<0.001
1915–1919	2.56 (2.11–3.09)	<0.001	2.27 (1.71–3.01)	<0.001
1920–1924	2.31 (1.97–2.71)	<0.001	2.23 (1.75–2.84)	<0.001
1925–1929	2.08 (1.81–2.39)	<0.001	2.19 (1.76–2.72)	<0.001
1930–1934	1.82 (1.59–2.08)	<0.001	2.05 (1.66–2.54)	<0.001
1935–1939	1.60 (1.40–1.84)	<0.001	1.85 (1.48–2.31)	<0.001
1940–1944	1.33 (1.13–1.57)	<0.001	1.61 (1.25–2.09)	<0.001
1945–1949	1.14 (0.93–1.40)	0.205	1.43 (1.05–1.94)	0.024
1950–1954	0.97 (0.76–1.25)	0.834	1.27 (0.88–1.83)	0.198
1955–1959	0.81 (0.61–1.09)	0.173	1.02 (0.66–1.58)	0.922
1960–1964	0.68 (0.48–0.96)	0.030	0.80 (0.48–1.33)	0.383
1965–1969	0.64 (0.43–0.95)	0.027	0.69 (0.38–1.23)	0.208
1970–1974	0.54 (0.33–0.87)	0.011	0.57 (0.28–1.17)	0.127
1975–1979	0.46 (0.26–0.84)	0.011	0.45 (0.18–1.13)	0.090
1980–1984	0.45 (0.21–0.96)	0.040	0.35 (0.10–1.26)	0.110
1985–1989	0.44 (0.16–1.25)	0.124	0.30 (0.05–1.72)	0.177
1990–1994	0.39 (0.05–3.06)	0.372	0.24 (0.01–7.32)	0.411
P trend	<0.001		<0.001	
Deviance	0.33		2.43	
AIC	3.32		5.47	
BIC	−170.74		−168.64	

RR denotes the relative risk of CVD death in particular age, period, or birth cohort relative to the average level of all ages, periods, or birth cohorts combined.
RR, relative risk; CI, confidence interval; AIC, Akaike Information Criterion; BIC, Bayesian Information Criterion.

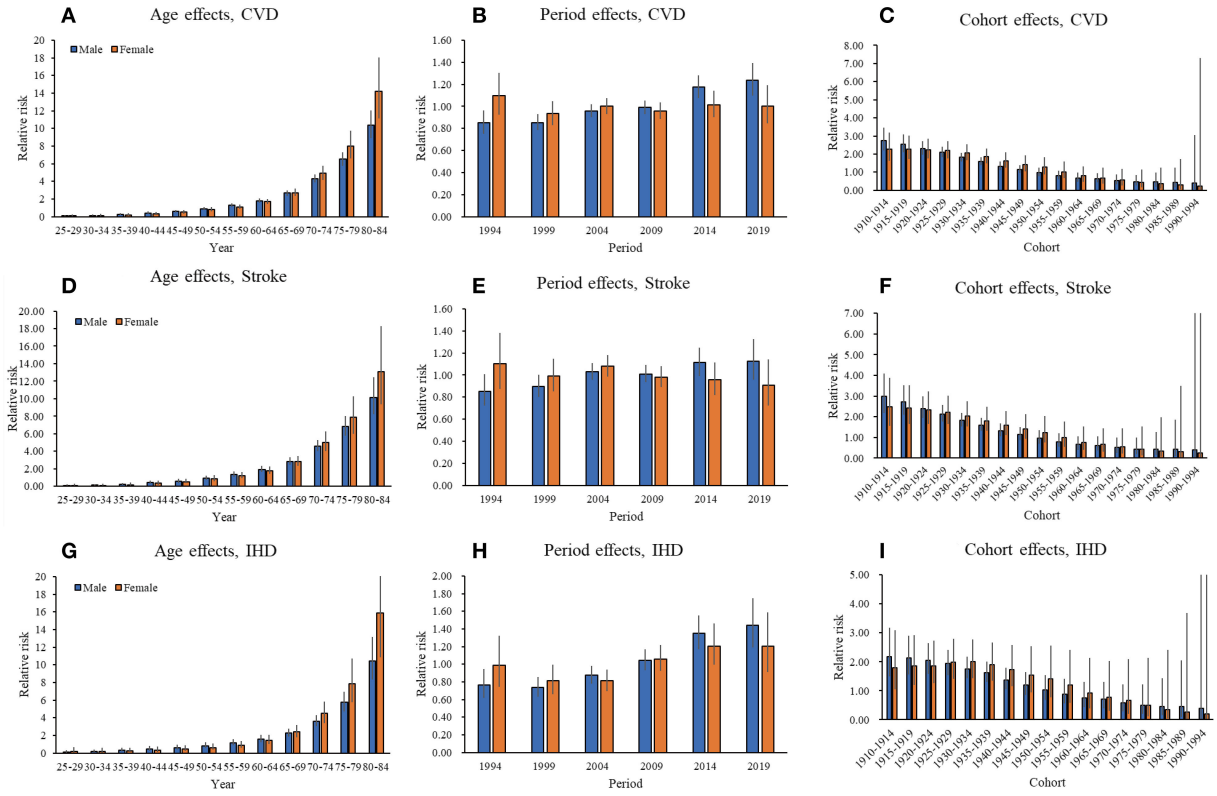

FIGURE 4
Parameter estimates of age, period, and cohort effects on CVD, stroke, and IHD mortality attributable to non-optimal temperatures from 1990 to 2019. **(A, D, G)** Age relative risk of CVD, stroke, and IHD mortality attributable to non-optimal temperatures and the corresponding 95% CI. **(B, E, H)** Period relative risks of CVD, stroke, and IHD mortality attributable to non-optimal temperatures and the corresponding 95% CI. The period relative risk was adjusted for age and nonlinear cohort effects. **(C, F, I)** Cohort relative risks of CVD, stroke, and IHD mortality attributable to non-optimal temperatures and the corresponding 95% CI. The cohort relative risk was adjusted for age and nonlinear period effects.

CVD. In addition, we found that the mortality of IHD caused by non-optimal temperature showed an upward trend. Over the past 30 years, the CVD death burden attributable to low temperature was higher than the high temperature, but it showed an increasing trend attributable to high temperature.

Previous studies had generally found that the death burden caused by low temperatures was higher than that of high temperatures (12–14, 16, 27). A study in China showed that 8.86% of CVD deaths were caused by low temperatures, and 0.17% were caused by high temperatures (16). Another study including 272 main Chinese cities reported that the PAFs attributable to low and high temperatures were 11.62 and 2.71%, respectively (14). Our results are consistent with previous studies. There are several possible reasons for this phenomenon. First, the duration of cold waves is typically greater than that of hot waves, which can lead to longer exposure to low temperatures than to high temperatures (28). Second, to date, the overall increase in ambient temperature is not very large, and the annual mean temperature increases slowly so that the CVD death burden due to high temperatures is relatively small. However, the global temperature has increased by about 1.25°C over the 20th century and the current emissions trajectory suggests that it will exceed 1.5°C in <10 years (29). It means that future global warming could exacerbate the adverse health effects of high temperature and increase the burden of disease caused by high temperature. In addition, one study showed a higher risk of myocardial infarction as a consequence of heat exposure compared to cold exposure (30, 31). This may explain why the IHD death burden attributable to high temperature has generally shown an increased trend among both sexes since 1990. Overall, we should not only focus on the impact of high temperatures on CVD under unavoidable global warming, but also develop a better plan to reduce the CVD death burden due to low temperatures.

We also found there were clear gender differences in CVD death burden attributable to non-optimum temperatures, which was higher in males regardless of high or low temperatures. In addition, we can observe that the AAPC mortality of CVD for females decreased greater than for males across all age groups from 1990 to 2019 in Table 1. Previous studies have shown gender differences in mortality risk patterns of CVD caused by non-optimal temperature; similar between males and females (32), higher in females (33), higher in males (34). The underlying reasons for these gender differences remain unclear and should be explored in future prospective studies. Some researchers argued that these differences may be related to factors such as thermoregulation, physiological responses, culture, and socioeconomic (35, 36). For example, influenced by economic structure and culture in China, men generally engage in more outdoor activities and physical labor in high or low temperature environments, while women tend to engage in light labor indoors. To some extent, this may explain the differences between males and females that we observed in our analysis.

The results of the age-period-cohort effect analysis concluded that age was a risk factor for CVD death, including IHD and

stroke, attributable to non-optimal temperature. Age effect showed continuously increasing with age among males and females. Similar to our findings, most previous studies consistently showed that the elderly are more vulnerable to the effects of temperature than younger populations (14, 33, 35, 37). This may be ascribed to senescence, which leads to impairments of thermoregulatory capacity and degeneration of physiologic functions, as well as the possibility of the existence of multiple chronic diseases (31). The findings suggest that as the population age, governments and health authorities should focus on these elderly populations and develop targeted measures to protect them from the effects of non-optimal temperature. For example, strengthening the prediction and monitoring of early cold and hot spells, timely issuing warnings to the public; giving full play to the role of community health, popularizing the knowledge of preventing non-optimal temperature to residents, and transforming the knowledge into behavioral adaptation.

The period effects showed an increasing trend in males CVD death, including stroke, attributable to non-optimal temperature across 30 years, whereas it was flat in females. The risk of death for IHD rises the most strikingly for both sexes in period effects. Among all environmental risk factors attributable to CVD mortality, the ranking and value of ASMR due to non-optimal temperature in stroke decreased, while increasing in IHD (Supplementary Figures 1–3). In addition, the ranking of IHD attributed to other risks also rose, such as poor diet, and an increasing prevalence of hypertension. The decline in stroke mortality was the main cause of the improvement in CVD mortality, largely offsetting the increases in IHD mortality. Previous studies reported that out-of-hospital IHD mortality in China was high, with only 11% receiving basic cardiopulmonary resuscitation when IHD occurred, reflecting inadequate knowledge of health first aid (21, 38). The irregularities of guideline-recommended treatments (e.g., β-blockers and angiotensin-converting enzyme inhibitors) remained common and had not significantly improved, which may also contribute to a lack of improvements over time in IHD mortality (39).

The cohort effects showed continuously decreasing trends as a whole, and the cohorts born later had lower mortality risk. This may be relevant to improvements in healthcare coverage, upgrades in early diagnosis of the disease, and advances in treatment techniques and disease management, as well as improvements in public health initiatives in CVD prevention. However, previous studies reported that CVD risk factors were more prevalent in the later birth cohort than in the earlier birth cohort such as air pollution, traffic noise, and bad urban city planning (4, 31, 40). In addition, modifiable risk factors for CVD death are increasing, including high BMI, hypertension, hypercholesterolemia, and poor diet, which are substantial gaps between recommended goals and warrant increased policy and health system attention (21).

This study has some advantages. To the best of our knowledge, this is the first study to investigate the temporal trends of CVD mortality attributable to non-optimal temperatures in China. Besides, data of the GBD 2019 uses the unified and standard methodology to provide consistent estimates of age- and sex-specific all-cause and cause-specific mortality for 369 diseases in 204 countries and territories, which could reduce the potential for misclassification of results and are comparable across time. Moreover, both alterations over the entire period (assessed by the AAPC) and each segmental period (assessed by the APC) were determined using a joinpoint regression model. Furthermore, the effects of age, period, and birth cohort were explored, allowing for the analysis of particular time periods on non-optimal temperature contribution to CVD mortality rather than the risk carried by the birth cohort. Lastly, the main clinical implications of this study were that patients with CVDs should be advised to minimize exposure to non-optimal temperatures and to enhance care for the target population, especially the elderly.

Our study has several limitations. First, the data of CVD death attributable to non-optimum temperatures was only an estimate, and temperature effects were defined as short-term effects that occur on the day of exposure and did not consider the lagged and cumulative effects, which may underestimate the burden of CVD associated with non-optimum temperatures. Second, there may be ecological fallacies because temperature exposure was assessed using ambient temperature and not based on individual-level exposure. Thirdly, due to the unavailability of data, we could not consider the effects of socioeconomic status, air conditioning and heating usage, and development levels of infrastructure and public health services on CVD mortality, which also may underestimate the effects of non-optimum temperatures on CVD death. Finally, the age-period-cohort analysis, which was based on cross-sectional GBD data from 1990 to 2019 rather than a cohort study, is subject to an ecological fallacy, since the interpretation of findings at the population level may not hold up at the individual level. Therefore, Large cohort studies are needed to confirm these findings in the future. Despite the limitations, the results presented here provide valuable information for public health policy by highlighting the secular trend in deaths attributable to CVD from non-optimum temperatures compared with other risk factors. It suggests that efforts to address vulnerability should support, focus on target populations (e. g., older people) and potential disease burden (e. g., IHD), or develop strategies to reduce exposure (e. g., housing insulation, air conditioning) or health education.

5. Conclusion

Although there have been reductions in CVD mortality attributable to non-optimum temperatures during the past 30 years, the mortality of IHD has increased in China, which indicates the burden remains high. Low temperatures have a greater death burden than high temperatures, but the death burden from high temperatures showed an increasing trend. With a reduction in CVD mortality attributable to non-optimum temperatures across all age groups over time, but generally, the decreases were smaller in men than in women and the death burden in men was greater than in women. In addition, although the cohorts born later had lower mortality risk, age and period effects showed unfavorable trends. In the context of global climate change, our results call for more attention and strategies to address climate change that protect human health from non-optimal temperatures.

Data availability statement

The original contributions presented in the study are included in the article/Supplementary material, further inquiries can be directed to the corresponding authors.

Author contributions

LC, TW, and JW contributed to the conception and study design, revised the article critically for important intellectual content, and interpreted the results. JW, PW, FX, JM, XZ, ZY, and ZG contributed to acquisition of data, analysis, and interpretation of data. JW drafted the manuscript. All authors read and approved the final manuscript.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fpubh.2022.1075551/full#supplementary-material

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The role of rare earth elements and dietary intake in tongue cancer: a mediation analysis in southeast China

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Objective: The current research aimed to examine how dietary intake and rare earth elements may affect the development of tongue cancer.

Methods: The serum levels of 10 rare earth elements (REEs) in 171 cases and 171 healthy matched controls were measured by inductively coupled plasma mass spectrometry (ICP-MS). The conditional logistic regression was used to examine the relationship between dietary intake, serum levels of 10 REEs, and tongue cancer. Mediation effect and multiplicative interaction analysis were then performed to estimate the potential contribution of REEs in dietary intake associated with tongue cancer.

Results: Compared with the control group, patients with tongue cancer consumed significantly less fish, seafood, fruit, green leafy vegetables, and non-green leafy vegetables, with higher serum praseodymium (Pr), dysprosium (Dy), and lanthanum (La) levels, and lower serum cerium (Ce) and scandium (Sc) levels. The interaction effect was observed between some REEs and food categories. Green vegetables' impact on the risk of tongue cancer is partially attributed to the La and Thorium (Th) elements ($P < 0.05$, the mediated proportion were 14.933% and 25.280%, respectively). The effect of non-green leafy vegetables for tongue cancer mediated via Pr, Dy, and Th ($P < 0.05$, the mediated proportion were 0.408%, 12.010%, and 8.969%, respectively), and the Sc components in seafood ($P < 0.05$, the mediated proportion was 26.120%) is partly responsible for their influence on the risk of tongue cancer.

Conclusion: The correlation between REEs and dietary intakes for tongue cancer is compact but intricate. Some REEs interact with food intake to influence tongue cancer, while others act as a mediator.

KEYWORDS

rare earth elements, tongue cancer, case-control study, dietary intake, food categories

1. Introduction

Tongue cancer, including oral tongue cancer (the anterior two-thirds of the tongue in the oral cavity) and the base of tongue cancer (the posterior third of the tongue in the oropharynx), accounts for the most familiar intraoral site for cancer globally (1, 2). Surgery is the primary treatment modality for patients with head and neck cancer, especially tongue cancer. However, larger tongue resection may limit tongue function and quality of later life for patients. The high aspiration rate and complications after surgery may also perplex tongue cancer patients more (3). So, the critical task is to find factors influencing the incidence of tongue cancer. Recent studies showed that tobacco smoking, drinking, poor oral hygiene, and oral disorders might affect the occurrence of tongue cancer in varying degrees (4, 5). Even though dietary intake was closely associated with the tongue, the relationship between dietary intake and tongue cancer was scarcely researched.

According to the International Union of Pure and Applied Chemistry (IUPAC), rare earth elements (REEs) are defined as a group of 17 chemical elements in the periodic table, specifically the 15 lanthanides, scandium, and yttrium (6). REEs are exploited with increasing annual amounts applied to agricultural, medical, zootechnical, and industrial fields (7, 8). REEs are commonly used in agriculture as forage additives and fertilizers and are consumed via the food chain. Approximately 90% of the global REEs are distributed in China, indicating more absorption hazards for the Chinese population (9). Although few studies focus on REEs, several studies still prompt that they might influence cancer. The levels of 15 REEs in lung tumor tissue were demonstrated to be different from those in healthy lung tissue (10). The relationship of REEs with a brain tumor, colorectal, and hepatic cancer was also reported (11). For tongue cancer, a previous study reported that lanthanum and praseodymium ions might affect the activity of the tongue carcinoma Tca8113 cell (12). Interestingly, the nucleotide-binding domain and leucine-rich repeat protein 3 (NLRP3) inflammasome were reported to have been activated by lanthanum, which played a key role in oral squamous cell carcinoma (including tongue cancer) (13, 14). Since the association between other REEs and the risk of tongue cancer is largely unknown, further investigations are needed.

Rare earth elements present in the environment can transfer from soil to edible parts and accumulate continuously (15, 16). Moreover, the accumulated concentration of REEs differed in various food categories, such as cereals, fresh aquatic products, fresh vegetables, fresh meats, and eggs (4). Strong evidence has been provided that a close relationship exists between dietary intake and malignant tumors (especially oral cancer) (17, 18). Thus, verifying whether the relationships between dietary intake and tongue cancer are mediated by the intake of REEs is necessary. This study aimed to evaluate the levels of 10 REEs, namely cerium (Ce), praseodymium (Pr), neodymium (Nd), lanthanum (La), samarium (Sm), europium (Eu), dysprosium (Dy), yttrium (Y), scandium (Sc), and thulium (Th), in serum by inductively coupled plasma mass spectrometry (ICP-MS) and explore the role of REEs and dietary-related factors in tongue cancer and further to access potential intricate effect (interaction and mediation) of them in tongue cancer.

2. Material and methods

2.1. Sample collection

A case–control study was conducted in the First Affiliated Hospital of Fujian Medical University (Fujian, China), which enrolled patients with primary tongue cancer diagnosed from December 2010 to September 2019. As described previously (19–21), 191 eligible patients were involved if they fulfilled the following criteria: patients who (a) histologically confirmed with primary tongue cancer; (b) aged between 20 and 80 years; and (c) had lived at least 10 years in Fujian Province. The patients were excluded if they fulfilled the following criteria: patients (a) with a long-term intake of dietary supplements; (b) who have experienced neoadjuvant chemotherapy or radiotherapy before surgery; and (c) who are suffering from severe systemic diseases (including liver and renal damage). Control participants without a history of cancer were enlisted throughout the same time frame from the same hospital's health assessment center with the following exclusion criteria: patients (a) who had worked with inorganic materials regularly, such as welders and potters; (b) who aged under 20 or over 80 years old; (c) who had lived in the Fujian Province less than 10 years; and (d) with a long-term intake of dietary supplements. Finally, 1,417 healthy subjects (682 men and 735 women) were recruited. Following propensity score matching (PSM), 171 patients and 171 healthy-matched controls were enrolled in this study.

We have obtained written informed consent from all participants. This research was performed in accordance with the ethical standards of the Helsinki Declaration, and ethics approval was obtained from the Ethics Committees of Fujian Medical University, Fuzhou, China (2011053).

2.2. Data collection

General features (age, weight, height, gender, family history of cancer, residence, tobacco smoking, alcohol, and tea drinking) and food categories (fruits, seafood, fish, green leafy vegetables, non-green leafy vegetables, milk, egg, meat, and processed meat consumption) were obtained via face-to-face interview using structured questionnaires. The following options were given to participants when asked about the frequency of each food category: <1 time per week or not at all, 1–2 times per week, 3–4 times per week, 5–6 times per week, 1 time per day, 2 times per day, and 3 times per day. The questions of food categories were about the diet 1 year before their diagnosis or interview (for controls).

2.3. Blood sample collection

Approximately 3–5 ml fasting blood sample of each subject was collected in a trace metal-free tube. The blood samples of cases were collected the day after patients were accepted to the hospital to avoid the impact of any drug treatment or examination. After collection, the blood samples were centrifuged at 1,509 rpm for 10 min at 4°C to separate the serum.

2.4. Sample digestion and detection

First, using microwave digestion equipment (PreeKem, China), 200 μl of blood samples were digested with 1 ml of nitric acid (HNO_3) and 4 ml of ultrapure water. After that, the acid-catching temperature was set to 140°C, and the digestion inner tank was placed on the acid catcher to drive acid until 0.5 ml. After flushing the digestive tube's inner wall more than three times, the flushing solution was added into a volumetric flask, and then ultrapure water was used to create a constant amount of 10 ml. Then, the concentrations of 10 rare earth elements (Ce, Pr, Nd, Sm, Eu, Dy, Sc, La, Y, and Th) were measured by inductively coupled plasma mass spectrometry (ICP-MS, NexION 350X; Perkin-Elmer, USA). The instrument parameters are shown in Supplementary Table 1. The limit of detection and the percentage below LOD (%) are described in Supplementary Table 2, and REEs were enrolled for further analysis with a detection rate above 50%.

2.5. The analytic quality controls

We used human hair powder (GBW07601a, China) as a standard reference material for maintaining method performance for quality control. The spike-and-recovery test also showed the validity of measurement (range: 80–105%) (Supplementary Table 2). For every batch, at least two standard reference materials and two blanks were measured. The variation coefficients were <5%, and 12.5% of each batch's samples were repeated.

2.6. Statistical analysis

A 1:1 propensity score matching (PSM) (22) was used to balance the potential confounding (age, gender, family history of cancer, residence, Body Mass Index (BMI), tea and alcohol drinking, and tobacco smoking) between case and control groups with the nearest-neighbor matching approach (maximum caliper distance, 0.02). The group differences before and after PSM were evaluated using the chi-square test or *t*-test. The distribution state of each REE was tested by the Shapiro–Wilk test method, while the Wilcoxon rank sum test was used in the case of non-normal distribution. The associations of each feature and each REE with tongue cancer were tested by univariate and multivariate conditional logistic regression based on the "stats" package (R software). Odds ratios (ORs) and 95% confidence intervals (CIs) were presented. Then, we evaluated the interaction effect of food intake and each REE in tongue cancer, the interaction term was multiplied by food categories, and each REE was included in the multivariable conditional logistic regression model. If the interaction term was significantly associated with tongue cancer, a dummy variable regression analysis (23) would then further be performed. Finally, based on the "mediation" package (R software), the mediation analysis was performed. All analyses were based on R software version 4.1.3. All *p*-values were two-sided, and a $P < 0.05$ was considered statistically significant.

3. Results

3.1. Patients' characteristics

The comparisons of general characteristics between case and control groups before and after PSM are presented in Supplementary Table 3. Age, gender, residence, family history of cancer, BMI, smoking, alcohol, and tea-drinking status distributions were different ($P < 0.05$), but the distributions of most general characteristics were uniform and comparable between the case and control groups after PSM ($P > 0.05$).

3.2. Relationship between dietary intake and tongue cancer

In total, 10 food categories from a food frequency questionnaire were used to assess the dietary intake of enrolled subjects. An increased diet of fish, seafood, fruit, green leafy vegetable, and non-green leafy vegetable was closely associated with decreased risk of tongue cancer and the adjusted OR (95% CI) which were 0.343 (0.181, 0.651), 0.270 (0.146, 0.497), 0.326 (0.175, 0.607), 0.304 (0.160, 0.580), and 0.141 (0.067, 0.295), respectively (Table 1, Model-1). The independent link between dietary intake and tongue cancer after further adjusting for REEs was also investigated in Table 1 (Model-2).

3.3. Relationship between rare earth elements and tongue cancer

Values of each REE below the detection limit were replaced by half of the detection limits (15). As shown in Figure 1, the Wilcoxon rank sum test showed that the distributions of Ce, Sc, and La were different between case and control groups ($P^{\#} < 0.05$), while the distributions of Pr, Sm, Eu, Y, and Th were similar between the two groups ($P^{\#} > 0.05$). Elements were dichotomized into low and high groups based on the median concentration value of healthy controls, and the cutoff values are presented in Supplementary Table 4. Inverse relationships were found between serum Ce, Sc, and tongue cancer [OR and 95% CI were 0.543 (0.318, 0.927) and 0.163 (0.081, 0.331), respectively], while direct relationships were found between Pr, Dy, La, and tongue cancer [OR and 95% CI were 3.490 (1.954, 6.235),4.510 (2.389, 8.576), and 2.700 (1.543, 4.733), respectively] after adjusting for gender, residence, family history of cancer, BMI, tea drinking, tobacco smoking, and alcohol drinking (Figure 1). As results presented in Figure 2, we found that after additional adjusting for dietary intakes, the relationships between some REEs and tongue cancer were changed.

3.4. Interaction effect of the REEs and dietary intake for tongue cancer

Significant interaction effects were observed between serum levels of La and dietary intake of non-green leafy vegetables;

TABLE 1 Food categories of enrolled subjects.

						Model-1		Model-2	
Variables	Categories	Control	Case	χ^2	*P**	*OR 95%CI*	*P*	*OR 95%CI*	*P*
Meat				1.42	0.233				
	<1 time/day	86	98						
	≥1 time/day	85	73			0.878 (0.484, 1.593)	0.670	0.772 (0.462, 1.672)	0.610
Processed meat				1.79	0.181				
	<1 time/week	152	160						
	≥1 time/week	19	11			0.439 (0.134 ,1.433)	0.170	0.375 (0.155, 0.546)	0.270
Fish				9.68	**0.002**				
	<2 times/week	101	129						
	≥2 times/week	70	42			0.343 (0.181 ,0.651)	**0.001**	0.221 (0.128, 0.276)	**0.006**
Seafood				27.08	**<0.001**				
	<1 time/week	55	104						
	≥1 time/week	116	67			0.270 (0.146, 0.497)	**<0.001**	0.361 (0.217, 0.518)	**0.045**
Milk				5.95	**0.015**				
	<1 time/week	93	116						
	≥1 time/week	78	55			0.623 (0.346, 1.119)	0.110	0.664 (0.418, 1.289)	0.380
Egg				0.61	0.434				
	<2 times/week	103	111						
	≥2 times/week	68	60			0.862 (0.475, 1.563)	0.620	0.602 (0.377, 1.099)	0.280
Green leafy vegetables				14.95	**<0.001**				
	<2 times/day	50	86						
	≥2 times/day	121	85			0.304 (0.160, 0.580)	**<0.001**	0.203 (0.120, 0.249)	**0.003**
Non-green leafy vegetables				43.53	**<0.001**				
	<2 times/day	56	118						
	≥2 times/day	115	53			0.141 (0.067, 0.295)	**<0.001**	0.055 (0.026, 0.058)	**<0.001**
Fruit				26.87	**<0.001**				
	<2 times/week	86	133						
	≥2 times/week	85	38			0.326 (0.175, 0.607)	**<0.001**	0.104 (0.054, 0.116)	**0.001**
Pickled food				0.01	0.904				
	<1 time/week	124	122						
	≥1 time/week	47	49			0.938 (0.756, 1.164)	0.560	0.951 (0.785, 2.461)	0.790
Total		171	171						

*P values of Chi-square test. Model-1: conditional logistic regression adjusted Gender, age, residence, family history of cancers, BMI, tobacco smoking, tea, and alcohol drinking. Model-2: conditional logistic regression adjusted Gender, age, residence, family history of cancers, BMI, tobacco smoking, tea, and alcohol drinking and each REE. Bolded values indicate statistical significance at $p < 0.05$.

between serum levels of Ce and Pr and dietary intake of green leafy vegetables; and between serum levels of Eu and dietary intake of seafood or non-green leafy vegetables for tongue cancer (Table 2 all $P_{interaction}$ <0.05). The results of the stratified analysis for the REEs and food categories were further discussed and are presented in Table 2.

3.5. Mediated effect of the REEs and dietary intakes for tongue cancer

We selected food categories that were significantly related to tongue cancer and assessed the potential mediation effect of each REE in the relationships between the food categories and the risk

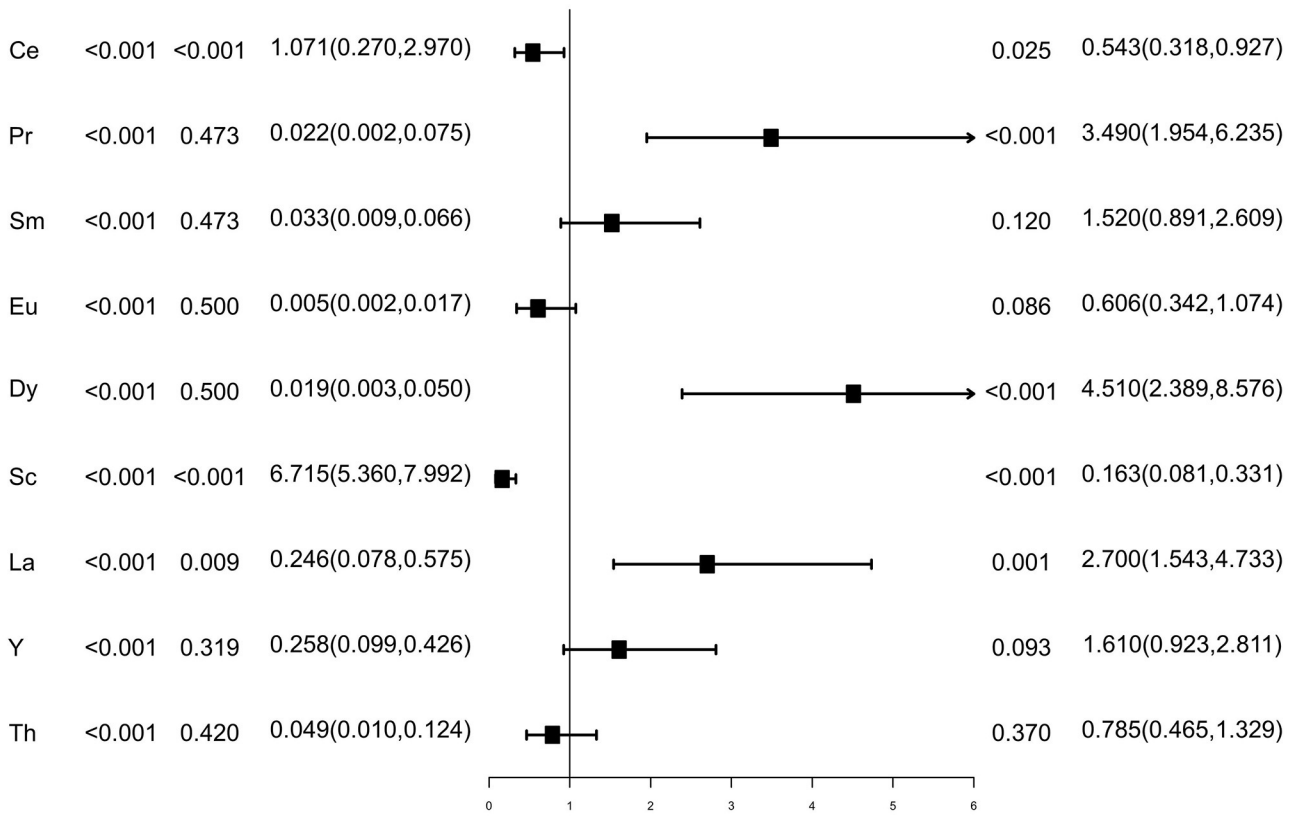

FIGURE 1
Rare earth elements of enrolled subjects. P*: P values of Shapiro-Wilk test. P#: P values of Wilcoxon rank sum test. P&: P values of logistic regression adjusted age, gender, residence, family history of cancers, BMI, tobacco smoking, tea, and alcohol drinking. M(Q): Median (quartile25, quartile75), μg/L.

of tongue cancer. The total effect, direct effect, mediated effect, and mediated proportions of each REE are shown in Table 3. We observed that some serum REEs were related to food intake ($P^* < 0.05$). Pr, Dy, and Th act partially as mediators between intake of non-green leafy vegetables and risk of tongue cancer ($P < 0.05$, the mediated proportion were 0.408%, 12.010%, and 8.969%, respectively). La and Th perform as mediators between intake of green leafy vegetables and tongue cancer with the mediated proportions of 14.933% and 25.280%, respectively. The relation between seafood consumption and tongue cancer was mediated by Sc with a mediated proportion of 26.120%.

4. Discussion

The relationship of diatery intake or REEs with varied types of cancer [oral and pharyngeal cancer (24), breast cancer (25, 26), thyroid cancer (27), pancreatic cancer (28), and lung cancer (10)] has been reported, but hitherto, their role and potential interaction in tongue cancer have still not been elucidated. After minimizing the potential confounding effects of PSM, our study supported that both serum REE levels and food categories were associated with the risk of tongue cancer, and potential multiplicative interaction and mediated effect existed between the two parameters.

Fruit, fish, seafood, green leafy vegetables, and other vegetable intake have been reported to be inversely related to oral squamous cell carcinoma, the pharynx, and larynx cancer risks, which were also observed in our study, and it may be ascribed to the abundant potential anticarcinogenic agents present in foods, such as carotenoids, dietary fiber, n-3 fatty acid, and vitamins C and E (29–32). However, the epidemiological evidence for a protective effect of food categories against cancer was inconsistent. A previous meta-analysis assessed the effect of fish intake on the risk of oral cancer and found a positive relationship in European populations rather than in other populations (33). The significant association between a higher intake of processed meat and the increased risk of oral cancer and oropharynx cancer has been suggested by numerous studies, partly owing to the potent mutagens caused during preservation or meat processing and high-temperature cooking [such as polycyclic aromatic hydrocarbons (PAHs) and N-nitroso compounds (NOCs)] (34–36). These mutagens will bind with DNA and produce PAH-DNA adducts, causing a growing risk tendency for many types of cancer (37, 38). However, other academics believed there was insufficient epidemiological evidence to substantiate an independent positive link between them (39). Existing research elucidates the role of food categories for oral cavity and pharynx cancer clearly, but few reports

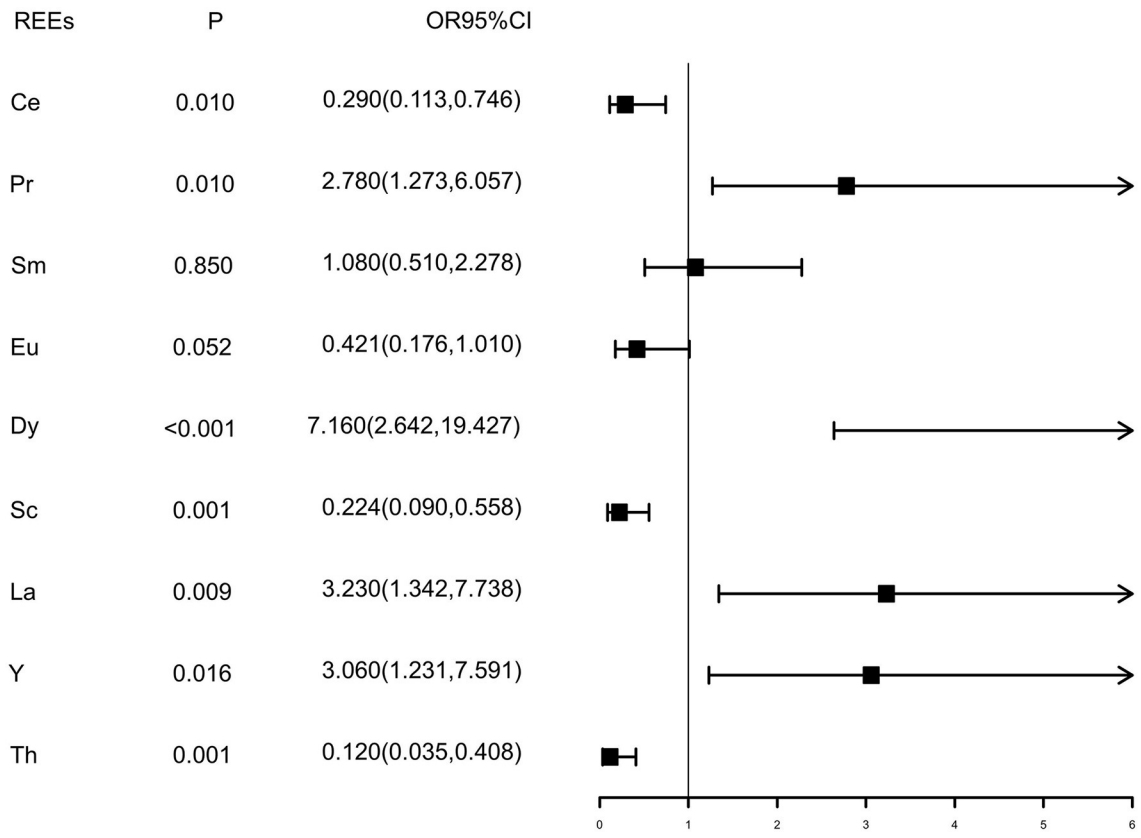

FIGURE 2
Multivariate conditional logistic regression results of REE elements. Pd: P-values of conditional logistic regression adjusted age, gender, residence, family history of cancer, BMI and alcohol drinking, tobacco smoking, tea drinking, and fruits, seafood, fish, green leafy vegetables, non-green leafy vegetables, milk, egg, meat, processed meat, and pickled food consumption.

directly explore the relationship between dietary intake and tongue cancer.

Our research revealed that the serum levels of Pr, Dy, and La increased the risk of tongue cancer, whereas Ce and Sc reduced it. Dysregulation of cellular apoptosis was a key promotion of tumorigenesis. Although no direct evidence implicated that some REEs may exert an effect on cell apoptotic, exposure to REEs could increase telomerase activity, which may be associated with DNA replication and cell apoptosis (40). Experiments proved that CeO_2 will make more cells stay in the G1 phase and decrease the production of reactive oxygen to inhibit the proliferation of tumor cells (41). Cerium oxide nanoparticles were also reported to inhibit the proliferation and promote the apoptosis of tumor cells selectively (42). Europium oxide nanorods metallocene complexes with scandium characterized anti-proliferative activity in several cancer cell lines, including triple-negative breast cancer cell line (MDA.MB231) and non-hormone sensitive prostate cancer cell line (DU145), which supported that Sc may perform as a protective factor in cancer (43). Clinical studies on Eu and Th have been conducted, which using them as new target therapies for a variety of malignancies (44, 45). To the best of our knowledge, Pr and La would induce adverse developmental effects in zebrafish embryos (especially neural and cardiovascular development) (46). Dy was shown to increase antioxidant defenses, oxidative stress, and cellular damage in mussels with a dose-dependent response, which may support our results to a certain degree (47).

Many reports highlighted that some pollutants including contamination of heavy metals and organic pollutants may pose a health risk (especially cancer) to humans via the food chain (48–51), but the REEs were almost unheeded, although it is an indisputable fact that those are widespread in various foods. Our study found that after adjusting for food categories, the effects of Y and Th for tongue cancer were covered up. Meanwhile, the ORs of food categories were modified after adjusting for REEs. This suggests that dietary intake and REEs interact intricately in tongue cancer. So, the latent combined or mediated effect of dietary intake and serum REEs for tongue cancer was then investigated. In this study, several serum REEs were associated with the intake of some food. The further stratified analysis results showed that people with lower serum levels of Eu and who consume less seafood have a higher risk of tongue cancer than others, indicating that the joint effect between serum Eu and seafood consumption under the multiplicative model was greater than expected. When people have higher serum Eu levels, the protective impact of intake of more non-green leafy vegetables is increased (OR was 0.041). The risk effect of tongue cancer was stronger in the group with low serum Ce level and intake of high green leafy vegetables or the group with high serum Pr level and intake of high green leafy

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TABLE 2 The combined effect of the REE and dietary intakes for tongue cancer.

Variables	Food categories	β	OR95%CI	*P*	$P_{interaction}$
Eu	Seafood				**0.036**
Low	<1 time/week				
High	<1 time/week	−1.831	0.160 (0.042, 0.606)	**0.007**	
Low	≥1 time/week	−2.345	0.096 (0.024, 0.389)	**0.001**	
High	≥1 time/week	−2.351	0.095 (0.023, 0.388)	**0.001**	
Eu	Non-green leafy vegetables				**0.017**
Low	<2 times/day				
High	<2 times/day	0.159	1.170 (0.342, 4.022)	0.800	
Low	≥2 times/day	−1.090	0.336 (0.080, 1.409)	0.140	
High	≥2 times/day	−3.207	0.041 (0.008, 0.214)	**<0.001**	
Ce	Green leafy vegetables				**0.039**
Low	<2 times/day				
High	<2 times/day	−0.084	0.920 (0.220, 3.839)	0.910	
Low	≥2 times/day	1.910	6.750 (1.110, 41.053)	**0.038**	
High	≥2 times/day	−0.224	0.799 (0.189, 3.375)	0.760	
Pr	Green leafy vegetables				**0.030**
Low	≥2 times/day				
High	≥2 times/day	0.575	6.160 (3.474, 10.952)	**0.002**	
Low	<2 times/day	0.736	1.770 (0.848, 3.697)	0.440	
High	<2 times/day	0.739	1.670 (0.798, 3.500)	0.490	
La	Non-green leafy vegetables				**0.046**
Low	<2 times/day				
High	<2 times/day	1.979	7.230 (2.096, 24.969)	**0.002**	
Low	≥2 times/day	−1.658	0.191 (0.046, 0.788)	**0.022**	
High	≥2 times/day	−1.268	0.281 (0.063, 1.255)	0.097	

P: The P values of stratified analysis adjusted age, gender, residence, family history of cancers, BMI and alcohol drinking, tobacco smoking, drinking tea, and fruits, seafood, fish, green leafy vegetables, non-green leafy vegetables, milk, egg, meat, processed meat, pickled food consumption. *Pinteraction*: The P values of interaction analysis of the REE and food categories for tongue cancer adjusted age, gender, residence, family history of cancers, BMI and alcohol drinking, tobacco smoking, tea drinking, and fruits, seafood, fish, green leafy vegetables, non-green leafy vegetables, milk, egg, meat, processed meat, pickled food consumption. Bolded values indicate statistical significance at $p < 0.05$.

vegetables. Compared with populations who eat fewer non-green leafy vegetables and have lower serum La levels, those with higher serum La levels have an increased risk (OR was 7.230), while those who eat more non-green leafy vegetables have a decreased risk (OR was 0.191). Those indicated that the interactions of the levels of some serum REEs and intake of some food categories played important roles in tongue cancer. The changes in tongue cancer risk due to dietary changes were influenced by the levels of some serum REEs.

In order to know whether dietary control may limit the intake of some REEs and further induce or inhibit tongue cancer, we tested the potential mediate effects of REEs. Our study suggests that green vegetables' impact on the risk of tongue cancer is partially attributed to the La and Th elements. The Pr, Dy, and Th components in non-green leafy vegetables and the Sc components in seafood are partly responsible for their influence on the risk of tongue cancer. According to a Chinese study in 2012, the concentrations of Ce, Dy, Y, Nd, and La in some food categories were higher than the concentrations of other REEs, whereas green vegetables and aquatic products have higher quantities of total rare earth element oxides than other food categories (52). Another report also noted that vegetables in mining regions contain higher levels of some REEs (including La, Pr, and Dy), and the southeast province of China investigated in our study is one of the key mining regions (26, 53). A study found that marine algae are effective natural adsorbents for some REEs, particularly for the Sc element (54). As the Fujian province is a significant coastal region, its residents would consume more marine algae than those in other locations, increasing their exposure to the Sc element. These studies help to explain some of our findings.

Our study creatively explored the mediated effect and multiplicative interaction of food categories and REEs on the development of tongue cancer. The findings of this research will offer direction for daily food and future mechanistic research on

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TABLE 3 The mediation effect of the REE and food categories for tongue cancer.

Variables	Total effect	Direct effect	Mediated effect	Proportion mediated (%)	*P*	*P**
Ce						
Seafood	−0.148 (−0.211, −0.070)	−0.150 (−0.213, −0.080)	0.003 (−0.006, 0.020)	17.267	0.700	0.375
Fish	−0.032 (−0.126, 0.070)	−0.036 (−0.138, 0.080)	0.004 (−0.006, 0.020)	3.048	0.540	0.248
Fruit	−0.164 (−0.242, −0.080)	−0.167 (−0.244, −0.080)	0.003 (−0.007, 0.010)	0.549	0.740	0.783
Green leafy vegetables	0.071 (−0.074, 0.180)	0.078 (−0.071, 0.190)	−0.007 (−0.026, 0.000)	4.750	0.260	**0.044**
Non-green leafy vegetables	−0.231 (−0.267, −0.180)	−0.233 (−0.273, −0.190)	0.002 (−0.005, 0.010)	0.408	0.700	0.291
Pr						
Seafood	−0.145 (−0.222, −0.060)	−0.121 (−0.207, −0.040)	−0.024 (−0.060, 0.000)	15.820	0.100	**0.015**
Fish	−0.038 (−0.139, 0.080)	−0.038 (−0.138, 0.090)	0.000 (−0.030, 0.030)	1.417	0.980	0.937
Fruit	−0.167 (−0.251, −0.060)	−0.163 (−0.248, −0.070)	−0.004 (−0.029, 0.020)	2.809	0.760	0.635
Green leafy vegetables	0.062 (−0.080, 0.160)	0.041 (−0.105, 0.150)	0.021 (−0.005, 0.040)	23.944	0.160	0.109
Non-green leafy vegetables	−0.232 (−0.265, −0.190)	−0.202 (−0.236, −0.150)	−0.030 (−0.057, −0.010)	0.408	**<0.001**	**0.003**
Sm						
Seafood	−0.138 (−0.201, −0.040)	−0.137 (−0.200, −0.040)	−0.002 (−0.015, 0.010)	0.571	0.800	0.637
Fish	−0.030 (−0.145, 0.070)	−0.032 (−0.143, 0.070)	0.002 (−0.007, 0.020)	0.092	0.660	0.683
Fruit	−0.163 (−0.230, −0.070)	−0.165 (−0.230, −0.070)	0.001 (−0.005, 0.010)	0.410	0.820	0.592
Green leafy vegetables	0.048 (−0.090, 0.160)	0.041 (−0.095, 0.160)	0.007 (−0.005, 0.030)	2.522	0.240	0.098
Non-green leafy vegetables	−0.231 (−0.269, −0.200)	−0.225 (−0.262, −0.190)	−0.007 (−0.024, 0.000)	7.417	0.420	**0.007**
Eu						
Seafood	−0.152 (−0.208, −0.070)	−0.156 (−0.211, −0.070)	0.004 (−0.007, 0.020)	2.327	0.440	0.358
Fish	−0.021 (−0.128, 0.100)	−0.028 (−0.137, 0.100)	0.007 (−0.006, 0.030)	3.769	0.340	0.131
Fruit	−0.162 (−0.227, −0.060)	−0.160 (−0.226, −0.060)	−0.002 (−0.016, 0.010)	0.550	0.740	0.359
Green leafy vegetables	0.069 (−0.049, 0.170)	0.078 (−0.044, 0.180)	−0.010 (−0.030, 0.000)	9.385	0.120	0.090
Non-green leafy vegetables	−0.228 (−0.264, −0.190)	−0.235 (−0.271, −0.200)	0.007 (−0.006, 0.020)	2.878	0.240	0.116
Dy						
Seafood	−0.147 (−0.210, −0.040)	−0.135 (−0.198, −0.030)	−0.012 (−0.036, 0.010)	7.200	0.260	0.123
Fish	−0.042 (−0.134, 0.060)	−0.046 (−0.140, 0.050)	0.005 (−0.027, 0.040)	0.365	0.720	0.501
Fruit	−0.157 (−0.243, −0.070)	−0.153 (−0.232, −0.070)	−0.004 (−0.027, 0.020)	2.049	0.760	0.613
Green leafy vegetables	0.054 (−0.070, 0.160)	0.029 (−0.091, 0.140)	0.025 (−0.001, 0.050)	27.974	0.080	**0.025**
Non-green leafy vegetables	−0.226 (−0.264, −0.180)	−0.198 (−0.243, −0.140)	−0.028 (−0.047, −0.010)	12.010	**<0.001**	**<0.001**
Sc						
Seafood	−0.146 (−0.225, −0.060)	−0.106 (−0.175, −0.030)	−0.040 (−0.069, −0.010)	26.120	**<0.001**	**0.004**
Fish	−0.039 (−0.126, 0.080)	−0.016 (−0.105, 0.090)	−0.023 (−0.060, 0.010)	31.410	0.120	0.176
Fruit	−0.166 (−0.242, −0.070)	−0.141 (−0.221, −0.040)	−0.025 (−0.057, 0.000)	15.590	0.140	0.074
Green leafy vegetables	0.059 (−0.074, 0.160)	0.077 (−0.045, 0.170)	−0.018 (−0.064, 0.020)	11.280	0.360	0.192
Non-green leafy vegetables	−0.231 (−0.270, −0.180)	−0.231 (−0.277, −0.180)	0.000 (−0.024, 0.030)	1.000	0.123	0.995

(Continued)

TABLE 3 (Continued)

Variables	Total effect	Direct effect	Mediated effect	Proportion mediated (%)	*P*	*P**
La						
Seafood	−0.140 (−0.207, −0.070)	−0.127 (−0.193, −0.050)	−0.013 (−0.031, 0.000)	8.360	0.100	0.044
Fish	−0.028 (−0.149, 0.070)	−0.031 (−0.161, 0.070)	0.004 (−0.018, 0.030)	1.693	0.680	0.570
Fruit	−0.166 (−0.244, −0.060)	−0.166 (−0.247, −0.070)	0.000 (−0.018, 0.020)	<0.001	0.140	0.980
Green leafy vegetables	0.057 (−0.080, 0.160)	0.041 (−0.097, 0.150)	0.016 (0.001, 0.040)	14.933	**0.020**	**0.044**
Non-green leafy vegetables	−0.227 (−0.265, −0.190)	−0.214 (−0.251, −0.170)	−0.013 (−0.032, 0.000)	5.247	0.060	**0.006**
Y						
Seafood	−0.147 (−0.205, −0.060)	−0.148 (−0.207, −0.070)	0.001 (−0.006, 0.010)	0.388	0.680	0.564
Fish	−0.030 (−0.136, 0.080)	−0.033 (−0.138, 0.080)	0.002 (−0.008, 0.010)	0.780	0.820	0.114
Fruit	−0.159 (−0.236, −0.050)	−0.160 (−0.234, −0.050)	0.001 (−0.007, 0.010)	0.149	0.820	0.839
Green leafy vegetables	0.065 (−0.062, 0.150)	0.063 (−0.060, 0.150)	0.002 (−0.008, 0.010)	1.205	0.740	0.304
Non-green leafy vegetables	−0.227 (−0.262, −0.180)	−0.225 (−0.265, −0.180)	−0.002 (−0.011, 0.010)	0.418	0.620	0.228
Th						
Seafood	−0.144 (−0.212, −0.060)	−0.151 (−0.216, −0.070)	0.007 (−0.006, 0.020)	4.332	0.420	0.293
Fish	−0.037 (−0.134, 0.070)	−0.032 (−0.143, 0.070)	0.002 (−0.007, 0.020)	0.360	0.660	0.922
Fruit	−0.174 (−0.242, −0.090)	−0.177 (−0.243, −0.090)	0.004 (−0.016, 0.020)	1.582	0.660	0.594
Green leafy vegetables	0.063 (−0.076, 0.150)	0.087 (−0.057, 0.170)	−0.023 (−0.043, 0.000)	25.280	**0.020**	**0.003**
Non-green leafy vegetables	−0.233 (−0.270, −0.190)	−0.254 (−0.295, −0.200)	0.021 (0.002, 0.040)	8.969	**0.040**	**<0.001**

P: The P values of mediated effect for tongue cancer adjusted for age, gender, residence, family history of cancers, BMI and alcohol drinking, tobacco smoking, tea drinking, and fruits, seafood, fish, green leafy vegetables, non-green leafy vegetables, milk, egg, meat, processed meat, pickled food consumption. P*: The P values of the relations of the REE and food categories adjusted for age, gender, residence, family history of cancers, BMI and alcohol drinking, tobacco smoking, tea drinking, and fruits, seafood, fish, green leafy vegetables, non-green leafy vegetables, milk, egg, meat, processed meat, pickled food consumption. Bolded values indicate statistical significance at $p < 0.05$.

the pathophysiology of tongue cancer. However, the limitation also should not be ignored, due to the relatively weak causal reference of the case–control studies with a small sample, we cannot elucidate the causal relationship between REEs and tongue cancer. Thus, more direct epidemiological evidence from a large-scale prospective study needs to be collected in future studies. Then, the cases enrolled in the study were only from one hospital, and the dietary information was recalled by each participant (precise and explicit quantifications are not available); the bias cannot be avoided. Furthermore, intentional drug usage history concealment by participants may have an impact on the levels of serum REEs. In addition to that, though many measures were taken by us to reduce the difference, we cannot deny the possibility that the case and control are from two different populations according to the present eligibility criteria. Finally, the concentrations of elements may change due to exposure to air, water, cooking, and storage techniques; that is the information we cannot access.

5. Conclusion

The correlation between REEs and dietary intake for tongue cancer is compact but intricate; the change in dietary intake may change the serum levels of several REEs and further influence the risk of tongue cancer. The joint effect between REEs and food categories in tongue cancer should not be overlooked. Further prospective studies are still needed in validating our findings and exploring the underlying mechanism.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Ethics statement

This research was performed in accordance with the ethical standards of the Helsinki Declaration, and the ethical approval was obtained from the Ethics Committees of Fujian Medical University, Fuzhou, China (2011053). The patients/participants provided their written informed consent to participate in this study. Written informed consent was obtained from the individual(s) for the publication of any potentially identifiable images or data included in this article.

Author contributions

NW: formal analysis, writing—original draft, and writing—review and editing. FL and MX: investigation and writing—review and editing. YC: methodology and writing—review and editing. BG: validation and writing—review and editing. YQ, LL, and BS: resources and writing—review and editing. BH: conceptualization, funding acquisition, and writing—review and editing. FC: conceptualization, funding acquisition, formal analysis, and writing—review and editing. All authors contributed to the article and approved the submitted version.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fpubh.2023.1058013/full#supplementary-material

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Bibliometric analysis of the association between drinking water pollution and bladder cancer

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Background: Bladder cancer has become an increasingly intractable health problem worldwide. Long-term drinking water pollution is known to promote its occurrence. This study aimed to analyze the research status, hot spots, and future trends of drinking water pollution and bladder cancer through extensive bibliometric examination to provide reference data for better prevention and management of bladder cancer.

Methods: The Scopus database developed by Elsevier was browsed for articles that met the predefined criteria using the search terms related to drinking water and bladder cancer. Included articles were further evaluated by year of publication, subject category, institution, article type, source journal, authors, co-authorship networks, and text mining of titles by R software packages tm, ggplot2 and VOSviewer software.

Results: In total, 687 articles were selected after a comprehensive literature search by the Scopus database, including 491 research articles, 98 review articles, 26 conference papers, 23 letters and 49 other documents. The total number of articles published showed an upward trend. The United States has the largest number of published articles (345 articles), institutions (7/10) and funding sponsors (top 5). The journal with the most publications was *Environmental Health Perspectives*, with 46 published. The highest number of citations up to 2330 times for a single article published in 2007 on the journal of *Mutation Research*. Professor Cantor K.P. was the highest number of publications with 35 articles and Smith A.H. was the most cited author with the number of citations reaching 6987 times overall and 225 times per article. The most frequent keywords excluding the search subject were "arsenic", "chlorination", "trihalomethane", and "disease agents".

Conclusion: This study is the first systematic bibliometric study of the literature publications on drinking water pollution and bladder cancer. It offers an overall and intuitive understanding of this topic in the past few years, and points out a clear direction research hotspots and reveals the trends for further in-depth study in future.

KEYWORDS

drinking water pollution, bladder cancer, bibliometrics analysis, co-occurrence, future direction

Introduction

Bladder cancer is a common urological malignancy in females and the sixth most common type of urological malignancy in males; approximately 573,000 new cases and 213,000 deaths were reported in 2020 globally (1). Diverging incidence trends of bladder cancer have been observed; there were stabilizing or declining rates in males, but some increasing trends were observed for females (2, 3). Clinical studies have shown that the 5-year survival rate is approximately 90% although several patients with a first diagnosis of bladder cancer are treated with surgical resection combined with regular postoperative bladder infusion of chemotherapy drugs and other modern advanced treatments. However, most patients experience tumor recurrence within a few years after surgery, and up to 20% of patients with bladder tumor recurrence have lymph node metastasis and distant multiple metastases (4). The 5-year survival rate of patients with muscle-invasive bladder cancer is less than 50%, and muscle-invasive bladder cancer is more likely to metastasize (5). However, the etiology and mechanism of bladder cancer occurrence, recurrence, and metastasis are still unclear, which further impacts the incidence, prognosis, and mortality rates.

Studies have shown that the risk factors for bladder cancer include age, race, obesity, family history, and environmental risk factors, among others (6). Environmental risk factors are important factors leading to increased risks of bladder cancer and have attracted the interest of researchers (7, 8). For example, smoking increases the risk of bladder cancer and significantly increases the risk of bladder cancer recurrence and mortality in patients with non-invasive bladder cancer (2, 9). Occupational carcinogens (including exposure to diesel exhaust, polycyclic aromatic hydrocarbons, certain pesticides, and herbicides) (10) and previous exposure to chemotherapeutic agents (11) are risk factors for the development of bladder transitional cell carcinoma.

The bladder is the main excretion organ for urine, and a large number of toxic substances accumulate in it. Therefore, drinking water pollution, another potential risk factor for bladder cancer, has also attracted the interest of researchers in recent years (12, 13). For example, the long-term consumption of chlorinated drinking water containing complex mixtures leads to a significant increase in the incidence of bladder cancer as a by-product of chlorinated bromination in water (14). According to the U.S. Environmental Protection Agency, the highest concentration of arsenic in drinking water is 10 μg/L, which increases the risk of bladder cancer (15). Therefore, lifestyle interventions may be useful for the prevention of bladder cancer recurrence. However, systematic analyses on the role of drinking water pollution and bladder cancer in preventing bladder cancer recurrence are limited.

Bibliometrics is a scientific method that analyzes the distribution, quantitative relationship, and change rules of previous documents based on the literature system and bibliometric characteristics by applying various quantitative methods, such as mathematics and statistics (16). Bibliometric analysis can record the breadth and depth of the current status of scientific literature research and predict future research trends. Given its robustness, clarity, and comprehensiveness, it plays an important role in scientific bibliometrics. However, to the best of our knowledge, there have been no published bibliometric studies on drinking water and bladder cancer.

In this study, we conducted a systematic review using the method of bibliometric analysis to establish a visual knowledge map by searching the literatures related to drinking water and bladder cancer in the Scopus database, and summarize and analyze the literature publication, research topics, research hotspots and future development trends in this field, so as to provide a reliable reference of drinking water pollution and bladder cancer.

Materials and methods

Literature search and data acquisition

We searched the world's largest Scopus database through the Wuhan University Library on January 7th, 2023, and the search strategy was set as the following: (1) search time: scheduled until January 7th 2023; (2) search terms: limited to "bladder cancer" and " drinking water "; (3) search scope: "TITLE-ABS-KEY"; (4) literature types: select all, including regular and review articles, and other forms. With no limitation in publication language, all other settings were as the default value, and duplicate or invalid documents were removed. Thus, we obtained 687 published articles with a comprehensive coverage of all the available literature, studies pertaining to fields other than medicine, studies on non-human subjects, and those without abstracts were also included. We download all the articles information including but not limited to

Author, Title, Cited by, Affiliations in RIS and CSV file formats for further analyses. In VOSviewer, we select the minimum number of documents of the nodes according to the needs of data visualization and set other documents as the default value.

Software and version

We used text-mining R packets such as "NLP", "tm", "wordcloud2", "ggplot2" to cluster title keyword and draw clustering diagrams. The version of R software is 4.1.0. The VOSviewer 1.6.18 software was used to visualize network of co-author, co-occurrence and co-citation. In addition, several tables in the study were generated in the Microsoft Excel program of Microsoft Corporation.

Statistical analysis and visualization

In this bibliometrics analysis, we created network visualization using VOSviewer version 1.6.18 (released on January 24, 2022, Centre for Science and Technology Studies, Leiden University, Leiden, the Netherlands) with nodes representing documents, affiliations, authors, or keywords/author-keywords and can be connected by co-authorship, citation, co-citation, and co-occurrence analysis. The size of a node was determined by the weight of the element, such as the number of publications, citations, or the frequency of occurrences. And cluster of the same color indicated the same category, which was a set of items in the network with similar properties. The thickness of the links and the total link strength (TLS) were used to quantitatively assess the links. Similar analyses and visualizations were performed to create network map of highly cited documents and the journals, keywords, authors with the publications. The workflow of this bibliometrics analysis is shown in Figure 1.

Results

Bibliometric analysis of basic information on drinking water pollution and bladder cancer

We retrieved 687 documents related to drinking water and bladder cancer. The number of published documents has been increasing, but the rate has reduced in recent years. Based on the number of published articles, the top five countries were the United States, Japan, China, Canada, and Spain. Among them, the United States published approximately four times the number of articles published by second-ranked Japan, reaching 345 articles (Figure 2A). The number of published research articles in China and other countries has increased significantly within the past 20 years. It also shows that more countries have begun to pay attention to drinking water safety. Among the top 10 institutions with the most publications, seven were in the United States (Supplementary Table 1). Moreover, the top five funding sponsors were all in the United States (Supplementary Table 2). As observed, the literature dominantly belonged to the categories of medicine (n = 402) and environmental sciences (n = 253), which accounts for more than 60% of the documents that had duplicate disciplines. Reports in the fields of biochemistry, genetics, molecular biology, pharmacology, toxicology, pharmacy, chemistry, and other disciplines were also found (Figure 2B). Researchers are looking for causal relationships and progress for several aspects of drinking water pollution and

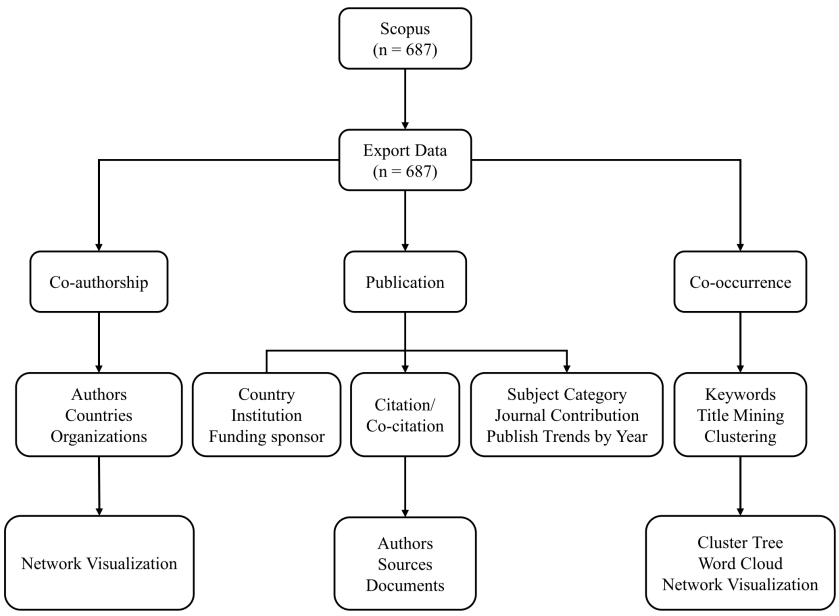

FIGURE 1
Workflow of the bibliometrics analysis.

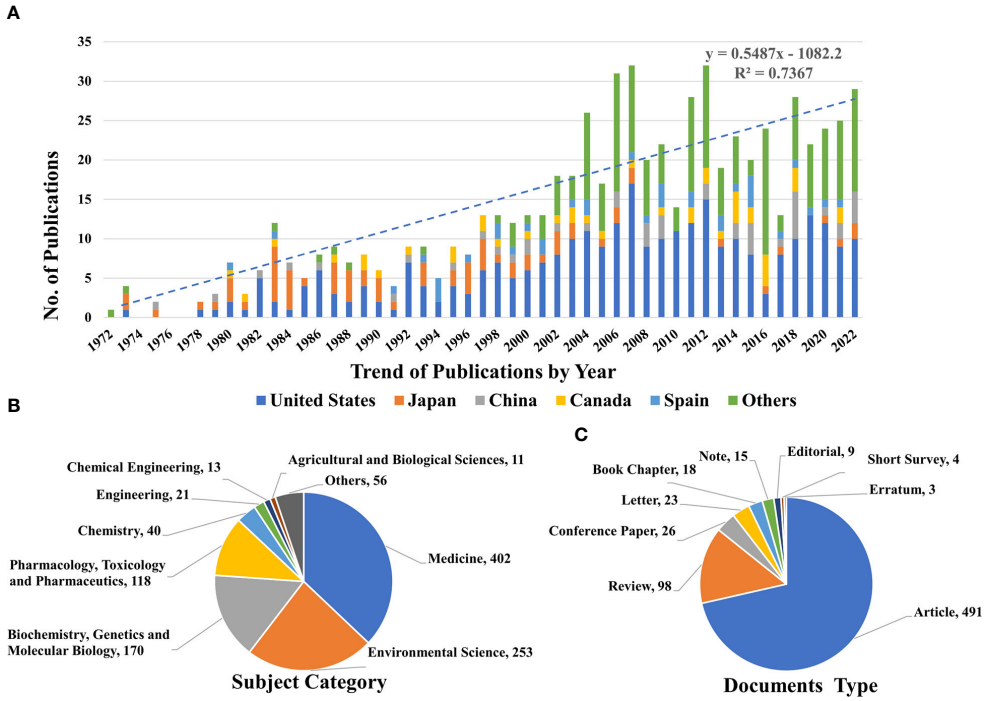

FIGURE 2
Basic Information of publishing trends and statistical results. **(A)** is the line graph and linear fitting curve showing the number of published documents each year. **(B)** shows the different subject categories of the published documents. **(C)** shows the documents types of the published documents.

bladder cancer. Most of the studies were research articles (n=491, 71.47%). There were also 98 review papers, 26 conference papers, and 23 letter papers; most of these were research-based statistical analysis documents (Figure 2C). With the development of the economy and society, the relationship between drinking water and bladder cancer has attracted increasing attention from scholars globally.

Distribution analysis of journals, authors, and co-authorship in the fields

To further investigate the data on journal publications and bibliographic citations, we performed a more specific bibliometric analysis and visualization of the source journals, authors, and co-authorship. As shown in Table 1, 152 journals were assessed, and 13 had published at least 10 papers. The journal with the most publications was *Environmental Health Perspectives*, with 46 published within half a century, which was 19 more than the number of publications of the second journal, the *American Journal of Epidemiology*. *Environmental Health Perspectives* was also the only journal with more than 15 publications. Eleven of the 13 journals that had published at least 10 papers belonged to the Q1 journal category according to the latest Thomson Web of Knowledge Journal Citation Reports Ranking, and their impact factors were within the range of 4–13. Six of these journals had impact factors exceeding 10. At the same time, 33 journals had published no less than five articles. Regarding the bibliometrics analysis of the author, Table 2 shows the data of 10 authors with the most published articles in this field. Professor Cantor K.P. from the National Cancer Institute published 35 articles in this field, which was the highest number of publications. The most cited author was Smith A.H., with the number of citations reaching 6987 times overall and 225 times per article. Co-authorship is an important part of scientific research, from which we can identify clusters of authors and their contributions to publications in this field. Figure 3 shows collaborative associations among 197 authors filtered from 2336 authors in total, with the minimum number of documents per author being 3. Focusing on the authors with the most published literature, 10 author groups were formed, among which the Cantor K.P. and Vilanueva C.M. cluster groups were the most closely related.

Literatures citation and co-citation analysis

Citation is the most important indicator for evaluating the influence of a journal or article, and we analyzed and visualized the citations of journals and literature to fully understand the research situation in the field of drinking water pollution and bladder cancer. As shown in Table 3, we collected the basic information of the 10 most-cited articles and found that the most-cited document was cited 2330 times. Three of the top 10 articles came from the Smith A.H. team, which also confirmed the result that the Smith A.H. team received the highest number of citations in the study (Table 2). There were also four journals with publication number rankings, as shown in Table 3. Proceeding with the study citations and co-citations, the citations were presented from the perspectives of literature, journals, and authors. The number of documents shown in Figure 4A is 433 instead of all 687 articles, with the conditions

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TABLE 1 Journals published no less than 10 items.

Sort	Journals	No. of documants	Impact Factor*	JCR*
1	**Environmental Health Perspectives**	46	11.035	Q1
2	**American Journal Of Epidemiology**	19	5.363	Q2
3	Toxicology And Applied Pharmacology	15	11.357	Q1
4	**Environmental Science And Technology**	14	4.46	Q2
5	Science Of The Total Environment	14	10.753	Q1
6	Journal Of The National Cancer Institute	13	11.816	Q1
7	Journal Of Urology	13	7.6	Q1
8	Cancer Research	12	13.312	Q1
9	International Journal Of Environmental Research And Public Health	12	4.614	Q1
10	Environmental Research	11	8.431	Q1
11	Epidemiology	11	4.86	Q1
12	**Environment International**	10	13.352	Q1
13	Toxicology	10	4.571	Q1

*Journal impact factor based on Thomson Web of Knowledge Journal Citation Reports Ranking(2022).

that the minimum number of citations of a document is 5 and the largest set of connected items is 433. The inclusion criteria for the 289 journals are that the minimum number of documents of a source is 1 and the minimum number of citations of a source is 10. Finally, 189 journals met the thresholds (Figure 4B). The filter rules for 2320 authors were also similar; the minimum number of documents and citations of an author were 1 and 50, respectively, and 843 authors were selected from the network (Figure 4C). Overall, only 223 authors out of 41618 had been cited more than 50 times. Figure 4D shows that the aggregation effect was significant.

Distribution analysis of keywords and network analysis of co-occurrence of the documents

We included two types of keywords, all keywords and author keywords, for the bibliometric analysis. The former included the high-frequency keywords filtered from the title and abstract, and the author keywords were those provided by the author, usually including 5–8 words. We performed clustering and visualization based on the frequency of the co-occurrence of the keywords. For future analysis, the title text mining clustering tree and word cloud

TABLE 2 Statistics of the 10 authors with the most published articles.

Author	No. of publications	Citations in 669-study	Publications in Web of Science	Sum of Times Cited*	Cited by articles in total*	H-index*
Cantor K.P.	35	3063	214	10245	7297	57
Smith A.H.	31	6987	212	15655	9872	69
Villanueva C.M.	25	2247	84	3883	2555	34
Fukushima S.	22	868	200	2935	2282	28
Kogevinas M.	21	2116	670	31007	23536	93
Cohen S.M.	17	979	182	4030	2724	38
Ferreccio C.	17	1436	236	18993	15799	58
Ito N.	17	715	17	95	94	5
Karagas M.R.	17	984	504	18513	13870	74
Steinmaus C.	17	1828	124	6342	4627	45

*Information from Author Profile webpage of Web of Science.

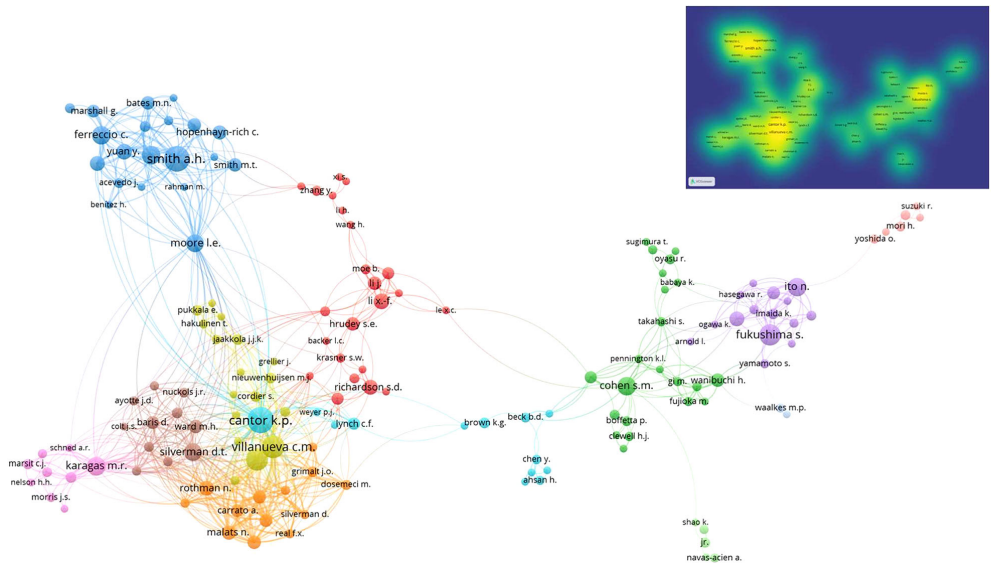

FIGURE 3
The network of co-authorship. In the network graph, there are ten colors representing different clusters, and the size of points represents the number. Some of the 197 items in the network are not connected to each other, so we replaced this 197 items network with 156 items that included the largest number authors. The upper left is the density map by publications.

were generated by R software through the text mining package "tm." The all-keyword analyses included 5433 keywords, with the filtering condition that the minimum number of occurrences of a keyword was 5. Finally, 734 keywords were selected. The highest-ranked contaminant was arsenic, which appeared 365 times, which is consistent with the reality of water pollution. The clustering results also showed that several toxicants associated with carcinogenicity in water pollution, such as arsenic, chlorination, and trihalomethane, and disease agents appeared in the bladder cancer-related clustering (Figure 5A). Of the total of 1167 author keywords, only 74 appeared more than five times, but the network clustering results showed important information. Based on the clustering results, drinking water pollution was highly associated with skin cancer, in addition to bladder cancer. Cluster analysis of the title keywords revealed more concentrated terms with causal associations (Figure 5B). Figure 5C shows the type of toxic substances involved in drinking water pollution and the highly related organ damage, such as the bladder, liver, lung, and kidney. Damage pathway analyses were also performed, which revealed methylation and genetic mutations. To show the frequency of title keywords more intuitively, we used the title text-mining results to generate a word cloud map (Figure 5D). We found the high-frequency entries and topics that had been studied in the field in this cloud map. Larger fonts denoted higher frequencies and importance. This visualization can help researchers quickly obtain the distribution of popular research results in this field.

Discussion

In this bibliometric analysis, we systematically analyzed the essential bibliometric parameters, such as the number and type of publication articles, institutions, funding sponsor distribution, and journals of study associated with the relationship between drinking water and bladder cancer, and identified the journals with the highest number of published manuscripts and/or citations. We created the co-authorship network to identify the collaborations among authors, countries, and organizations. We drew a citation/co-citation point-like relationship diagram to describe the linkage of authors, documents, and journals. Citation/co-citation precedes the interconnection between studies in this field. To identify the hot topics related to the relationship between drinking water pollution and bladder cancer, we generated the term cloud. In short, through the above analysis, we gained a comprehensive and in-depth understanding of research in this field and assessed its development and current situation, which will be useful in further promoting the depth and breadth of future research (17).

The number of published articles in a particular field is an important indicator of the development of scientific research. This reflects the change in the knowledge of scholars on the subject, as well as the latest research progress in this field (18). We collected 687 articles related to drinking water and bladder cancer published between 1972 and 2023 from the largest Scopus literature database, from which the number of research studies on drinking water and bladder cancer is on the rise, and the subject areas and research hotspots are constantly expanding. For the first report on drinking water and bladder cancer from 1972 to 2001, there were no more than 13 publications in the field annually. However, since 2002, the number of documents published in the field has grown rapidly; there were 32 publications in 2007. In addition, we established a simple growth trend model ($y=0.515x-1015.2$, $R^2 = 0.6939$) that could predict the publication of articles, and it showed that as many as 28 articles on drinking water pollution causing bladder cancer will be published in 2023. Overall, the number of published

TABLE 3 Basic information of the top 10 documents in total citations.

Authors	Title	Year	Source Journal	Impact Factor*	Cited by	Affiliations
Richardson S.D. et al.	Occurrence, genotoxicity, and carcinogenicity of regulated and emerging disinfection by-products in drinking water: A review and roadmap for research	2007	Mutation Research - Reviews in Mutation Research	3.151	2330	National Exposure Research Laboratory, US Environmental Protection Agency, Athens, GA 30605, USA.
Smith A.H. et al.	Contamination of drinking-water by arsenic in Bangladesh: A public health emergency	2000	Bulletin of the World Health Organization	13.831	1557	Department of Epidemiology, School of Public Health, University of California, Berkeley, Berkeley, CA 94720-7360, United States.
Camargo J.A. et al.	Ecological and toxicological effects of inorganic nitrogen pollution in aquatic ecosystems: A global assessment	2006	**Environment International**	13.352	1367	Departamento de Ecología, Edificio de Ciencias, Universidad de Alcalá, 28871 Alcala de Henares, Madrid, Spain.
Smith A.H. et al.	Cancer risks from arsenic in drinking water	1992	**Environmental Health Perspectives**	11.035	947	Biomedical/Envtl. Health Sci. Dept., 314 Warren Hall, University of California, Berkeley, CA 94720, United States.
Hughes M.F. et al.	Arsenic exposure and toxicology: A historical perspective	2011	Toxicological Sciences	4.109	836	Office of Research and Development, National Health and Environmental Effects Research Laboratory, U.S. Environmental Protection Agency, Research Triangle Park, NC 27711, United States.
Chen C.-J. et al.	Cancer potential in liver, lung, bladder and kidney due to ingested inorganic arsenic in drinking water	1992	British Journal of Cancer	9.075	675	Institute of Public Health, National Taiwan University College of Medicine, Taipei, 10018, Taiwan.
Kitchin K.T.	Recent advances in arsenic carcinogenesis: Modes of action, animal model systems, and methylated arsenic metabolites	2001	**Toxicology and Applied Pharmacology**	4.46	672	Environmental Carcinogenesis Division, National Health and Environmental Effects Research Laboratory, U.S. Environmental Protection Agency, Research Triangle Park, NC 27711, United States.
Smith A.H. et al.	Marked increase in bladder and lung cancer mortality in a region of northern chile due to arsenic in drinking water	1998	**American Journal of Epidemiology**	5.363	652	School of Public Health, University of California, Berkeley, CA, United States.
Costa M.	Toxicity and carcinogenicity of Cr(VI) in animal models and humans	1997	Critical Reviews in Toxicology	6.184	494	Nelson Inst. of Environ. Medicine, Kaplan Cancer Center, 550 First Avenue, New York, NY 10016, United States.
Mohammed Abdul K.S.	Arsenic and human health effects: A review	2015	Environmental Toxicology and Pharmacology	5.785	455	Department of Zoology, Faculty of Science, University of Ruhuna, Matara, 81000, Sri Lanka

*Journal impact factor based on Thomson Web of Knowledge Journal Citation Reports Ranking (2022).

documents has increased, although the growth rate has decreased in recent years. Several countries have recently appeared on the list of posts and their publications are increasing every year. This also shows that an increasing number of countries are focusing on the safety of drinking water. This may be due to the fact that researchers in countries that initially studied drinking water pollution are still limited to the identified pollutants, and no breakthrough has been made in other possible drinking water pollutants, pathogenic mechanisms, and improvement of exposure assessment and accurate characterization of individual factors, while developing countries pay more and more attention to the relationship between drinking water pollution and diseases.

The level of national emphasis and scientific research on sanitation and health are often closely related to local economic and social development and public health status (19, 20). The present study demonstrated that the United States, Japan, Canada, and Spain, as traditionally developed countries, have been the main countries involved in the study of the relationship between drinking water pollution and bladder cancer. Surprisingly, China (21) and other countries (22, 23) have also paid more attention to it within the past 20 years, accounting for more than half of the articles published every year. For example, the Chinese government has recently revised the Environmental Quality Standards for Surface Water (EQSSW) (GB3838-2002) to address the challenges of environmental protection by refining water function zoning, establishing priority pollutants, and improving the protection of drinking water sources (24). Furthermore, the United States is still at the forefront based on the perspective of research institutions and fund sponsors, which means that American research institutions have a strong scientific output in this field. This may be related to the high incidence of bladder cancer in North America according to the global cancer statistics (1, 25), and they are more concerned and eager to determine the relationship between water pollution and bladder cancer, as well as prevent it.

From the perspective of research by discipline category, current research is mainly concentrated in the field of medicine and environmental science, accounting for more than 60% of the

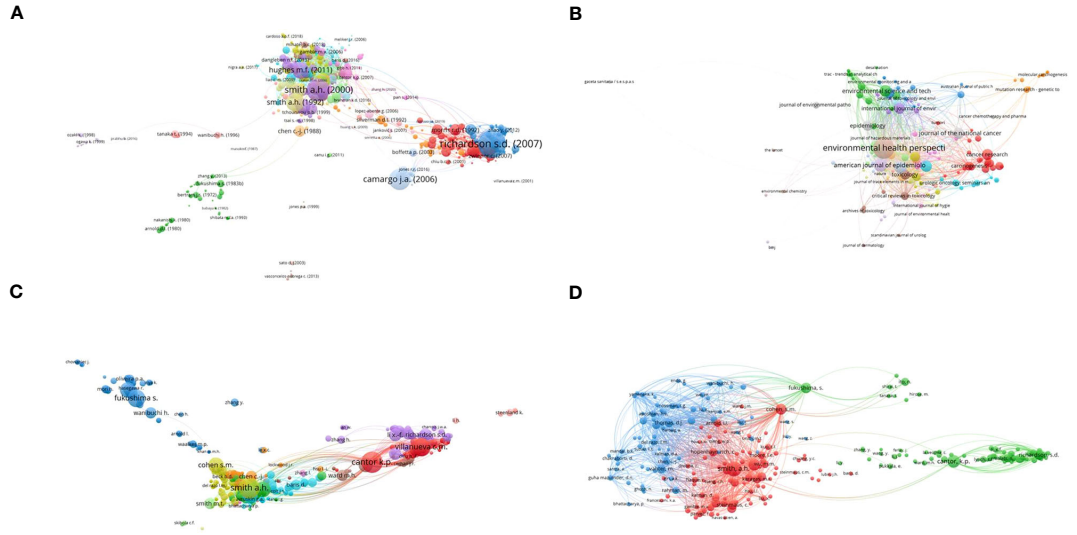

FIGURE 4
Network of citation of documents, journals and authors by VOSviewer. **(A–C)** represent the citations of documents, journals, and authors, respectively. **(D)** is the co-citation network of the cited authors. Document network shows the citation relationship between 420 documents in the name of authors. 170 journals appear in **(B)** D graph is a subset of C graph.

subject double-counting literature. This is consistent with our prediction of the association between drinking water pollution and bladder cancer development. Additionally, there are research reports in the fields of biochemistry, genetics, molecular biology, pharmacology, toxicology, pharmacy, chemistry, and other disciplines. This suggests that researchers around the world are searching for causal relationships and progress on several aspects of this association (12, 13).

From the perspective of research output, the number of studies on drinking water and bladder cancer is on the rise, and the subject areas and research hotspots are constantly expanding. Among the high-yield journals, *Environmental Health Perspectives* published most of the articles. This indicates that research in the field of drinking water pollution and bladder cancer has been widely recognized, and corresponding preventive measures have been focused on. However, in general, the number of publications in high-quality core journals remains small. The authors with the high number of publications were Professor Cantor K.P. from the National Cancer Institute and Professor Smith A.H. from the University of California, who have long been engaged in epidemiological (26) and risk factor (27) research on the relationship between pollutants in drinking water and cancer. This field has several groups of authors with high yields and some high-yield research teams have a certain degree of cooperation. However, in-depth cooperation is lacking.

To better understand the current situation and research development for drinking water pollution and bladder cancer, a visual analysis of drinking water pollution and bladder cancer was performed and the research hotspots in this field were determined. The clustering results also showed that several toxicants associated with carcinogenicity in water pollution, such as arsenic (28), chlorination (29), trihalomethane (30), and disease agents (31), appeared in the bladder cancer-related clustering. From the clustering results, drinking water pollution was highly associated with skin cancer (32), in addition to bladder cancer. Cluster analysis of the title keywords showed more concentrated terms with causal associations; drinking water pollution and highly related damage to organs, such as the bladder, liver, lung, and kidney. There are also damage pathway analyses, with methylation and genetic mutations appearing, which provide a guide for future research.

Future ahead, with continuous research in this field, the studies on drinking water pollution and bladder cancer will steadily increase. Several experimental, clinical, and evidence-based studies have elucidated the association between drinking water pollution and bladder cancer from different perspectives. However, there remains a regional imbalance in available information for the study of drinking water pollution and bladder cancer. Additionally, there is an insufficient collaboration between research groups. Therefore, we suggest that researchers can combine data mining (21), artificial intelligence (33), and other computer technologies or meta-analysis (34) to conduct interdisciplinary research, which will further improve the research methods and broaden ideas, provide a scientific basis for epidemiological and clinical oncology research on bladder cancer decision-making, and provide a new direction for the discussion of drinking water pollution and bladder cancer prevention and treatment.

Our study has some limitations. First, we only used the Scopus database to search publications related to drinking water and bladder cancer. Although Scopus database, with tremendous information on authors, countries, journals, and citations distribution, is the most widely used tool for bibliometric analysis, some influential literatures from other databases (namely WoSCC, SCI, EI and SSCI) may have been excluded, resulting in a small amount of bias. Second, the literature in Scopus database is predominantly published in English language. Therefore, this

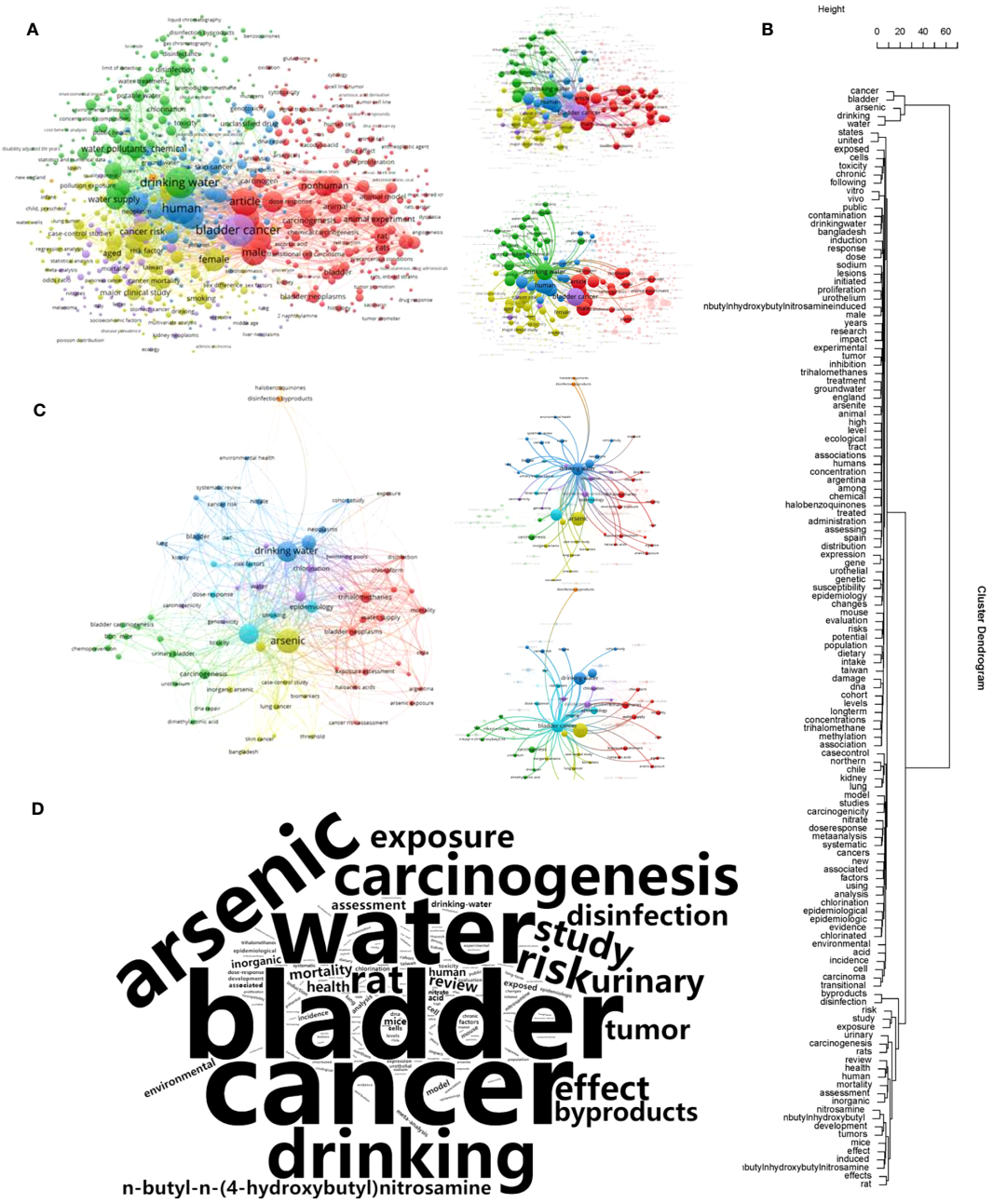

FIGURE 5
Keywords, author keywords and title test mining. **(A)** is the network of all keywords network and A right are two cluster based on 'bladder cancer' and 'drinking water'. **(B)** shows the network of author keywords, and the B right are two diagrams displaying a more accurate clustering relationships based on 'drinking water' and 'bladder cancer'. **(C)** is the cluster tree of all 669 titles by title test mining. **(D)** is the word cloud of the title keywords.

study may lack high-impact articles written in non-English languages. Third, this study only conducted a preliminary bibliometric analysis of drinking water and bladder cancer; however, the research methodology of existing literatures and carcinogenic mechanism of pollutants in drinking water were not thoroughly studied. Hence, we will conduct a further in-depth analysis. Despite the above shortcomings, we believe that our findings can provide valuable suggestions for future research development of this field.

Conclusion

Bibliometric analysis of drinking water and bladder cancer has a significant guiding role in the research of water resources, water environment, and incidence of bladder cancer. We drew landscapes of publication year, affiliation, citation/co-citation, source journal, author/co-authorship, keywords, and title text to find key research hotspots. The most popular research direction is disinfection by-products causing bladder cancer, which confirms the importance of

the bibliometric analysis of drinking water pollution in epidemiological and clinical oncology research.

Data availability statement

The original contributions presented in the study are included in the article/Supplementary Material. Further inquiries can be directed to the corresponding authors.

Author contributions

JW and SL: study conception and design. YZ, ML, JJW and SX: data acquisition. KXH, FYH and BCW: analysis and data interpretation. YZ and JW: drafting of the manuscript. JW, SL, MDZ and CHY: review and revise the manuscript. All authors contributed to the article and approved the submitted version.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fonc.2023.1170700/full#supplementary-material

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Association between per- and polyfluoroalkyl substances and risk of hypertension: a systematic review and meta-analysis

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Background: Existing evidence indicates that exposure to per- and polyfluoroalkyl substances (PFASs) may increase the risk of hypertension, but the findings are inconsistent. Therefore, we aimed to explore the relationship between PFASs and hypertension through this systematic review and meta-analysis.

Methods: We searched PubMed, Embase, and the Web of Science databases for articles published in English that examined the relationship between PFASs and hypertension before 13 August 2022. The random effects model was used to aggregate the evaluation using Stata 15.0 for Windows. We also conducted subgroup analyses by region and hypertension definition. In addition, a sensitivity analysis was carried out to determine the robustness of the findings.

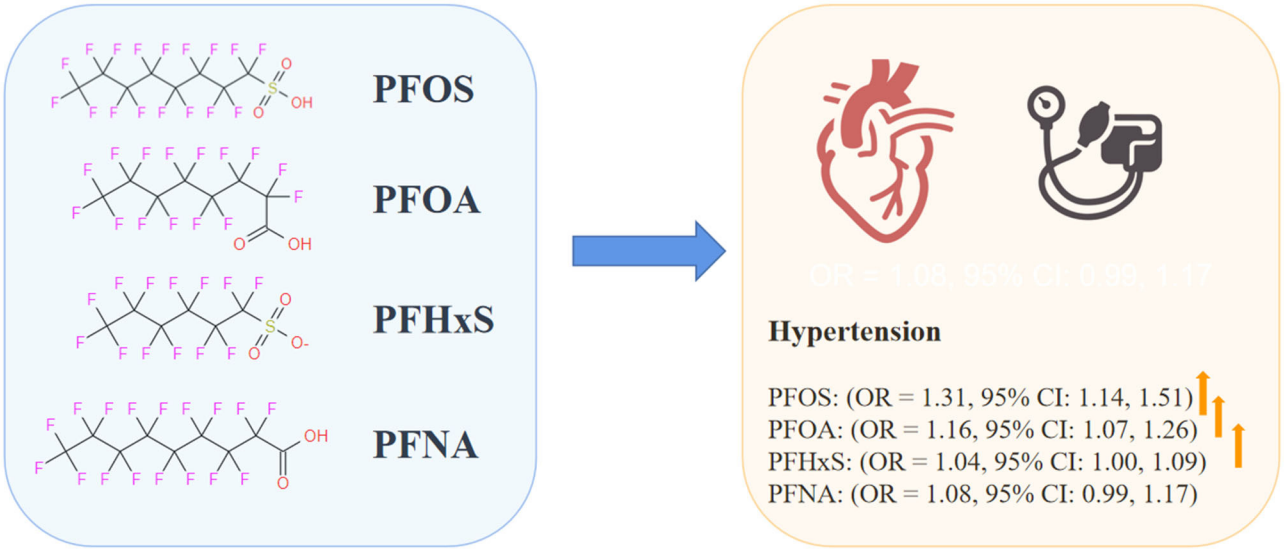
Results: The meta-analysis comprised 15 studies in total with 69,949 individuals. The risk of hypertension was substantially and positively correlated with exposure to perfluorooctane sulfonate (PFOS) (OR = 1.31, 95% CI: 1.14, 1.51), perfluorooctanoic acid (PFOA) (OR = 1.16, 95% CI: 1.07, 1.26), and perfluorohexane sulfonate (PFHxS) (OR = 1.04, 95% CI: 1.00, 1.09). However, perfluorononanoic acid (PFNA) exposure and hypertension were not significantly associated (OR = 1.08, 95% CI: 0.99, 1.17).

Conclusion: We evaluated the link between PFASs exposure and hypertension and discovered that higher levels of PFOS, PFOA, and PFHxS were correlated with an increased risk of hypertension. However, further high-quality population-based and pathophysiological investigations are required to shed light on the possible mechanism and demonstrate causation because of the considerable variability.

Systematic review registration: https://www.crd.york.ac.uk/prospero/ PROSPERO, registration number: CRD 42022358142.

KEYWORDS

per-and polyfluoroalkyl substances, hypertension, high blood pressure, meta-analysis, environmental pollutants



Graphical Abstract

1. Introduction

Since the 1940s, per- and polyfluoroalkyl substances (PFASs) have been extensively used because of their surfactant and stability qualities in industrial processes and goods such as aerospace and military, automotive, aviation, textiles, leather, clothing, construction and household goods, electronics, fire protection, food processing, and medical supplies (1–3). Persistent organic pollutants (POPs), including PFASs are now widely found in the environment, animals, plants, and humans worldwide because of their extensive usage (4). Due to the production of fluorocarbon bonds, PFASs have a long half-life and biopersistence in humans. The typical serum half-life of PFASs ranges from 2.3 to 8.5 years (5). Due to their structural similarity to fatty acids, PFASs may interfere with the function of peroxisome proliferator-activated receptors (PPARs) and the signaling pathways that connect them to metabolic processes (6). Meanwhile, toxicological investigations have also indicated that PFASs exposure is connected with oxidative stress and endothelial dysfunction (7). Thus, the cardiovascular system is especially susceptible to the toxicity of PFASs. PFASs exposure is associated with an increased risk of cardiovascular disease (CVD) and peripheral artery disease (PAD) (8, 9), in addition to other CVD risk factors such as thyroid disease (10, 11), high total cholesterol and low-density lipoprotein (LDL) levels (12), a higher body mass index (13), and impaired glucose homeostasis.

According to the data from the World Burden of Disease (GBD), the increasing incidence of hypertension has emerged as a major contributor to global mortality (14–17). Hypertension is also a significant contributor to the development of cardiovascular disease and renal failure (18). Different environmental exposures, including nutrition, alcohol consumption, lifestyle, and environmental contaminants, have been found to have variable impacts on blood pressure (19–21) and have been implicated as essential and changeable risk factors for hypertension (22). Toxicological evidence shows that PFASs may contribute to hypertension by increasing oxidative stress and the generation of reactive oxygen species (23). Several cross-sectional studies have shown that there is a positive correlation between PFASs exposure (24–29) and hypertension, while some researchers have reported no correlation or even a negative correlation (30–38). Conclusions vary depending on the population studied and the specific PFASs, and substantial inconsistencies have been observed between multiple studies on the same type of PFASs. Furthermore, PFASs exposure has occurred worldwide but still varies among countries due to the diversity of potential sources and approaches (39). These results show that more research is needed to gather data and quantify the impact of lingering, alternative, and emergent fluorinated chemicals on the blood pressure health of the population.

A systematic review and meta-analysis were conducted to (1) review the evidence for the effect of PFASs exposure on hypertension in the population and (2) quantitatively assess the relationship between the concentration of specific PFASs in the blood and the risk of hypertension.

2. Methods

2.1. Data sources and search strategies

The review has been registered in the International Prospective Register of Systematic Reviews (PROSPERO; registration number: CRD 42022358142) (https://www.crd.york.ac.uk/prospero/), which was conducted under the guidance of the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) statement (40). All the relevant studies in the database were searched from its establishment to 13 August 2022 to obtain all relevant articles from the PubMed, Embase, and Web of Science databases. Search keywords include exposure (per- and polyfluoroalkyl substances,

PFOS, PFOA, PFNA, and PFHxS) and result (hypertension). A specific search strategy is added to the Supplementary material. Given the complexity and growing number of PFASs homologs, we manually scanned all the references in the collected research to obtain more relevant articles and ensure that all investigations were included.

2.2. Selection criteria

We preliminarily screened the titles and abstracts, evaluated the full articles, and independently identified articles that met the criteria. The following epidemiological studies are included: (1) observational study design, such as case–control studies, cohort studies, and cross-sectional studies; (2) at least one type of PFASs exposure (such as PFOS, PFOA, PFNA, and PFHxS) is observed; (3) hypertension results; (4) a risk assessment is provided, including a 95% confidence interval (CI), ORs, RRs, or HRs. Exclusion criteria included studies that (1) are not full-text; (2) have pregnant women as participants; (3) have repetitive data; (4) take the form of laboratory research, non-human animal research, a letter, or a review; and (5) are of low quality.

2.3. Data extraction and quality assessment

Lv and An separately extracted data and evaluated the quality of each research project. Disagreements were discussed and resolved amicably. We retrieved the following data from every qualifying study: first author; publication year; research design; population characteristics (distribution of region, age, and gender); sample size; categories of PFASs; definition and diagnostic criteria of hypertension; maximum adjusted ORs, RRs, or HRs, 95% CI (41) corresponding adjustment covariates and so on.

The Newcastle–Ottawa Scale (NOS) was used in order to assess the level of methodological rigor present in case–control studies and cohort studies (42). The study's quality was evaluated based on its selection, comparability, exposure (in case–control studies), or result (in cohort studies). The highest score was 9, and research that scored $\geq$ 7 was considered high quality. The cross-sectional scale recommended by the Agency for Healthcare Research and Quality (AHRQ) was used to evaluate cross-sectional research (43). The scale consists of 11 items, with a maximum score of 11. The evaluation criteria are as follows: 0–3 = low quality, 4–7 = medium quality, and 8–11 = high quality.

The NTP/OHAT Risk of Bias Rating was also used to assess the quality of the included studies (44). Seven main domains were included: selection bias, confounding bias, attrition/exclusion bias, exposure characteristics, outcome representation, selective reporting bias, and conflict of interest. The criteria for risk of bias assessment are reported in Supplementary Table 3.

The Grading of Recommendations Assessment, Development, and Evaluation (GRADE) guideline was used to assess the confidence in the body of evidence (45), which evaluates eight criteria (risk of bias, indirectness, inconsistency, imprecision, publication bias, large magnitude of effect, dose–response, and confounding effect) to systematically assess the overall confidence in the evidence derived from the meta-analysis. Based on the overall assessment of reviewers, the method assigns the evidence a quality rating of "high," "moderate," "low," or "very low."

2.4. Statistical analysis

The ORs, RRs, and HRs, together with their respective 95% CIs, were derived using the maximum adjusted models in each research study. For these studies in which the categorical PFASs exposure dosage was variable and split into tertiles or quartiles, the fixed effect model combined the data, and the meta-analysis used the final pooled findings (46). The relevant effect values, such as ORs, RRs, or HRs, which may be integrated into the meta-analysis, were included. The effect size (ES) was calculated by ES = ln (OR), and the standard error (SE) of the effect size was calculated as SE = [ln (UC)–ln (LC)]/3.92 (UC and LC represent upper and lower confidence limits, respectively). The % weight represented the size of the information (i.e., sample size, number of events, and confidence interval) and was calculated as weight = $1/(SE^2)$ (41). Heterogeneity in the research was tested using the I^2 and *P*-values. A *P*-value of <0.05 was regarded as heterogeneous. I^2 statistics > 50% showed high heterogeneity, 25–50% moderate heterogeneity, and <25% low heterogeneity. The fixed effect model was utilized for analysis when there was no significant heterogeneity ($I^2 < 50\%$ or $P > 0.05$), otherwise, a random effect model was employed (46).

To determine the cause of the variation, a subgroup analysis was performed, stratified by geographic area and hypertension threshold. To evaluate the impact of missing studies and to identify the source and size of any heterogeneity in the results, sensitivity analyses were conducted by eliminating studies one by one from the analysis. Publication bias was evaluated using funnel plots, and the predicted findings were confirmed using Egger's test (47). The meta-analysis used Stata version 15.0 for Windows.

3. Results

3.1. Study selection

Following the search strategy, a total of 6,784 articles from these three online resources were reviewed. After removing the duplicates, we were left with 4,276 research articles. After evaluating the titles and abstracts, 34 articles were chosen for further consideration. Researchers discarded 19 articles because they did not meet the inclusion or exclusion criteria. These included one review, 10 studies without an applicable exposure or result, four studies conducted on pregnant women, and four studies that simply replicated previous findings. A total of 15 publications were included in the meta-analysis, as shown in Figure 1. There were a total of 71,059 participants in the studies that quantified PFASs levels in blood samples from 154 to 32,254 individuals. Table 1 shows the detailed article information.

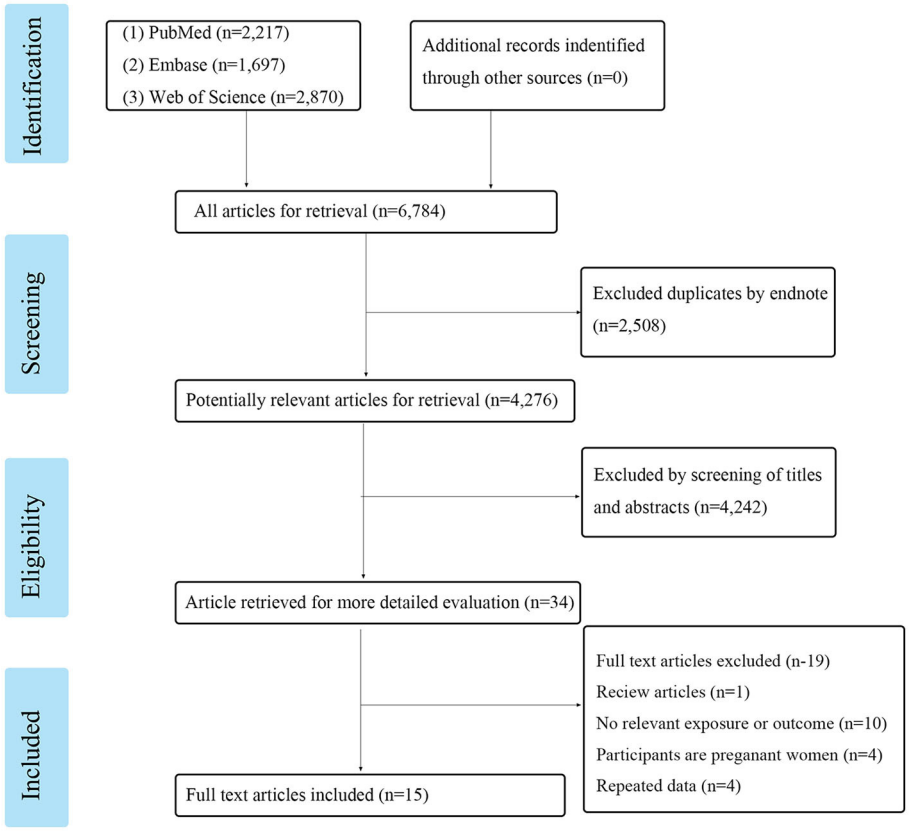


FIGURE 1
PRISMA flow diagram of study selection.

3.2. Definition of hypertension

The term "hypertension" alone has a wide range of interpretations. The majority of studies (10 of 15 included studies) use a blood pressure reading of 140/90 mm Hg as the diagnostic threshold for hypertension (24–27, 29, 30, 32, 35–37), which is in line with the guidelines of the American Heart Association and the Joint National Committee (Seventh Report of the Joint National Committee on Prevention, Detection, Evaluation, and Treatment of High Blood Pressure). Four studies use a blood pressure reading of 130/85 mm Hg as the cutoff for hypertension (28, 33, 38), while another (31) uses a reading of 130/80 mm Hg since its subjects are adolescents. An individual study did not definitively establish the cutoff for hypertension, with the condition being defined as one that requires a medical professional's diagnosis (34).

3.3. Assessment of quality

The quality of the 15 studies that were suitable for inclusion was analyzed. The results showed that 11 of the cross-sectional studies were of high- or medium-quality (25–31, 33–36, 38), three of the cohort studies scored 7 (24, 29, 37), and one case–control study scored 8 (32), indicating that none of these studies was of poor quality. Supplementary Tables 1, 2 include further information.

The results of the risk of bias assessment are shown in Table 2. The risk of bias regarding attrition/exclusion, confounding, selection (exposure), and conflict was rated as "probably low" in all the included studies. Of the 15 studies, five were rated as "definitely low risk of bias", seven were rated as "probably low", and two were rated as "definitely high risk of bias" due to the use of self-reported cases. Selection bias was rated as "probably low" in all but one study. Overall, 13 studies and two studies were grouped as tier 1 and tier 2, respectively.

Based on cross-sectional and case–control studies, the overall strength of evidence for the association between PFASs and hypertension was "limited", and the direction of effect was inconsistent across most studies. However, for PFASs combined with hypertension events, we rated the overall strength of evidence as "moderate". The majority of PFASs–hypertension combinations assessed exhibited consistent statistically significant positive evidence of an association, and all studies included in the meta-analysis were "moderate." These results provide some epidemiologic proof that PFASs may increase the risk of hypertension.

3.4. Meta-analysis

To determine whether PFASs exposure was associated with hypertension, 15 studies were analyzed, as shown in Figure 2. This included 15 outcomes for PFOA exposure, 14 outcomes for PFOS

TABLE 1 Characteristics of studies included in the meta-analysis.

References	Year	Country	Research type	Age range or mean age (years)	Male subjects (%)	Exposure to substances	Estimated size (95%CI)	*N*	Covariates
Min et al. (26)	2012	United States	Cross-sectional study	≥20	NR	PFOA	Q2-Q1: 1.21 (0.86, 1.70) Q3-Q1: 1.60 (1.15, 2.22) Q4-Q1: 1.71 (1.23, 2.36)	2,934	Age, sex, ethnicity, education, income, smoking status, alcohol use, obesity status, total saturated fatty acid intake, physical activity, serum PFOS concentrations, total cholesterol, and poor kidney function
Geige et al. (30)	2014	United States	Cross-sectional study	15 ± 0.1	51	PFOS PFOA	Q2-Q1: 0.99 (0.55, 1.78) Q3-Q1: 0.73 (0.36, 1.48) Q4-Q1: 0.77 (0.37, 1.61) Q2-Q1: 0.89 (0.53, 1.49) Q3-Q1: 0.96 (0.53, 1.73) Q4-Q1: 0.69 (0.41, 1.17)	1,655	Age, sex, ethnicity, body mass index, annual household income, physical activity, total cholesterol, and serum cotinine
Winquist and Steenland (24)	2014	United States	Cohort study	≥20	46	PFOA	Q2-Q1: 1.10 (1.02, 1.19) Q3-Q1: 1.10 (1.02, 1.18) Q4-Q1: 1.05 (0.97, 1.12) Q5-Q1: 0.98 (0.91, 1.06)	32,254	Sex, ethnicity, education, smoking status, alcohol use, body mass index, and diabetes
Christensen et al. (34)	2016	United States	Cross-sectional study	≥50	100	PFOS PFOA PFNA PFHxS	0.99 (0.96,1.01) 0.74 (0.52,1.01) 0.74 (0.53,0.98) 1 (0.88,1.13)	154	Age, BMI, work status, and alcohol consumption
Bao et al. (36)	2017	China	Cross-sectional study	55.1 ± 16.4	74.69	PFOS PFOA PFNA PFHxS	1.24 (1.08, 1.44) 1.12 (0.97, 1.30) 1.19 (1.04, 1.36) 0.99 (0.95, 1.03)	1,612	Age, sex, BMI, education, income, exercise, smoking status, alcohol consumption, and family history of hypertension
Chen et al. (38)	2019	Croatia	Cross-sectional study	55 ± 15.8	42.10	PFOS PFOA PFNA PFHxS	1.24 (0.67, 2.31) 0.72 (0.32, 1.63) 0.89 (0.39, 2.04) 1.14 (0.63, 2.05)	1,430	Age, sex, education, socio-economic status, smoking status, diet, physical activity
Donat-Vargasa et al. (32)	2019	Sweden	Case–control study	56 ± 6	54	PFOS PFOA PFNA PFHxS	Q2-Q1: 0.82 (0.48, 1.40) Q3-Q1: 0.73 (0.41, 1.30) Q2-Q1: 0.79 (0.47, 1.33) Q3-Q1: 0.87 (0.50, 1.52) Q2-Q1: 0.96 (0.59, 1.56) Q3-Q1: 0.90 (0.52, 1.57) Q2-Q1: 1.16 (0.58, 2.33) Q3-Q1: 0.54 (0.25, 1.18)	370	Age, sex, education, year of sampling, body mass index, smoking status, alcohol consumption, physical activity, and healthy diet score
Liao et al. (25)	2020	United States	Cross-sectional study	49.9 ± 18	50.60	PFOS PFOA PFNA PFHxS	Q2-Q1: 1.16 (0.99, 1.35) Q3-Q1: 1.14 (0.97, 1.34) Q3-Q1: 1.04 (0.90, 1.21) Q3-Q1: 1.35 (1.16, 1.58) Q2-Q1: 1.18 (1.01, 1.37) Q3-Q1: 1.18 (1.01, 1.38) Q2-Q1: 1.07 (0.92, 1.24) Q3-Q1: 1.19 (1.02, 1.38)	6,967	Age, sex, education level, ethnicity, diabetes mellitus, consumption of at least 12 drinks/year, current smoking status, body mass index, and waist circumference

(Continued)

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TABLE 1 (Continued)

References	Year	Country	Research type	Age range or mean age (years)	Male subjects (%)	Exposure to substances	Estimated size (95%CI)	*N*	Covariates
Pitter et al. (35)	2020	Italy	Cross-sectional study	20-39	48.57	PFOS PFOA PFHxS PFNA	Q2-Q1: 0.99 (0.85, 1.16) Q3-Q1: 1.06 (0.91, 1.24) Q4-Q1: 1.12 (0.95, 1.32) Q2-Q1: 1.00 (0.85, 1.16) Q3-Q1: 1.02 (0.87, 1.20) Q4-Q1: 1.16 (0.99, 1.37) Q2-Q1: 1.01 (0.86, 1.19) Q3-Q1: 1.08 (0.92, 1.27) Q4-Q1: 1.19 (1.00, 1.41) 1.10 (0.96, 1.26)	1,430	Age, sex, education, socioeconomic status, smoking, diet, physical activity
Mi et al. (27)	2020	China	Cross-sectional study	61.98 ± 14.40	54.85	PFOS PFOA	2.52 (1.91, 3.33) 1.72 (1.27, 2.31)	1,238	Age, sex, ethnicity, occupation, education, smoking, alcohol consumption, physical activity, annual household income, and seafood consumption
Lin et al. (37) (1)	2020	United States	Cohort study	NA	34.70	PFOS PFOA PFNA PFHxS	Q2-Q1: 1.09 (0.76, 1.54) Q3-Q1: 1.18 (0.84, 1.66) Q4-Q1: 1.19 (0.85, 1.67) Q2-Q1: 1.15 (0.84, 1.58) Q3-Q1: 1.08 (0.79, 1.48) Q4-Q1: 1.24 (0.91, 1.68) Q2-Q1: 0.78 (0.54, 1.11) Q3-Q1: 1.02 (0.78, 1.34) Q4-Q1: 1.00 (0.76, 1.32) Q2-Q1: 1.42 (0.94, 2.17) Q3-Q1: 1.49 (0.98, 2.25) Q4-Q1: 1.59 (1.05, 2.41)	957	Age, sex, ethnicity, treatment assignment, education, income, marital status, alcohol consumption, smoking, and DASH diet score
Lin et al. (37) (2)	2020	United States	Cohort study	NA	34.70	PFOS PFOA PFNA PFHxS	Q2-Q1: 1.65 (0.94, 2.88) Q3-Q1: 1.58 (0.91, 2.74) Q4-Q1: 1.45 (0.83, 2.52) Q2-Q1: 1.15 (0.78, 1.68) Q3-Q1: 0.96 (0.65, 1.41) Q4-Q1: 0.95 (0.65, 1.38) Q2-Q1: 1.07 (0.78, 1.48) Q3-Q1: 0.96 (0.70, 1.31) Q4-Q1: 0.93 (0.68, 1.28) Q2-Q1: 1.08 (0.83, 1.42) Q3-Q1: 1.09 (0.83, 1.41) Q4-Q1: 0.84 (0.60, 1.18)	956	Age, sex, ethnicity, treatment assignment, education, income, marital status, alcohol drinking, smoking, and DASH score

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TABLE 1 (Continued)

References	Year	Country	Research type	Age range or mean age (years)	Male subjects (%)	Exposure to substances	Estimated size (95%CI)	*N*	Covariates
Averina et al. (31)	2021	Norway	Cross-sectional study	16.30 ± 1.24	52.66	PFOS PFOA PFHxS	Q2-Q1: 1.40 (0.78, 2.51) Q3-Q1: 1.01 (0.56, 1.80) Q4-Q1: 1.86 (1.08, 3.19) Q2-Q1: 1.28 (0.74, 2.22) Q3-Q1: 1.45 (0.85, 2.49) Q4-Q1: 2.08 (1.17, 3.69) Q2-Q1: 1.63 (0.90, 2.94) Q3-Q1: 1.25 (0.69, 2.28) Q4-Q1: 2.06 (1.16, 3.65)	940	Sex, age, BMI, and physical activity outside of school
Zare Jeddi et al. (33)	2021	Italy	Cross-sectional study	30 ± 5.8	48.61	PFOS PFOA PFNA PFHxS	1.10 (1.03, 1.17) 1.05 (1.01, 1.08) 1.07 (1.03, 1.12) 1.10 (0.99, 1.21)	15,876	Age, gender, time between study entry and blood sampling center where BP was measured, education, number of deliveries, physical activity, country of birth, diet, alcohol intake, smoking status, HDL-C, BMI ≥ 25, diabetes
Yu et al. (28)	2021	China	Cross-sectional study	61.8 ± 14.4	55.30	PFOS	Q2-Q1: 4.19 (2.89, 6.08) Q3-Q1: 3.29 (2.27, 4.75) Q4-Q1: 5.53 (3.72, 8.23)	1,228	Age, sex, annual income, smoking, alcohol consumption, physical activity, and seafood consumption
Ding et al. (29)	2022	United States	Cohort study	49.2	0	PFOS PFOA PFNA PFHxS	Q2-Q1: 1.11 (0.93, 1.33) Q3-Q1: 1.42 (1.19, 1.68) Q2-Q1: 1.37 (1.15, 1.63) Q3-Q1: 1.47 (1.24, 1.75) Q2-Q1: 0.93 (0.79, 1.09) Q3-Q1: 1.00 (0.83, 1.19) Q2-Q1: 0.84 (0.71, 1.00) Q3-Q1: 1.06 (0.89, 1.25)	1,058	Ethnicity, study site, education, financial strain, smoking status, environmental tobacco smoke, alcohol consumption, total caloric intake, and menopausal status

BMI, body mass index; HDL-C, high-density lipoprotein cholesterol; DASH, dietary approaches to hypertension; PFOS, perfluorooctane sulfonate; PFOA, perfluorooctanoic acid; PFNA, perfluorononanoic acid; PFHxS, perfluorohexane sulfonate; Q1, first or lowest quartile; Q2, second quartile; Q3, third quartile; Q4, fourth quartile; Q5, fifth quartile; DASH score, Dietary Approaches to Stop Hypertension score; NA, not applicable; NR, not reported.

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TABLE 2 Summary of risk of bias domains for individual studies examining associations between PFAS and hypertension.

RESPONSE LEVEL		Min et al. (26)	Geige et al. (30)	Winquist and Steenland (24)	Christensen et al. (34)	Bao et al. (36)	Chen et al. (38)	Donat-Vargasa et al. (32)	Liao et al. (25)	Pitter et al. (35)	Mi et al. (27)	Lin et al. (37)	Averina et al. (31)	Zare Jeddi et al. (33)	Yu et al. (28)	Ding et al. (29)
++	Definitely low risk of bias															
+	Probably low risk of bias															
-	Probably high risk of bias															
–	Definitely high risk of bias															
BIAS MAIN																
CONFOUNDING BIAS. [Key domain] Did the study design or analysis account for important confounding and modifying variables?		+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
ATTRITION/EXCLUSION BIAS Were outcome data incomplete due to attrition or exclusion from the analysis?		+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
DETECTION BIAS Can we trust the exposure characterization? [Key domain]		+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Can we be confident in the outcome assessment? [Key domain]		+	++	-	-	++	+	++	++	+	++	+	+	+	++	+
SELECTIVE REPORTING BIAS Were all measured outcomes reported?		+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
SELECTION BIAS Did the selection of study participants result in appropriate comparison groups?		+	+	+	-	+	+	+	+	+	+	+	+	+	+	+
CONFLICT OF INTEREST		+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
SUMMARY TIERED CLASSIFICATION		T1	T1	T2	T2	T1	T1	T1	T1	T1	T1	T1	T1	T1	T1	T1

"T1" is Tier 1: the study must be rated as "definitely low" or "probably low" risk of bias for key criteria AND "definitely low" or "probably low" risk of bias for most other applicable criteria. "T2" is Tier 2: the study does not meet the criteria for tiers 1 or 2. Tier 3: the study must be rated as "definitely high" or "probably high" risk of bias for key criteria AND "definitely high" or "probably high" risk of bias for most other applicable criteria. Tier 3: the study must be rated as "definitely high" or "probably high" risk of bias for key criteria AND "definitely high" or "probably high" risk of bias for most other applicable criteria.

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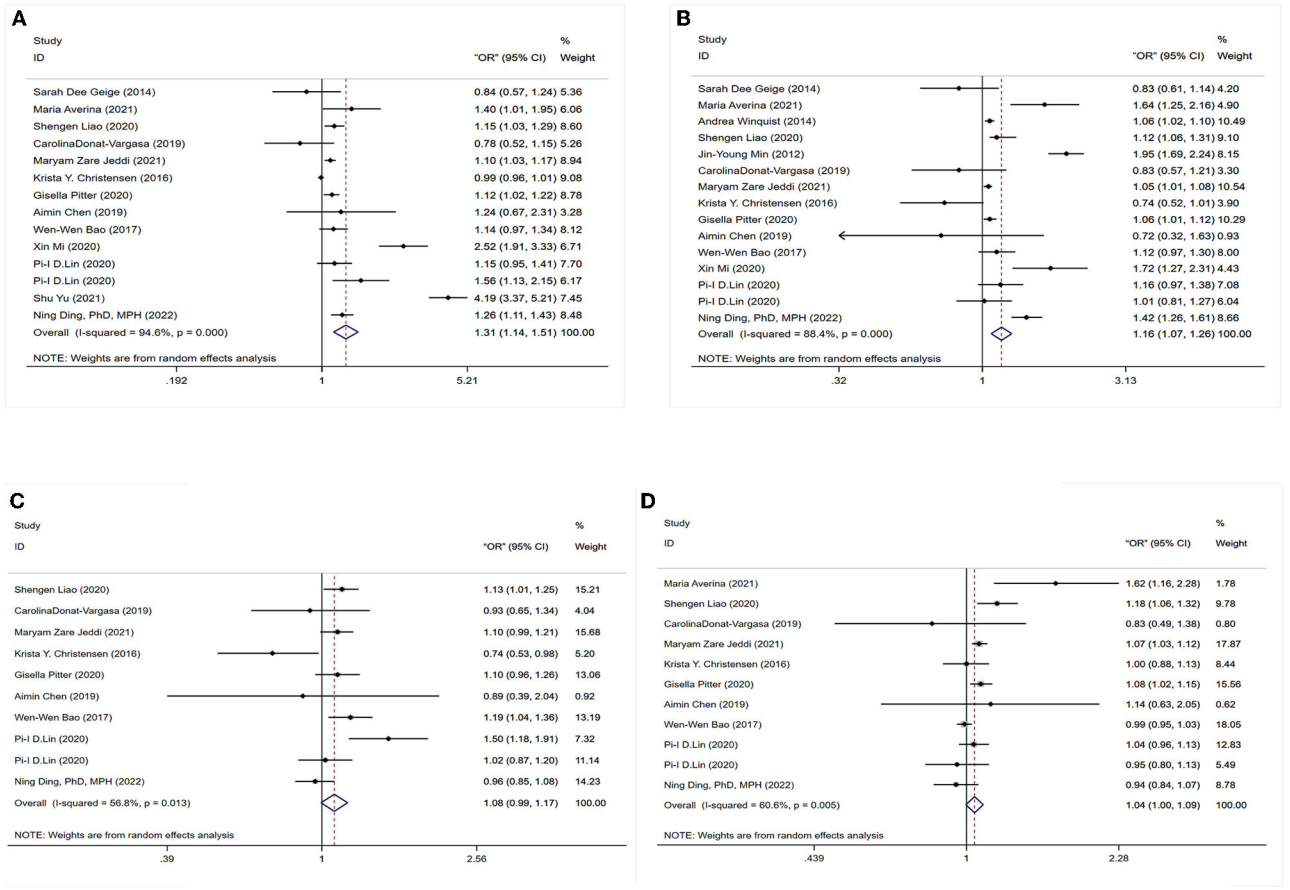

FIGURE 2
Forest plot of the association between exposure to PFAS with hypertension. **(A)** PFOS; **(B)** PFOA; **(C)** PFNA; **(D)** PFHxS.

exposure, 11 outcomes for PFHxS exposure, and 10 outcomes for PFNA exposure.

3.4.1. Association between PFOS exposure and hypertension

The association between PFOS exposure and hypertension was investigated in 13 studies (10 cross-sectional, one case–control, and two cohort studies). A combined OR estimate of 1.31 (95% CI: 1.14, 1.51) was calculated. We employed a random effects model to examine the connection between PFOS exposure and hypertension due to the significant heterogeneity of the studies ($I^2 = 94.6\%$, $P < 0.05$), as shown in Figure 2A.

3.4.2. Association between PFOA exposure and hypertension

In total, 11 cross-sectional studies, one case–control study, and three cohort studies were retrieved to examine the correlation between PFOA exposure and hypertension. The overall findings revealed that being exposed to PFOA increased the risk of hypertension (OR = 1.16, 95% CI: 1.07, 1.26). A random effects model was used due to the significant heterogeneity of the included studies ($I^2 = 88.4\%$, $P < 0.05$), as shown in Figure 2B.

3.4.3. Association between PFNA exposure and hypertension

No statistically significant association between PFNA exposure and hypertension was found in six cross-sectional studies, one case–control study, and two cohort studies. As a whole, we found a merged evaluation of 1.08 (95% CI: 0.99, 1.17). In addition, a random effects model was adopted since the studies had high heterogeneity ($I^2 = 6.8\%$, $P < 0.05$), as shown in Figure 2C.

3.4.4. Association between PFHxS exposure and hypertension

The association between PFHxS exposure and hypertension risk was examined in seven cross-sectional investigations, one case–control study, and two cohort studies. A positive relationship (OR = 1.04, 95% CI: 1.00, 1.09) was found between PFHxS exposure and the risk of hypertension with a random effect model because of high heterogeneity ($I^2 = 60.6\%$, $P < 0.05$), as shown in Figure 2D.

3.5. Subgroup analysis

Exposure to PFOS, PFOA, and PFHxS was observed to have a positive and statistically significant connection with hypertension, but exposure to PFNA did not. We conducted a further subgroup

analysis based on geography and hypertension thresholds to delve deeper into the correlation studies. Stratified by region, the pooled evaluated OR of PFOA and hypertension was 1.13 (95% CI: 1.03, 1.24) for Non-American region and 1.15 (95% CI: 0.97, 1.35) for American region. Then, the pooled estimate OR of PFNA and hypertension was 1.11 (95% CI: 1.04, 1.19) for non-America and 1.05 (95% CI: 0.90,1.23) for America. In addition, a subgroup analysis by hypertension threshold revealed a positive association between PFOS and PFOA exposure and the development of hypertension (OR = 1.19, 95% CI: 1.05, 1.28; OR = 1.15, 95% CI: 1.03, 1.28) for 140/90 mmHg, but no statistically significant association (OR = 1.58, 95% CI: 0.90, 2.78; OR = 1.28, 95% CI: 0.83, 1.98) for non-140/90 mmHg. All results are shown in Table 3.

3.6. Sensitivity analysis and publication bias

The correlation between PFOA, PFNA, and hypertension was investigated, and the results showed no substantial publication bias (P = 0.26 for PFOA, P = 0.56 for PFNA, P = 0.67 for PFHxS). However, publication bias was present in the meta-analysis evaluating the association between PFOS and hypertension (P = 0.028). A sensitivity analysis was conducted by eliminating specific articles one by one to assess the stability of the findings and establish that any research did not influence them. The results for PFOA, PFNA, and PFHxS are consistent to some degree. The odds ratio (OR) between PFOS exposure and hypertension increased but remained positively associated (OR = 1.20, 95% CI: 1.16, 1.25), except for the research by Christensen et al. (34). As shown in Figure 3, however, when the trimming and filling approach was used, the result was reversed (OR = 1.04, 95% CI: 0.89, 1.20), indicating that the robustness of the meta-analysis between PFOS and hypertension is poor, and the source of the disagreement must be explained.

4. Discussion

In this systematic review and meta-analysis, we first summarize all the current evidence on the risk of PFASs exposure for hypertension. The same type of research on PFASs was collected for analysis in order to summarize their relevance in this study. According to our results, there was a significant positive association between exposure to PFOS, PFOA, and PFHxS and an increased risk of hypertension in the population, but no association between PFNA and hypertension. Our study is the first meta-analysis to investigate the association between PFASs exposure and the risk of hypertension in a population. It has implications for reducing the risk of hypertension in populations living in areas contaminated with PFASs.

The results revealed a substantial positive relationship between PFOA (OR = 1.31, 95% CI: 1.14, 1.51) and PFOS (OR = 1.16, 95% CI: 1.07, 1.26) exposure and the risk of hypertension. Our findings are consistent with the nine previously published studies (25, 27–29, 31, 33, 35–37) that found a positive connection between PFOS or PFOA exposure and hypertension. A negative link between PFOS and hypertension was found in three studies (30, 32, 34), although the results were not statistically significant. Uncontrolled variables, such as diet, comorbidities, physiological features of distinct subpopulations, and family history of hypertension, may be to blame for this discrepancy. For PFHxS, we found a relationship between exposure and hypertension (OR = 1.04, 95% CI: 1.00, 1.09). Our findings are consistent with those of previous research showing that prolonged exposure to high levels of PFHxS increases blood pressure in the general population (25, 27, 31, 35). Although not statistically significant, some studies have shown an inverse correlation between PFHxS and hypertension (29, 33, 34, 36, 37). It is essential to consider the possibility that this discrepancy is due to random chance or confounding factors in the study design or treatment pharmacokinetics. In light of this discrepancy, more studies are needed to establish a causal relationship between PFHxS and hypertension. Contrary to expectations, we found no evidence that PFNA exposure increased the risk of developing hypertension (OR = 1.08, 95% CI: 0.99, 1.17). Studies by Pitter et al. (35) (OR = 1.1, 95% CI: 0.96, 1.26), Lin et al. (37) (OR = 1.00, 95% CI: 0.76, 1.32), Donat-Vargas et al. (32) (OR = 0.9, 95% CI: 0.52, 1.57), and Ding et al. (29) (OR = 1.00, 95% CI: 0.83, 1.19) were consistent with our findings. Three other studies reported positive associations between hypertension and PFNA: one by Liao et al. (25) (OR = 1.18, 95% CI: 1.01, 1.38), one by Zare Jeddi et al. (33) (OR = 1.07, 95% CI: 1.03, 1.12), and one by Bao et al. (36) (OR = 1.19, 95% CI: 1.04, 1.36). However, one study using the NHANCE database found that PFNA exposure was linked to a reduced incidence of hypertension (34) (OR = 0.74, 95% CI: 0.53, 0.98). This finding was not consistent with ours. These discrepancies may be related to the failure to consider other confounding factors that may be strongly associated with hypertension, such as ethnicity, diet, family history, exercise habits, and the local prevalence of hypertension. In addition, this discrepancy highlights the need for further investigation of the effects of PFNA exposure on hypertension.

The association between hypertension and any PFASs (PFOS, PFOA, PFNA, and PFHxS) (OR = 1.26, 95% CI: 1.12, 1.41) was reported in a cohort-based study based on electronic health records (48). Higher blood PFASs concentrations were also related to an increased risk of hypertension (HR = 1.71, 95% CI: 1.15, 2.54) (29), according to a recently published cohort study with a mean follow-up of 12.4 years. At the same time, a cross-sectional study of adolescents in northern Norway showed that total PFASs were positively associated with hypertension, with OR=2.24 95% CI: 1.10, 4.54 (31). Moreover, a study based on the Study of Women's Health Across the Nation reported that in a mixed model, there were positive associations between n-PFOS (β = 0.051), Sm-PFOS (β = 0.115), n-PFOA (β = 0.032), PFNA (β = 0.086), and PFHxS (β = −0.074) (29). This is not entirely consistent with the results of our study. The choice of the statistical model, the definition of hypertension, the degree of exposure, and the susceptibility of different populations may have contributed to this difference (49, 50).

It was shown that in the further stratified analysis, grouping according to the hypertension threshold, PFASs exposure, and hypertension were not significantly correlated with the non-140/90 group, which may be due to the lack of studies in the non-140/90 group (PFOS, N = 3; PFOA, N = 2; PFNA, N = 1; PFHxS, N = 2). Studies were categorized by country in order to identify regional variations in the association between PFASs exposure and hypertension. Notably, PFOA, PFNA, and PFHxS

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TABLE 3 Subgroup analysis of PFASs (PFOA, PFOS, PFNA, and PFHxS) exposure and risk of hypertension.

Subgroup	Regions		Threshold for hypertension	
	American	Non-Amercian	140/90 mmHg	Non-140/90 mmHg
PFOS				
Studies (*N*)	7	7	11	3
Pooled ORs	**1.28**	**1.24**	**1.19**	1.58
(95% CI)	**(1.05, 1.55)**	**(1.06, 1.46)**	**(1.05, 1.34)**	(0.90, 2.78)
Heterogeneity (I^2, *P*)	$I^2 = 93.0\%$, $P < 0.05$	$I^2 = 84.1\%$, $P < 0.05$	$I^2 = 87.2\%$, $P < 0.05$	$I^2 = 95.6\%$, $P < 0.05$
PFOA				
Studies (*N*)	8	7	13	2
Pooled ORs	1.15	**1.13**	**1.15**	1.28
(95% CI)	(0.97, 1.35)	**(1.03, 1.24)**	**(1.03, 1.28)**	(0.83, 1.98)
Heterogeneity (I^2, *P*)	$I^2 = 92.4\%$, $P < 0.05$	$I^2 = 74.1\%$, $P < 0.05$	$I^2 = 88.8\%$, $P < 0.05$	$I^2 = 90.1\%$, $P < 0.05$
PFNA				
Studies (*N*)	5	5	9	1
Pooled ORs	1.05	**1.11**	1.17	1.10
(95% CI)	(0.90, 1.23)	**(1.04, 1.19)**	(0.97, 1.18)	(0.99,1.21)
Heterogeneity (I^2, *P*)	$I^2 = 77.1\%$, $P < 0.05$	$I^2 < 25.0\%$, $P > 0.05$	$I^2 = 61.4\%$, $P < 0.05$	–, –
PFHxS				
Studies (*N*)	5	6	9	2
Pooled ORs	1.03	1.06	1.03	1.27
(95% CI)	(0.95, 1.11)	(0.99, 1.13)	(0.98, 1.08)	(0.85, 1.90)
Heterogeneity (I^2, *P*)	$I^2 = 55.1\%$, $P < 0.05$	$I^2 = 69.6\%$, $P < 0.05$	$I^2 = 48.6\%$, $P < 0.05$	$I^2 = 82.5\%$, $P < 0.05$

Bold indicates statistical significance. PFOS, perfluorooctane sulfonate; PFOA, perfluorooctanoic acid; PFNA, perfluorononanoic acid; PFHxS, perfluorohexane sulfonate.

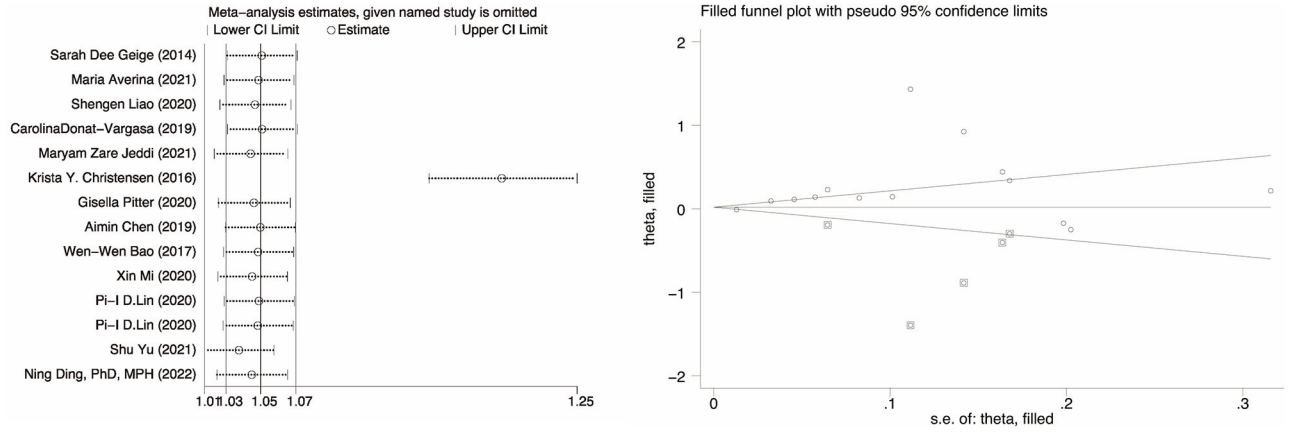

FIGURE 3
Sensitivity analysis of studies that evaluate the ORs of PFOS.

were not associated with hypertension in the United States. Regional differences in lifestyle, socioeconomic level, local diets, the local incidence of hypertension, average blood pressure, and ethnic adaptability to PFASs exposure may obscure the association between PFASs exposure and hypertension.

While there are various potential processes linking community exposure to PFASs to an increased risk of hypertension, the mechanism of the relationship between PFASs and blood pressure is still unclear. PFASs have been linked to increased oxidative stress in the liver and endothelial cells (23, 51, 52). Inadequate production of nitric oxide and increased production of superoxide, both byproducts of oxidative stress, may contribute to an increase in blood pressure in the process of attenuating vasodilation. Consequently, PFAS-induced oxidative stress may

increase the need for homocysteine methyl donors, which in turn may reduce the effectiveness of the body's natural ability to dilate blood vessels (51, 52). The presence of PFASs may also have a secondary effect on blood pressure, according to another theory. Numerous human (53, 54) and animal studies have revealed that reduced nephron endowment and glucocorticoid excess contribute to hypertension. In a recent animal model of hypertension, prenatal exposure to PFASs was shown to reduce nephron endowment and increase renal glucocorticoid receptor (GR) gene expression in the offspring of mothers who were exposed to the investigated chemicals during pregnancy (55). Upregulation of GRs augments the action of glucocorticoids, and the sclerotic and stress-induced natriuretic cycle initiated by a reduction in nephrons may contribute to hypertension. These mechanisms may cooperate with angiotensin II to boost proximal tubular sodium reabsorption (56). As a result, elevated serum PFASs levels may contribute to an indirect increase in blood pressure, especially in the presence of elevated glucocorticoids and diminished nephrons. In conclusion, PFASs exposure has been associated with a possible increase in the incidence of hypertension. Nevertheless, its mechanism in the human body remains unclear and needs further research.

The research has several strengths. First, the number of hypertension patients in our study is greater than that in smaller studies. With such large samples, we could thoroughly explore the link between PFASs and hypertension risk and conduct a nuanced subgroup analysis. Second, to more thoroughly evaluate the association between exposure to hypertension and various PFASs, we used a fixed effects model to pool data from studies in which the PFASs exposure dosage was categorically variable, such as split into tertiles or quartiles. The findings of this meta-analysis can be trusted since none of the 15 studies used to compile it were of poor quality.

Despite these benefits, there are several caveats to our research. First, many studies looked at "residual PFASs" (PFOS and PFOA) and found that the possible relationship could be completely recognized. However, there is a lack of research on "surrogate PFASs", or polyfluoroalkyl compounds such as F-53B and OBS, which results in a high degree of variability or a hampered capacity to discover possible correlations. Therefore, the lack of association we observed calls for a thorough explanation and further research. At the same time, PFASs exposure occurs worldwide but differs from country to country due to the wide variety of potential sources and routes of exposure. In addition, because each study's population, models, statistical techniques, and adjustments for numerous confounding variables are unique, each study's conclusions may differ. Finally, a recent study highlighted the importance of PFASs isomers and enantiomers (57). Due to their structural differences, different PFASs isomers and enantiomers may have different harmful health consequences. However, due to the need for data, we instead focused on the general direction of previous studies in this area. As the volume of research continues to grow, we will be able to better categorize our results using more precise diagnostic methods. Despite these obstacles, a meta-analysis can answer numerous questions and shed light on the causes of variability in study findings, pointing the way to new avenues of inquiry.

5. Conclusion

Our understanding of the relationship between PFASs exposure and hypertension has been strengthened by this meta-analysis, which demonstrates a positive association between PFOS, PFOA, and PFHxS exposure and hypertension but no relationship between PFNA and hypertension. In order to manage hypertension and further lower the prevalence of cardiovascular disease and stroke, people should seriously consider reducing environmental PFASs pollution and PFASs exposure. To further understand these mechanisms, further research should be encouraged.

Data availability statement

Publicly available datasets were analyzed in this study. This data can be found here: Pubmed, Embase, and Web Of Science.

Author contributions

FX and XS: integrity of the data, the accuracy of the data analysis, and study concept and design. FX, ZA, and JL: data extraction and analysis. FX: drafting of the manuscript. ZA and JL: study supervision. HG, XL, ZA, JL, XS, HS, and YL: critical revision. All authors reviewed and revised the manuscript, approved the final version for publication, and accepted responsibility for all aspects of the manuscript.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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The findings, interpretations, and conclusions expressed in this study do not necessarily reflect the views of the agencies mentioned.

Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fpubh.2023.1173101/full#supplementary-material

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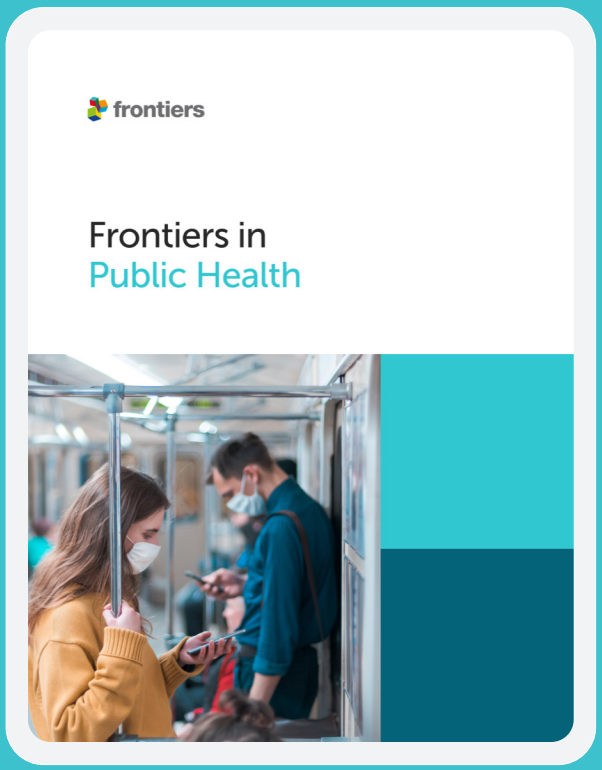

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