

The role of essential trace elements in health and disease

Edited by

Chunguang Yang, Dong-Xing Guan and Jerome Nriagu

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The role of essential trace elements in health and disease

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Editorial: The role of essential trace elements in health and disease

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Editorial on the Research Topic

The role of essential trace elements in health and disease

Research during the last half century has clearly established that trace elements, whether essential or non-essential, play important roles in a wide variety of biological processes of living systems and can also be a defining factor in the outcome of parasitic infections (1). Until recent years, most of the public health attention and funding were directed at the worldwide contamination of the environment with toxic but non-essential trace metals, especially the so-called “big four” of arsenic, cadmium, lead, and mercury. The bioaccumulation and biomagnification of these heavy metals oftentimes increase their potential for toxicity further up the food chain, thereby increasing the level of the public’s concern. Anthropogenic trace elements are non-degradable and are deemed to pose a continuous risk for human and animal health for a long time. Since the sources of pollution are well-defined, they are amenable to study and remediation.

In recent decades, attention is increasingly being focused on the essential trace elements (often called micronutrients) that occur naturally in small amounts and play critical roles in numerous physiological and metabolic processes in both plants and animals. The role of essential trace metals in health and disease is enigmatic considering that their exposure-disease relationships have evolved over time to be U-shaped mostly. Very low levels of an essential trace element in biological systems give rise to symptoms of deficiency. This is followed by a range in tissue concentrations where an organism is able to maintain biological functions at the optimum level of the trace element. Finally, at some higher tissue level of the trace element, the normal regulatory mechanisms are overloaded, resulting in toxic symptoms. Each element has its own characteristic exposure-response curve while the optimum concentration range can differ by as much as several orders of magnitude depending on the chemical form of the element and the specific compound in the exposure dose. Most toxicological studies assume simple linear exposure-response relationships and ignore the fact that exposures in the natural environment oftentimes fall outside the optimum concentrations to maintain physiological and metabolic processes. Failure to account for the U-shaped form of the exposure-disease relationships is believed to be

responsible for much of the confusing and conflicting results that have been published on the interconnection of trace elements with human health.

This Research Topic is not intended to be a comprehensive coverage of the huge volume of literature on the diverse roles that essential trace elements play in human health and disease. Rather, the articles have been selected to illustrate areas of emerging interests within the broad field. The papers cluster into three broad themes or public health problems of global concern, namely, the role of trace elements in metabolic diseases; maternal and perinatal health; and human cancers.

Trace elements have been identified for long time as potential candidates for improving metabolic disorders including insulin resistance, obesity, metabolic syndrome, and diabetes. Studies of the past few years clearly show that micronutrient nutrition is one of the most important modifiable lifestyle factors for preventing disease and maintaining health. As a consequence, this topic has been an active field of investigation for a long time.

Wu et al. used the Guangdong Provincial Residents' Chronic Disease and Nutrition Surveillance Survey data (2015) to extend the understanding of the relationship between exposure to multiple metals and hypertension. They found that higher levels of manganese, zinc and selenium are associated with increased risks for hypertension and elevated blood pressure readings in the general population of southern China. Wu et al. found synergistic interactions of the essential micronutrients with the non-essential toxic elements (arsenic, cadmium, and lead) in the mediation of hypertension by obesity. This finding is important since essential and non-essential trace elements tend to co-occur in many environmental media. Evidence for the association of obesity with levels of lead and cadmium in blood was found to be weak in this report. In another multiple-metal study, González-Domínguez et al. explored the role of dietary trace elements on the etiology of obesity and related disorders. They found that alterations in concentrations of trace elements in peripheral blood were closely correlated with the characteristic metabolic complications behind childhood obesity, namely hyperglycemia, hyperinsulinemia, and dyslipidemia in a cohort of Spanish children and adolescents.

The effects of specific trace elements alone on metabolic disorders are discussed in several articles. The paper by Ren et al. contributes to the growing fund of knowledge on the relationships between iron markers (including ferritin, transferrin, and soluble transferrin receptor) and metabolic obesity phenotypes. Non-alcoholic fatty liver disease (NAFLD) is a multistage condition that affects 30% of the global population, and is causally linked to end-stage liver disease. Growing evidence indicates that NAFLD is the hepatic manifestation of metabolic syndrome—obesity being a well-known predisposing factor for NAFLD (Zhu et al.). The article by Zhu et al. contributes to the growing body of evidences that increasingly show that dietary fiber intake from plant foods or supplements could confer a greater benefit in reducing the NAFLD risk and improving liver function. The role of trace elements in the association of obesity with NAFLD remains equivocal according to a meta-analysis of pooled data of 2,607 NAFLD patients and

1,441 non-NAFLD normal individuals. This review concluded that there was no significant association between serum copper or ceruloplasmin with NAFLD, even although NAFLD patients had low hepatic copper concentrations (Chen Y. et al.).

The effects of trace elements on maternal and perinatal health have been a matter of public health interest for a long time. One topic in this area that is receiving increased attention is preeclampsia, a leading cause of maternal and perinatal mortality especially in the low- and middle-income countries. Disruptions in metabolic cycles of trace elements are suspected as playing a vital role in developing preeclampsia. One of the metal suspects is copper. In pregnancy, aberrant maternal copper levels may give rise to early spontaneous miscarriage, fetal structural anomalies, gestational diabetes, small-for-gestation babies, and preterm birth—hallmarks of preeclampsia. From their systematic review of the published literature, Zhong, Yang, Sun et al. concluded that disruptions in maternal copper levels are correlated with risks of preeclampsia, but the resulting pathologies present variously across different geographical and economic contexts. Equally important, the experimental protocols used in most studies made it impossible to relate the exposure dose to the bounding concentrations for copper deficiency vs. copper toxicity. Similar conclusions were reached in a review and meta-analysis of maternal serum zinc level as a predisposing factor for preeclampsia (Jin et al.). By contrast, a meta-analysis of pooled data from 21 studies in different countries showed that that maternal lead exposure is unequivocally associated with preeclampsia during pregnancy, even at very low levels (Zhong, Yang, Li et al.).

A large volume of literature exists to show that trace elements play critical roles, both good and bad, in human carcinogenesis but the underlying mechanisms are still not well understood. The discovery of the so-called “esophageal cancer belts” in South Africa, France, Iran, and China has recently generated a lot interest on the etiology of esophageal cancer, the seventh leading cause of cancer death worldwide. Smoking, alcohol consumption, exposure to environmental pollutants and diet are the generally recognized risk factors for esophageal carcinoma. Strong associations with exposure to trace elements have been reported but the results are not coherent. The timely article by Yang et al. provides a good overview of the connections between zinc, copper, iron, and selenium and esophagus cancer with emphasis on the likely underlying mechanisms.

Individual papers in this Research Topic offer comprehensive reviews of current knowledge or present the results of cutting-edge research. The articles come from a range of disciplines and hopefully should add to the growing effort to integrate human health and ecosystem health under the emerging field of One Health. The papers together make it clear that a better understanding of the roles of the essential trace elements would be instrumental in the development of new dietary intervention measures to improve global health. We thank the authors of the articles who deserve the credit for the quality of this Research Topic. We also express our sincere appreciation to the reviewers who contributed immeasurably to the betterment of the papers.

Author contributions

D-XG: Writing—original draft, Writing—review & editing. CY: Writing—original draft. JN: Writing—original draft, Writing—review & editing.

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The authors declare that the research was conducted in the absence of any commercial or financial relationships

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A Global Perspective of Correlation Between Maternal Copper Levels and Preeclampsia in the 21st Century: A Systematic Review and Meta-Analysis

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Background: Preeclampsia (PE) is a common multi-system disorder in pregnancy and a major cause of maternal and perinatal morbidity and mortality globally. Copper is a crucial micronutrient for human health.

Methods: A systematic review was performed according to Preferred Reporting Item for Systematic Reviews and Meta-analysis (PRISMA) guidelines to synthesize the best available evidence regarding the correlation between maternal copper levels and PE from women with different geographical and economic backgrounds.

Results: A total of 34 studies containing 2,471 women with PE and 2,888 healthy pregnant controls across 16 countries were included for research. All studies were systematically reviewed and assessed with the Newcastle-Ottawa Scale (NOS), The Agency of Healthcare for Research and Quality (AHRQ) assessment tools according to the study types. Globally, there was no significant difference in maternal serum copper levels between women with PE and control (Mean difference 5.46, 95% CI -9.63, 20.54). Sub-group analysis from geographical and economic perspectives revealed contrasting results. In conclusion, copper is associated with PE, but the levels of copper leading to increased risk of PE varied across regions and economic development.

Conclusions: The deranged maternal copper levels are correlated with risks of PE, but it presents variously across different geographical and economic contexts.

Systematic Review Registration: https://www.crd.york.ac.uk/prospero/display_record.php?RecordID=306536. Identifier: CRD42022306536.

Keywords: copper, Cu, trace elements, hypertensive disorder complicating pregnancy, preeclampsia (PE), systematic review, meta-analysis

INTRODUCTION

Preeclampsia (PE) is a subset of hypertensive disorder complicating pregnancy (HDCP) that occurs in around 5% of all pregnancies worldwide (1). It is the leading cause of maternal and perinatal mortality, which has caused particular economic and healthcare burdens in low- and middle-income countries (LMICs) owing to insufficient medical resources.

This maternal disorder often presents in many ways across different organs and systems after 20 weeks of gestation. New-onset hypertension with proteinuria with or without edema is the most common clinical manifestation. However, the etiology of PE is still little known by far. Many candidate factors, such as genetic predisposition, lifestyle factors, and socioeconomic status, can contribute to the pathogenesis of PE (1–3).

Some researchers have proposed that the imbalanced micronutrients in women can increase the risk of PE (3–5). There are discrepancies in the human body status of micronutrients across people from different regions and ethnic backgrounds. Opinions vary to explain the causes. One argument holds that ethnic and genetic background is the main contributor to different serum micronutrient levels, predisposing pregnant women to varying risks of PE (2, 6). However, other academicians believe that the local environment and lifestyle mainly determine the levels of trace elements in inhabitants, which further affects the regional prevalence of PE (3).

Copper is an essential trace element for human health. It functions as a cofactor in enzymes, involved in cell oxidative balance and inflammatory and immune processes. Its deficiency is associated with diabetes and dyslipidemia, while its excessive storage could lead to tumorigenesis (7, 8). In pregnancy, aberrant maternal copper levels may lead to early spontaneous miscarriage, fetal structural anomalies, gestational diabetes, small-for-gestation babies, and preterm birth (9–11). However, its role in the development of PE seems controversial. Some studies reported that maternal Cu-deficiency is associated with an increased risk of copper, while others reported the opposite (5, 11–14). Whether aberrantly high levels of copper or marked low levels are more prone to develop PE is unknown by far. In this article, we searched extensive studies with no limitation of countries and ethnic backgrounds to determine: (1) whether pregnant women from different countries have varied copper levels; (2) whether aberrant serum copper levels are associated with higher maternal risk of PE; (3) how copper levels lead to a higher risk of PE.

METHODS

Literature Searches

The analysis was performed according to the Preferred Reporting Item for Systematic Reviews and Meta-analysis (PRISMA) statement. The review was registered with PROSPERO (ID: CRD42022306536). Two independent reviewers (ZX-Z and QM-Y) searched six electronic databases (PubMed, Embase, Web of Science, ClinicalTrials.gov, and two Chinese databases, namely, Wanfang and the Chinese National Knowledge Infrastructure, CNKI) from database inception to 26 Jan 2022. The language

is limited to English and Chinese. We used a combination of Medical Subject Headings (MeSH) terms and free text words such as “preeclampsia or pre-eclampsia,” “copper or Cu.” We use Chinese words equivalent to the MeSH to search in the Chinese database of Wanfang and CNKI. The search strategy is given in detail in **Supplementary 1**. We also manually searched the references cited in each included article to complement our initial search.

Selection Criteria

The criteria for inclusion and exclusion of studies were established prior to the literature search. Studies that fulfilled the following were included: (1) observational studies involving copper and preeclampsia; (2) the exposure of interest should include copper while the outcome of interest should include preeclampsia; (3) the control should be pregnant women instead of non-pregnant women.

A study was excluded if it (1) did not contain serum copper levels, e.g., only described the placental and/or umbilical copper levels; (2) was conducted before the year 2000; (3) the unit of serum copper or the copper levels it gave cannot be converted to mean \pm SD.

Study selection was made by two independent investigators (ZX-Z and QM-Y) who carefully reviewed all the articles and determined the inclusion and exclusion criteria by fully discussing them with each other. A third researcher (QQ-W) would resolve any disagreement between the two.

Quality Assessment and Data Extraction

Quality assessment of each study was based on the Newcastle-Ottawa Scale (NOS, http://www.ohri.ca/programs/clinical_epidemiology/oxford.asp) for case-control and cohort studies, a nine-star rating system to assess the three domains, i.e., selection, comparability, and outcome of a study. A score of 7–9 is considered high quality, 4–6 as fair quality, 0–3 as poor quality. The Agency for Healthcare Research and Quality (AHRQ) checklist (<http://www.ncbi.nlm.nih.gov/books/NBK35156/>) was used for cross-sectional studies. An item would score 1 point if the study being assessed clearly answers the question, which is marked as a “yes;” the answer of no or not accessible (NA) has no score in the item. A combined score between 8 and 11 indicates good quality, 4–7 fair quality, and 0–3 poor quality. Two individual reviewers (ZX-Z and QM-Y) did the data extraction while disagreements were fully discussed until all three researchers (ZX-Z, QM-Y, and QQ-W) reached a conclusion.

Sub-group Analysis and Meta-Regression

The influence of various factors, such as the year, location, and type of the study, and the economic level, fasting status, and methods of measurement for copper levels in the study population were explored by performing subgroup analysis and meta-regression.

Statistical Analysis

The statistical analysis was performed *via* Review Manager 5.4.1 (The Nordic Cochrane Center, Copenhagen, Denmark) and Stata version 15.0 (StataCorp, College Station, TX, USA). The pooled

outcomes of serum copper levels were calculated to assess the correlation with PE. All different units of serum copper levels were converted to ug/dl according to the website (<http://www.scymed.com/en/smnxtb/tbcdb1.htm>) with mean \pm SD put in the table of the software. The I^2 was used to test the heterogeneity ($I^2 \geq 50\%$ indicates significant heterogeneity), then visualized *via* a forest plot. The random-effect model (REM) was adopted to calculate the combined results if the heterogeneity is considered significant. A sensitivity analysis was performed with the removal of each study once to assess whether any single study could affect the whole outcome. Publication bias was visualized *via* funnel plot with Begg's test and tested with Egger's linear regression.

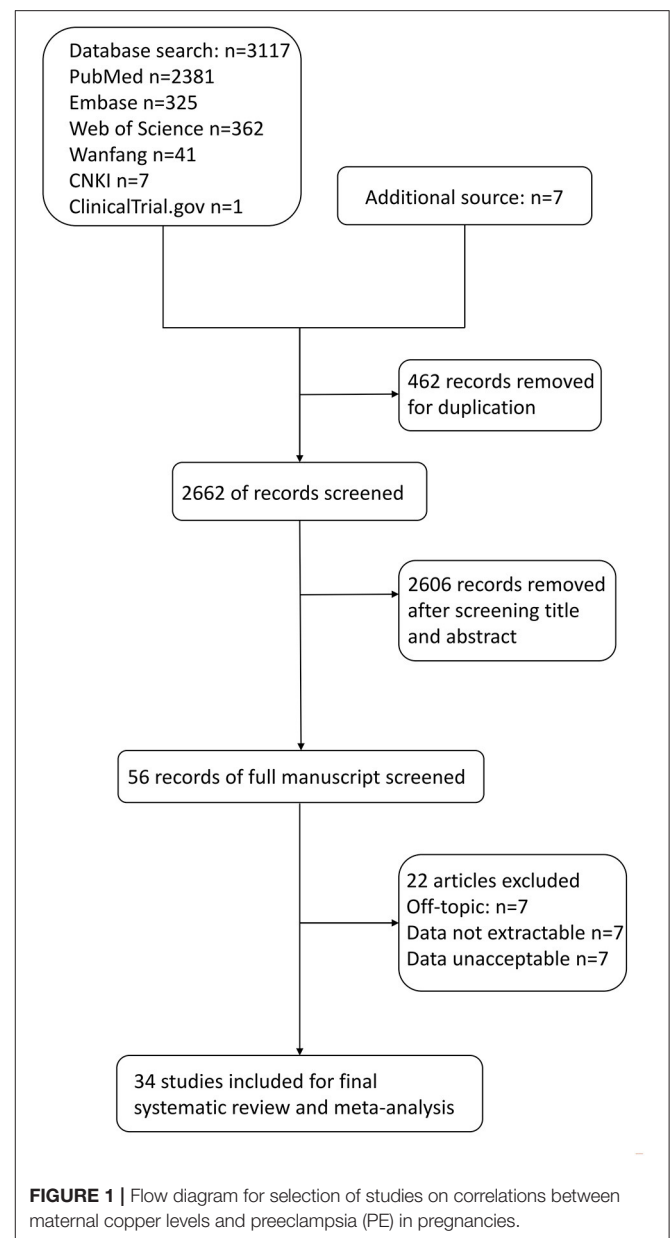
RESULTS

Literature Search

A total of 3,117 articles were identified through the six electronic databases mentioned above (**Figure 1**). The other 7 studies were manually added from the reference of some included articles to the study group for further analysis. Of the total 3,124 articles, we excluded 462 duplication reports. A total of 2,606 references were further excluded based on the title and abstract. After the full-text assessment, another 22 articles were excluded, leaving 34 studies in the final group for qualitative and quantitative analysis. Of the 22 articles, seven were excluded for not answering the research question. Two were excluded because the data of PE cannot be extracted separately (15, 16). In comparison, five articles were excluded for inaccessible data. Shaikh et al.'s study was excluded because part of its participants overlapped with an included study (17, 18). The rest of the seven studies were excluded for unacceptable levels of serum copper compared with the normal range in pregnancy updated in an article by Alvarez et al. in 2007 and Zhang et al. in 2013 or not giving units in the article (16, 19–26).

Characteristics of Included Studies

Thirty-four studies published between 2002 and 2022 were eligible for final review. Twenty-five studies are case-control, six are cross-sectional studies, and three are cohort studies. The detailed characteristics of included studies are presented in **Supplementary Table 1**. The diagnostic criteria were most commonly referred to as ACOG guidelines in different editions due to the study's years. Some use the local diagnosis of PE which was similar to ACOG's diagnostic criteria (14, 27–31). The number of participants with PE across different studies ranged from 14 to 427, while the number of healthy pregnant control ranged from 23 to 472. Serum copper levels were measured *via* atomic absorption spectrophotometer (AAS) or flame atomic absorption spectrophotometer (FAAS) in 24 studies, while inductively coupled plasma mass spectrometry (ICP-MS) was conducted in four studies (4, 5, 11, 32). The conventional method, "inductively coupled plasma optical emission spectrometer (ICP-OES)," was applied in two studies (33, 34). Bai's study did not clearly state the method of measurement (31).



Results of the Systematic Review

Thirty-four studies were further categorized as case-control, cross-sectional, and cohort studies, and evaluated *via* Newcastle-Ottawa Score (NOS) and the Agency for Healthcare Research and Quality (AHRQ). After the final evaluation, 19 studies (16 case-control and three cohort studies) were assessed as high quality, while the rest of the 15 studies (nine case-control and six cross-sectional studies) were of fair quality. No study was evaluated as poor quality. The detailed score can be accessible in **Supplementary Tables 1, 2**, and the publication bias can be seen in **Supplementary Figure 1**.

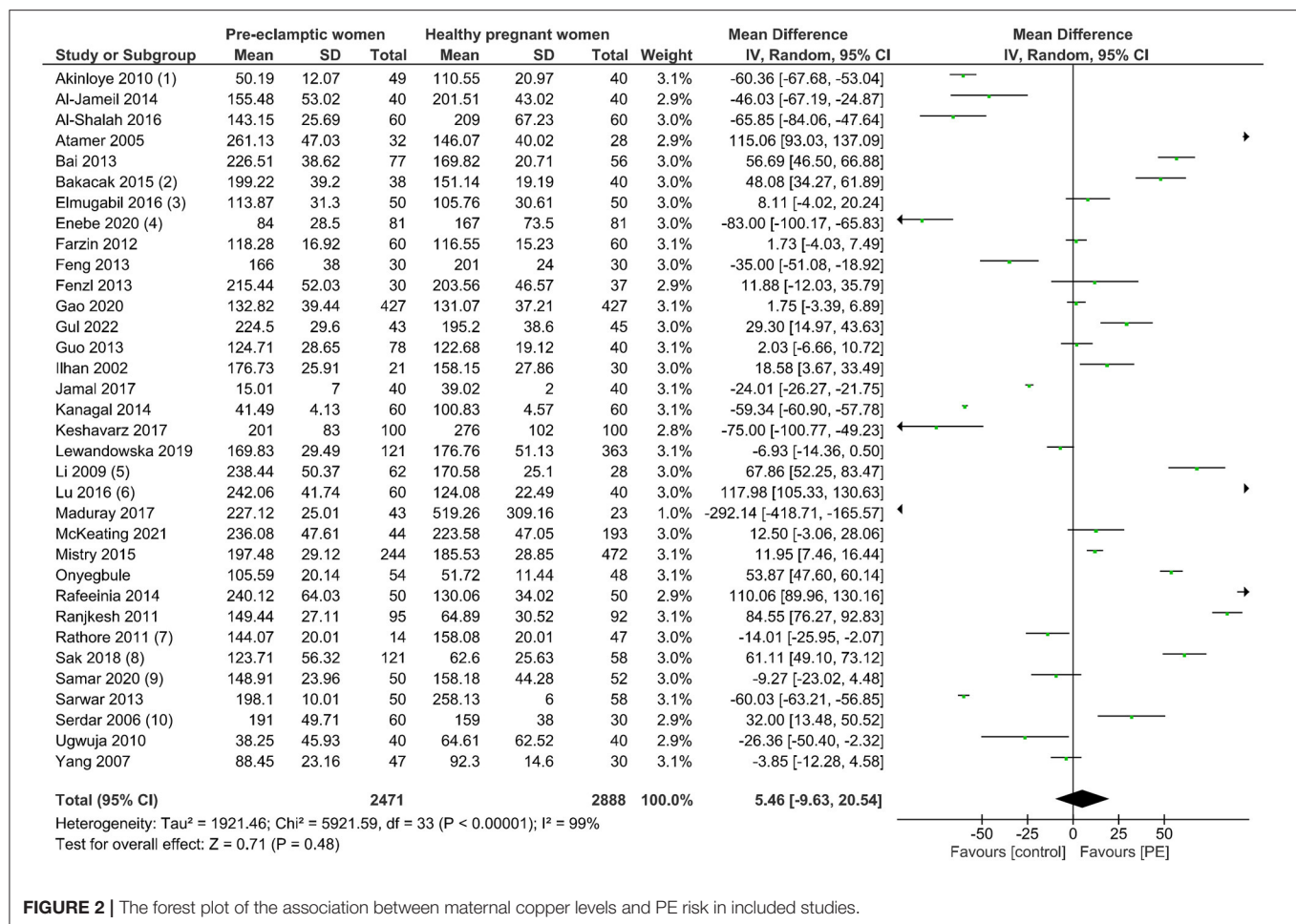


FIGURE 2 | The forest plot of the association between maternal copper levels and PE risk in included studies.

Results of the Meta-Analysis

A total of 5,359 participants were enrolled in this analytic study with 2,471 women with PE and 2,888 healthy pregnant controls compared to maternal serum copper levels. From the data we had harmonized from studies across 16 countries, no significant difference in serum copper levels was observed in women with PE compared with normotensive pregnant women (Mean difference 5.46, 95% CI -9.63, 20.54). Details can be seen in **Figure 2**. Begg's test and Egger's test were applied to assess publication bias, and no significant publication bias was identified ($z = 1.33$, $p = 0.182$; $t = -0.52$, $p = 0.609$; **Supplementary Figure 2**). Sensitivity analysis showed no single study had influenced the overall effect.

Results of Meta-Regression

A high level of heterogeneity was observed between studies and in sub-group analysis. Meta-regression was conducted to identify possible causes. The year of the study, study type, geographical location, national income level, fasting status of women upon blood test, and method of measurement were tested for potential contributors of heterogeneity. However, none of the aforementioned variables was significantly associated with the heterogeneity we detected. The P -value was 0.51 for the year of the study, 0.48 for study type, 0.47 for the location of the

study, 0.87 for economic status, 0.92 for fasting status, and 0.70 for variances in measurement. Detailed results can be seen in **Supplementary Figure 3**.

Results of Sub-Group Analysis

Sub-Group Analysis From a Geographical Perspective

From a geographical perspective, four studies were from South Asia (two from India, one from Bangladesh, and one from Pakistan, respectively) with 164 participants and 245 controls. The pooled result showed significantly lower levels of copper with a mean difference of -30.45 (95% CI -52.59, -8.31). Twelve studies, including 1,353 participants were originally conducted in the Middle East. A higher level of serum copper was observed in women with PE (mean difference 26.46, 95% CI -2.26, 55.18), the trend would be more prominent after the exclusion of the research from Saudi Arabia, the only regional high-income country in this sub-group (mean difference 32.95, 95% CI 3.57, 62.33). Six studies from Africa (599 participants, 317 with PE vs. 282 controls) also failed to demonstrate a statistically significant difference in copper levels in pregnant women (mean difference -0.82, 95% CI -5.04, 3.41). However, during the sensitivity test, the pooled result can be significantly different (mean difference

−46.23, 95% CI −51.95, −40.52) after removing Onyebule et al.'s study. Only two studies purely came from Europe (551 participants all from East Europe), one from Australia (237 participants), and an additional international study from women across Australia, New Zealand, and the UK (4). Detailed results can be seen in **Supplementary Figures 4–6**.

Single Country Sub-group Analysis (China vs. Turkey vs. Iran)

China, Turkey, and Iran ranked top three for most single-country studies. Eight studies focused on Chinese women, six on Turkish, and four on Iran (**Supplementary Figure 2**). The Chinese and Iranian studies shared a trend of higher levels of serum copper in women with PE (mean difference 24.72 vs. 30.90, 95% CI −3.58, 53.03 vs. −30.79, 92.58, respectively). In Turkey, the copper levels were more pronounced in women with PE than in healthy normotensive mothers-to-be. The mean difference of the national pooled result was 49.95 (95% CI 27.53, 72.37). The forest plot of each of the three countries is shown in **Supplementary Figure 7**.

Sub-group Analysis From an Economic Perspective

We then further sub-grouped all the studies according to economic development. The income levels of each country obtained from the World Bank Country classification determined how they were allocated to different sub-groups (35). The sub-groups are low-income economies (36), lower-middle-income economies (Bangladesh, India, Iran, Nigeria, Pakistan), upper-middle-income economies (China, Iraq, South Africa, Turkey), and high-income economies (Australia, Croatia, New Zealand, Poland, Saudi Arabia, UK) (35). No significant difference was observed in copper levels between preeclamptic and non-preeclamptic pregnancies in low-and-lower-middle income economies. However, higher maternal copper levels (mean difference of 17.30, 95% CI 3.60, 30.99) were revealed in women with PE in high-and-upper-middle income economies. The detailed data from the forest plot can be seen in **Figure 3** and the funnel plot of this sub-group analysis can be seen in **Supplementary Figure 8**.

DISCUSSION

This systematic review and meta-analysis, including 5,359 pregnant women, has shown a correlation between maternal serum copper levels and the risk of PE. However, this is not a unilaterally positive or negative association, and it contradicts all the existing meta-analyses available.

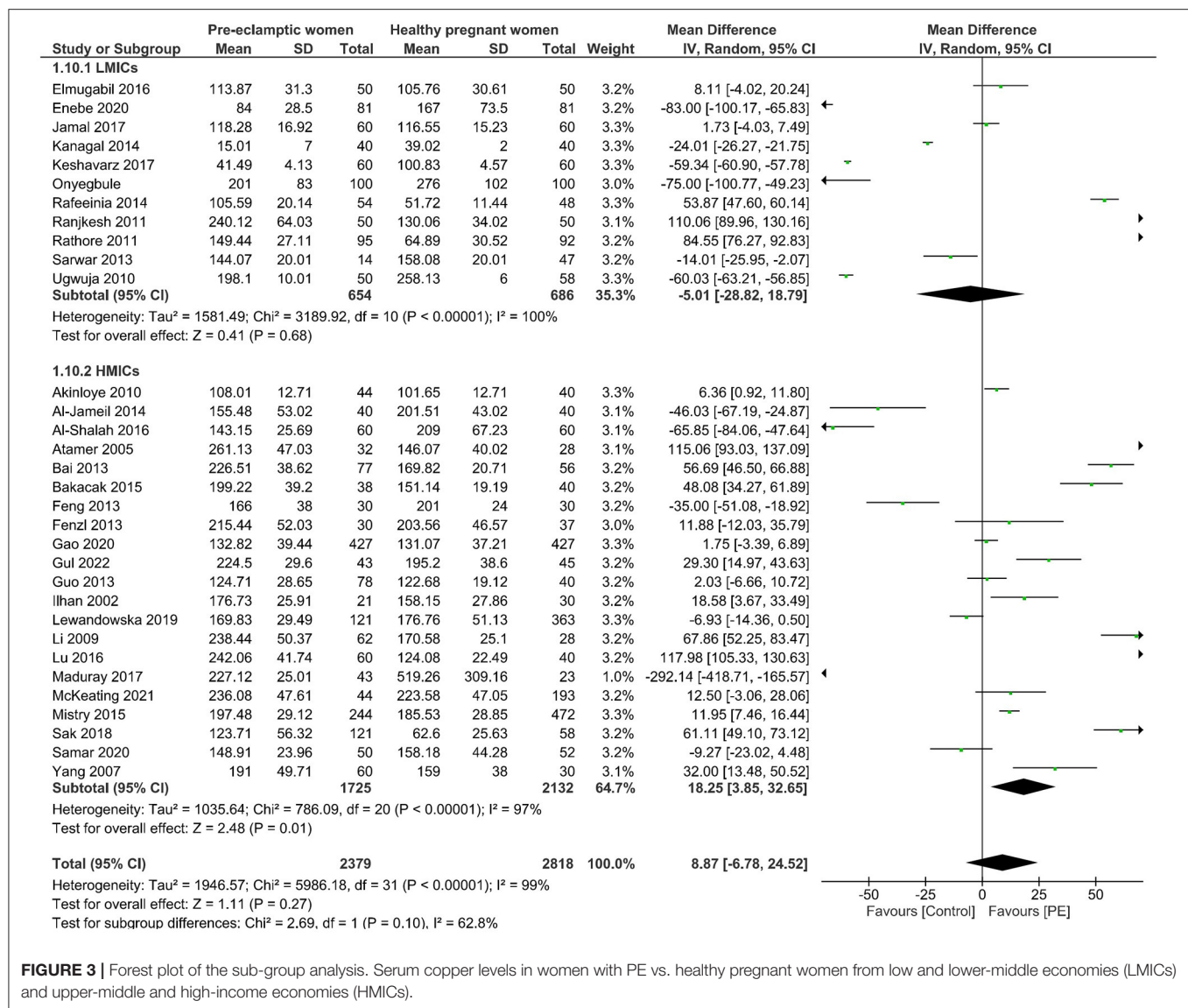
By far, this study has been the most up-to-date systematic review and meta-analysis and has included the largest population and almost all relative studies worldwide since 2000. We consider that the environment, food choices, lifestyle, and the techniques for measuring copper levels are way different compared to that of the 20th century, and this is why we only focus on research in the 21st century. We are the first to combine economic factors and geographic differences concurrently in analyzing the relationship between trace elements and risks of maternal complications during pregnancy. We considered lifestyle factors,

socioeconomic status, geographical locations, and ethnicity as contributing factors for PE, and these are supported by current evidence (1, 3, 37). The sub-group analysis was performed accordingly. The results suggested multilateral correlations, which should be interpreted cautiously. In general, a higher level of copper is associated with PE. Women with higher levels of copper from China, Iran, Turkey, the Middle East, and other higher-income level regions are more susceptible to developing PE. In contrast, South Asian and African women seem to experience an opposite trend. This might be explained by a U-shape effect, which suggests that either abnormally low or high levels of copper can lead to a higher risk of PE. Another cause could be that low copper levels indicated the holistic deficiency of trace elements in women in these regions. The imbalanced micronutrient environment makes women susceptible to PE.

Trace elements have long been an essential part of PE research. Calcium has been the hottest topic in preeclamptic research since the 1980s (38, 39). However, copper has not been fully elucidated for its role in the pathogenesis of PE, despite being the most active antioxidant among biological ions in the human body. It has been found to take an active part in the oxidative stress process in the form of enzymes (40). The altered oxidative stress was observed in the early stage of PE, which results in dysfunctional re-modeling of the uterine spiral arteries, predisposing women to develop preeclamptic symptoms after 20 wks. The studies presenting lower serum copper levels held that a deficiency of copper and its corresponding antioxidant effects in the initial phase of placental formation and formation predispose women to PE later on (5). However, this is not contradicted by some other study findings of higher levels of copper upon the diagnosis of PE or at delivery as copper can be either antioxidant or oxidant (41).

There are two meta-analysis studies in 2016 and 2017 that evaluated the correlation between serum copper level and PE during pregnancy (42, 43). Both showed a higher level of copper associated with an increased risk of PE. However, they included fewer studies, with Song et al. focusing on Asia's population while the other study (42) drew evidence from only 12 articles. Both have missed some articles even in their focus, making the conclusion limitedly valid. In addition, six studies from Turkey were included in the Asian population in Song's meta-analysis. However, the data we analyzed found that Turkey's data are similar to the data extracted from the Middle East. This is consistent with the geographical feature of Turkey, as it is more closely related to Iran, Iraq, and Saudi Arabia than countries such as China, India, and Bangladesh. Furthermore, both the two studies only conduct meta-analyses without systematic review. By contrast, we included more studies with a broader population to avoid selection and publication bias to the minimum level. The extensive work on the systematic review for each article would further consolidate the evidence in our study. The conclusion or the evidence picked up from the sub-group analysis can be of essential importance for researchers with an interest in studying the relationship between copper levels and PE.

However, there are limitations to our study. The between-study heterogeneity is significant even after subgroup analysis and sensitivity analysis. A range of factors could contribute to



it. Firstly, the definition of PE varies across studies. Most studies follow the ACOG definition but in different editions owing to the study's year, while others follow the local textbooks (14, 27–30, 32). Some studies have not disclosed how they diagnose PE (18, 44). Secondly, not all studies have clearly stated the timing of blood sampling in women diagnosed with PE. The timings are different in those studies that articulate how and when they did the venipuncture. For example, Lewandowska et al. conducted early-trimester serum copper levels and followed up with late-stage maternal complications, while others performed blood sampling upon the diagnosis or at delivery. Thirdly, we group the nations according to the up-to-date data from World Bank, but not the data of the year of the study, which might cause a minor derange to the overall result from an economic perspective. Fourthly, the unit and method for measuring serum copper levels are different. Various units are used in the measurement, such as $\mu\text{g/dL}$, mg/L , and $\mu\text{mol/L}$. The blood sample of copper can best

reflect the body status of its levels, but the different techniques for measurement can vary across studies, leading to differences in the results of copper levels in women. In some studies, PE was further classified as mild and severe type, or sub-grouped as gestational hypertension, Hemolysis, Elevated Liver enzymes, Low Platelets (HELLP) syndrome, and eclampsia. The results need to be pooled for comparative analysis, which further adds to the heterogeneity of this study.

CONCLUSION

In summary, our study demonstrates that maternal serum copper levels and the risk of PE are not simply positively correlated but in complex associations among women from different ethnic and economic backgrounds. Hence, large cohort studies or meta-analyses with individual patient data in the future would identify the role of copper in the pathogenesis of PE.

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

ZZ and QW: conceptualization, investigation, and resources. ZZ and QY: methodology and formal analysis. ZZ, QY, and TS: software. TS and QW: validation and supervision. QY and TS: data curation. ZZ: writing—original draft preparation and funding acquisition. QW: writing—review and editing and

project administration. TS: visualization. All authors have read and agreed to the published version of the manuscript.

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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.2022.924103/full#supplementary-material>

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Maternal serum zinc level is associated with risk of preeclampsia: A systematic review and meta-analysis

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Background: Preeclampsia (PE) is a multi-organ syndrome that onsets in the second half of pregnancy. It is the second leading cause of maternal death globally. The homeostasis of zinc (Zn) levels is important for fetomaternal health.

Objective: We aimed to collect all studies available to synthesize the evidence regarding the association between maternal Zn levels and the risk of preeclampsia.

Methods: A systematic review and meta-analysis was conducted via searching seven electronic databases [PubMed, Web of Science, Embase, African Journals Online (AJOL), ClinicalTrials.gov, and two Chinese databases: Wanfang and Chinese National Knowledge Infrastructure, CNKI]. Studies reporting maternal serum Zn levels in pregnant women with or without preeclampsia were included. Eligible studies were assessed through Newcastle-Ottawa Scale (NOS) and the meta-analysis was performed via RevMan and Stata. The random-effects method (REM) was used for the meta-analysis with 95% confidence interval (CI). The pooled result was assessed using standard mean difference (SMD). The heterogeneity test was carried out using I^2 statistics, and the publication bias was evaluated using Begg's and Egger's test. Meta-regression and sensitivity analysis was performed via Stata software.

Results: A total of 51 studies were included in the final analysis. 6,947 participants from 23 countries were involved in our study. All studies went through the quality assessment. The pooled results showed that maternal serum Zn levels were lower in preeclamptic women than in healthy pregnant women (SMD: -1.00 , 95% CI: -1.29 , -0.70). Sub-group analysis revealed that geographical, economic context, and disease severity may further influence serum Zn levels and preeclampsia.

Limitations: There are significant between-study heterogeneity and publication bias among included studies.

Conclusions: A lower level of maternal Zn was associated with increased risks of preeclampsia. The associations were not entirely consistent across countries and regions worldwide.

Systematic review registration: https://www.crd.york.ac.uk/prospero/display_record.php?RecordID=337069, Identifier: CRD42022337069

KEYWORDS

zinc, Zn, trace elements, hypertensive disorder complicating pregnancy (HDCP), systematic review, meta-analysis, preeclampsia (PE)

Introduction

Preeclampsia (PE) is a multi-system disorder that onsets at 20 wks or later in pregnancy. It can affect pregnant women in many ways, such as causing hypertension, proteinuria, liver dysfunction, placental abruption, fetal growth restriction. It is a subtype of the hypertensive disorder spectrum in pregnancy, complicating around 5% of pregnancies worldwide. It remains one of the major causes of maternal, fetal and neonatal mortality, particularly in low-income and middle-income countries (LMICs) (1, 2). Despite many researchers devoted to this field, the etiology of preeclampsia is still largely unknown.

Some scholars proposed that trace elements may play a vital role in developing preeclampsia (3–5). In spite of making up <0.1% in the human body, trace elements have a disproportional function in maintaining health. Overall, evidence suggests that micronutrient imbalances are associated with various disorders (4, 6). This will be more prominent in pregnancy when maternal requirements are usually increased. Optimal supplementation can reduce a range of pregnancies complications, e.g., anemia, gestational diabetes, thyroid disorders, and miscarriage (4).

Zinc (Zn) is one of the essential trace elements. As a micronutrient, it functions as a cofactor for up to 10% of proteins in living organisms, playing a vital role in a range of biological processes in the human body (7). Zn is involved in a range of signaling pathways, e.g., Nuclear Factor Kappa B (NF- κ B) signaling, and Toll-like Receptor 4 (TLR4) signaling, carrying considerable clinical implications (8, 9). Diseases such as breast cancer, tuberculosis, and cardiovascular diseases were associated with aberrant levels of serum Zn (10–12). Maternal serum Zn levels are usually measured *via* blood sampling from the maternal antecubital vein, reflecting the maternal homeostasis of Zn (13). During pregnancy, Zn exerts a key role in both maternal physiological adaptations and fetal development. The demand for Zn in fetal growth and placental function increases during the third trimester, and may lead to a lower level of Zn in maternal serum compared to healthy non-pregnant women (14). A significant lower levels of Zn may cause a series of dysfunctions in the biological process and higher risks of developing fetomaternal complications, such as gestational diabetes, preterm pre-labor rupture of membrane (PPROM), preterm birth, and low birth weight. The relationship

between maternal serum Zn and preeclampsia were studied as micronutrients imbalance is believed a contributing factor of preeclampsia (3, 15, 16).

In recent years, many studies have explored the association between maternal serum Zn levels and preeclampsia, but the results were inconsistent (17–20). There are geographical, economic and ethnic differences that may explain such disagreement (21–25). In this study, we conducted a systematic review and meta-analysis, including all studies covering the maternal serum Zn levels in preeclamptic and healthy pregnant women (1) to confirm that maternal serum Zn levels were correlated with their preeclamptic risks during pregnancy; (2) to analyze any clues of how geographical locations, economic and ethnic context affect maternal Zn status.

Methods

Protocol and registration

This study followed the Preferred Reporting Item for Systematic Reviews and Meta-analysis (PRISMA) Statement. We registered at the National Institution for Health Research with the registration identifier: CRD42022337069, https://www.crd.york.ac.uk/prospero/display_record.php?RecordID=337069

Search strategy

We searched seven electronic databases [PubMed, Web of Science, Embase, African Journals Online (AJOL), ClinicalTrial.gov, and two Chinese databases: Wanfang and Chinese National Knowledge Infrastructure (CNKI)] from the inception of the databases to May 31st 2022. Two independent reviewers (YM-Z and SJ-J) used a combination of Medical Subject Headings (MeSH) terms and free text words such as “preeclampsia or pre-eclampsia,” “zinc or Zn.” The Chinese databases were approached with equivalent Chinese medical terms. We have manually checked the references of all the full-text articles we had read to complement our study. There were no other restrictions. The detailed search strategies can be accessed in [Supplementary Table 1](#).

Eligibility criteria and study selection

Studies were included if they were: (1) Observational studies that report maternal serum Zn levels in preeclamptic and healthy pregnant women; (2) the control should be healthy pregnant women instead of gestational diabetic women or non-pregnant women.

Studies were excluded if they were conference papers, editorials, reviews, systematic reviews, or interventional studies.

Study selection was performed by YM-Z and SJ-J. A third reviewer, CZ-H was to resolve any disagreement between the two in study selection.

Data extraction and quality assessment

Following data were independently extracted by two investigators (YM-Z & SJ-J): Name of the authors, year of publication, types of study design, country of the study population, the number of subjects in the studies, the mean \pm standard deviation (SD) of maternal age and serum Zn level in each study. The third investigator (CZ-H) would be consulted once there was disagreement in data extraction or scoring of the quality of studies.

Case-control and cohort studies were assessed according to The Newcastle-Ottawa Scale (NOS, http://www.ohri.ca/programs/clinical_epidemiology/oxford.asp). A score between seven and nine indicates the high quality of a study. Scores ranging from four to six were considered fair quality. A score of three or less suggests that the study was poorly designed. For cross-sectional studies, we applied an adapted form of NOS for structural assessment. The scale ranged from zero to ten. A score of seven to ten indicates good quality. Four to six were considered fair, while a score of three or less was graded as poor quality (26). Rating studies were accomplished by YM-Z and SJ-J, and discussed with CZ-H once there was disagreement in evaluation.

Sub-group analysis and meta-regression

Sub-group analysis and meta-regression were conducted to evaluate the influence of geographic location, economic development, and disease severity on maternal Zn status, and their corresponding effect on risks of preeclampsia. We categorized four geographical groups primarily based on continents, but Asia was sub-divided into Asia and the Middle-East as there are huge differences in terms of demographical features between the two groups. The final groups were: Africa (Egypt, Kenya, Nigeria, South Africa, Sudan, Zambia), Asia (Bangladesh, China, India, Indonesia, Pakistan), Middle-East (Iran, Iraq, Jordan, Saudi Arabia, Turkey), and others (Australia, Brazil, Croatia, Italy, New Zealand, Poland, and the

UK). From an economic perspective, we form two groups, cited from the World Bank classification (27). Group 1 is Low-income and Lower-middle-income economies (LMICs), including Bangladesh, India, Indonesia, Iran, Kenya, Nigeria, Pakistan, Sudan, and Zambia. The countries that were rated as Upper-middle-income economies and High-income economies were allocated to the second group (HMICs), which included Australia, Brazil, Croatia, China, Egypt, Iraq, Italy, Jordan, New Zealand, Poland, Saudi Arabia, South Africa, Turkey, and the UK. Disease severity was applied to those studies with inherent groups of mild and severe disease. We also inspected whether Zn levels were associated with the measurement methods, study types, or geographical locations *via* meta-regression.

Statistical analysis

This study used Review Manager 5.4.1 (The Nordic Cochrane Center, Copenhagen, Denmark) and Stata version 16.0 (StataCorp, College Station, TX, USA). The serum Zn levels were pooled by standardized mean difference (SMD) with 95% CI to assess the correlation with preeclampsia. The I^2 was used to test the heterogeneity ($I^2 \geq 50\%$ indicates significant heterogeneity), then visualized *via* the forest plot. The random-effect model (REM) was adopted to calculate the combined results if the heterogeneity is considered significant. A sensitivity analysis was performed with the removal of each study once to assess whether any single study could affect the whole outcome. Publication bias was visualized *via* funnel plot with Begg's test and tested with Egger's linear regression.

Results

Study selection

One thousand four hundred seven articles were identified after screening seven databases mentioned above. No additional studies were found after checking the references of full-text articles. One hundred fifty-five articles were excluded for duplication. One thousand one hundred eighty-two papers were further ruled out based on title and abstract. After full-text checks, another 19 articles were excluded for the following reasons: (1) Nine were excluded for not answering the research questions; (2) Three were excluded for improper comparison; (3) Two were excluded for improper study types; (4) Five were excluded for inaccessible data. Fifty-one studies were left for quality assessment and data extraction (13, 21–25, 28–72) See Figure 1.

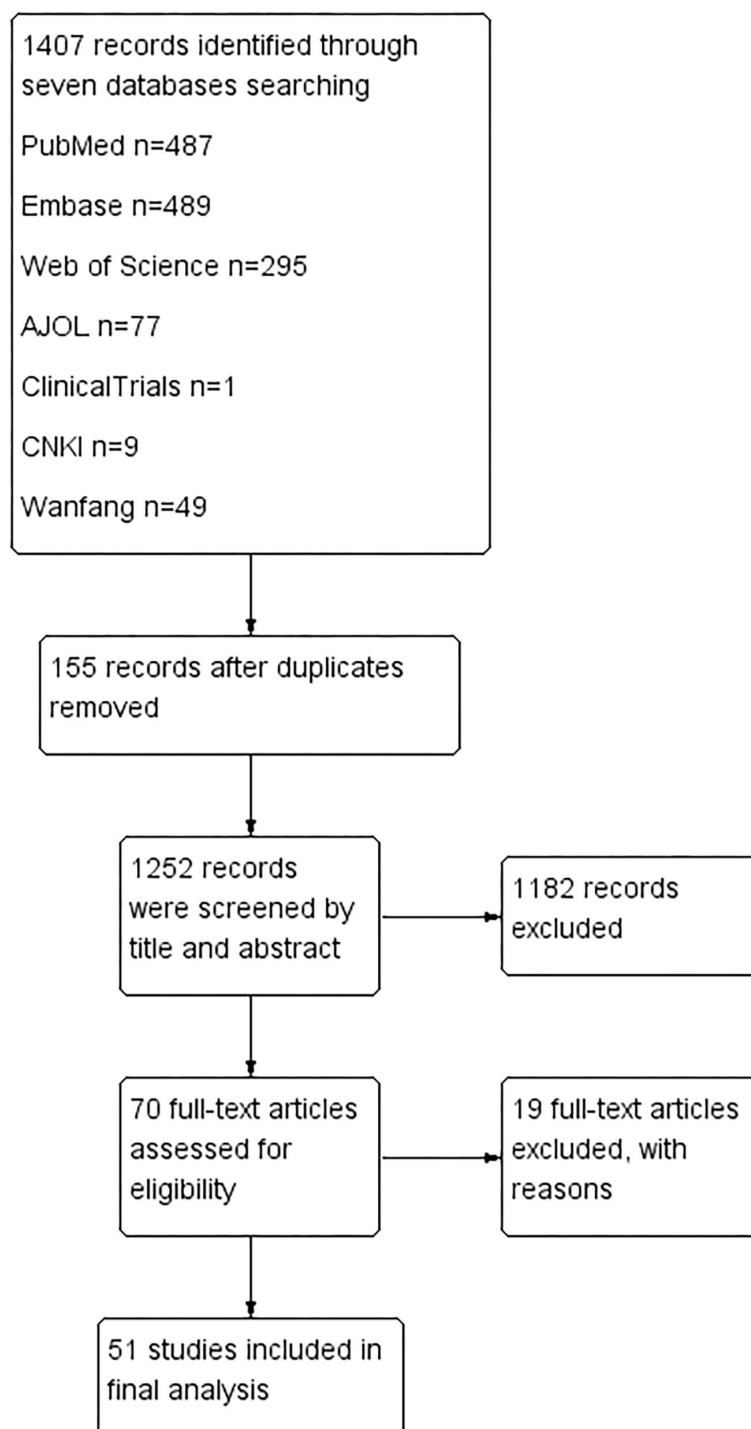


FIGURE 1
PRISMA flow diagram for study selection process.

Basic features of included studies

The 51 studies were conducted across 23 countries over a period of 32 years (1990–2022). Forty-one studies were

case-control, seven were cross-sectional and three were cohort studies. The numbers of preeclamptic women in a single study ranged from 14 to 427 (21, 67). Although not clearly stated in some articles, there was no significant difference in age between

the preeclampsia group and the control (21, 30, 37, 39, 40, 46–48, 51, 52, 63, 72). Maternal serum Zn levels were most frequently measured *via* atomic absorption spectrophotometer (AAS) or flame atomic absorption spectrophotometer (FAAS). Other details can be found in [Supplementary Table 1](#).

Results of systematic review

All studies were classified according to their study designs and further assessed *via* NOS quality assessment tools. Thirty-six (3 cross-sectional, 3 cohort studies and 30 case-control studies) articles were rated as high quality after structured evaluation, while 15 were rated as fair qualified. Detail scores can be accessible in [Supplementary Tables 2.1–2.3](#).

Results of meta-analysis

The total number of preeclamptic women involved in this research was 3,162, while the number of participants in the control group was 3,785. The pooled result showed that maternal serum Zn level in preeclamptic women was lower than in healthy control (SMD: -1.00 , 95% CI -1.29 , -0.70 , see [Figure 2](#)). The funnel plot can be seen in [Figure 3](#). Begg's test and Egger's test were also performed to assess publication bias, and significant bias was discovered ($z = 2.88$, $p = 0.004$; $t = -3.89$, $p = 0.000$; [Supplementary Figure 1](#)). Sensitivity analysis demonstrated that no single study had an overall influence ([Supplementary Figure 2](#); [Supplementary Table 3](#)).

Results of meta-regression

The heterogeneity between studies and across sub-groups was significant. Meta-regression was then performed to explore possible causes. The method of measurement, the geographical locations and the designs of the study types were assessed but the results revealed that the geographical location, study type and different measurement methods were not the causes for heterogeneity (the P -value were 0.399 for geographical location, 0.864 for study type, 0.277 for measurement, respectively). Detailed results can be seen in [Supplementary Figures 3A–C](#).

Results of sub-group analysis

Sub-group analysis from a geographic view

Africa has seen the largest number of recent studies. All 12 studies were published since 2010, with 1,465 participants involved. The pooled result was non-significant (SMD -0.65 , 95% CI: -1.52 , 0.21). Asia is currently home to the largest number of studies and participants (20 studies with 2,665

pregnant women involved), and the result was generally consistent between studies and revealed a negative significance (SMD -1.60 , 95% CI -2.13 , -1.07). In the Middle-East region, the maternal serum Zn levels were also consistently lower in preeclamptic women compared with normotensive pregnant women, but the difference were not as significant as the population in Asia (SMD -0.93 , 95% CI -1.36 , -0.49 vs. -1.60 , -2.13 , -1.07 in Asia). The rest studies were primarily from Europe: one from Italy, one from Croatia, one from Poland, one from Australia, one from Brazil, and one from multi-centers in the UK, Australia and New Zealand. The results were non-significant (SMD 0.02 , 95% CI -0.14 , 0.18). More detailed results were available in [Supplementary Figure 4](#).

Sub-group analysis from an economic view (HIMCs vs. LMICs)

The studies were divided into High-and-Upper-middle-Income countries (HIMCs, 29 studies from 13 countries were included) and Low-and Lower-middle-Income countries (LMICs, 22 studies from 10 countries were included) from an economic perspective. The pooled results of SMD were -0.84 in HIMCs vs. -1.23 in LMICs, respectively. The negative association of maternal Zn levels was more notable in developing countries with details accessible in [Supplementary Figure 5](#).

Disease severity and Zn levels

Only ten studies have sub-divided the disease of preeclampsia into mild and severe types. The pooled results demonstrated that maternal Zn levels were more negatively associated with disease severity. The SMD was -0.75 (95% CI: -1.36 , -0.15) in mild preeclamptic women and -1.32 (95% CI: -2.02 , -0.63) in severe form. Other details can be seen in [Supplementary Figure 6](#).

Discussion

This systematic review and meta-analysis mainly focus on whether there is an association between maternal serum Zn levels and preeclampsia. The overall result demonstrates that a lower level of maternal serum Zn was observed in preeclamptic women than in normotensive pregnant women worldwide. The trend was more prominent in Asian ethnicity, low-income economies and severe patients. This is generally consistent with findings from other reports (17–20).

Zn has many roles in the body, including maintaining the catalytic activity of a range of enzymes, protein synthesis, cell division. It is also involved in the immune system, nerve function, and fertility (4). Its role in the immune system has been well-known for several decades (73). T-lymphocytes activation requires the presence of Zn. Even a mild degree of

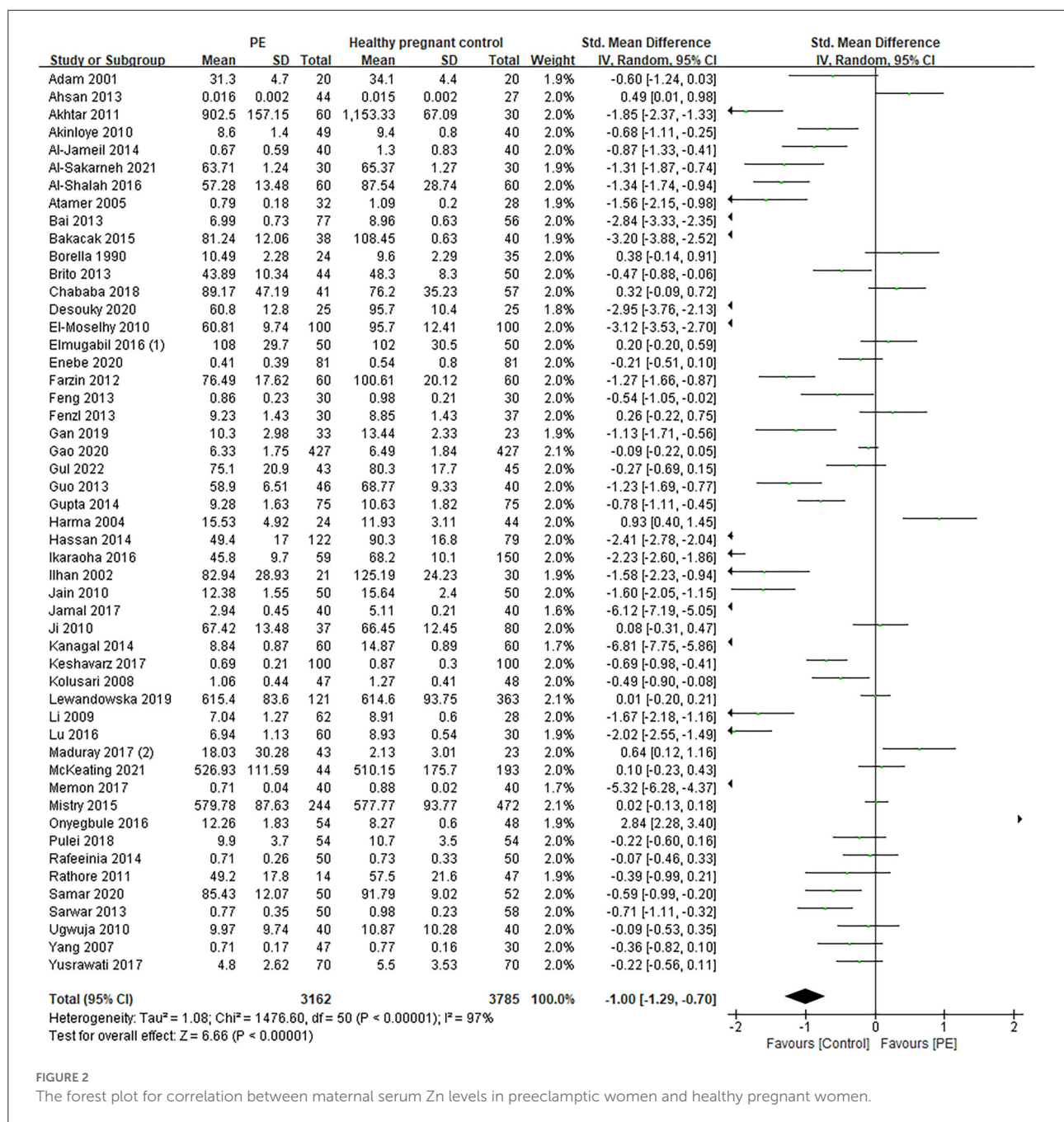


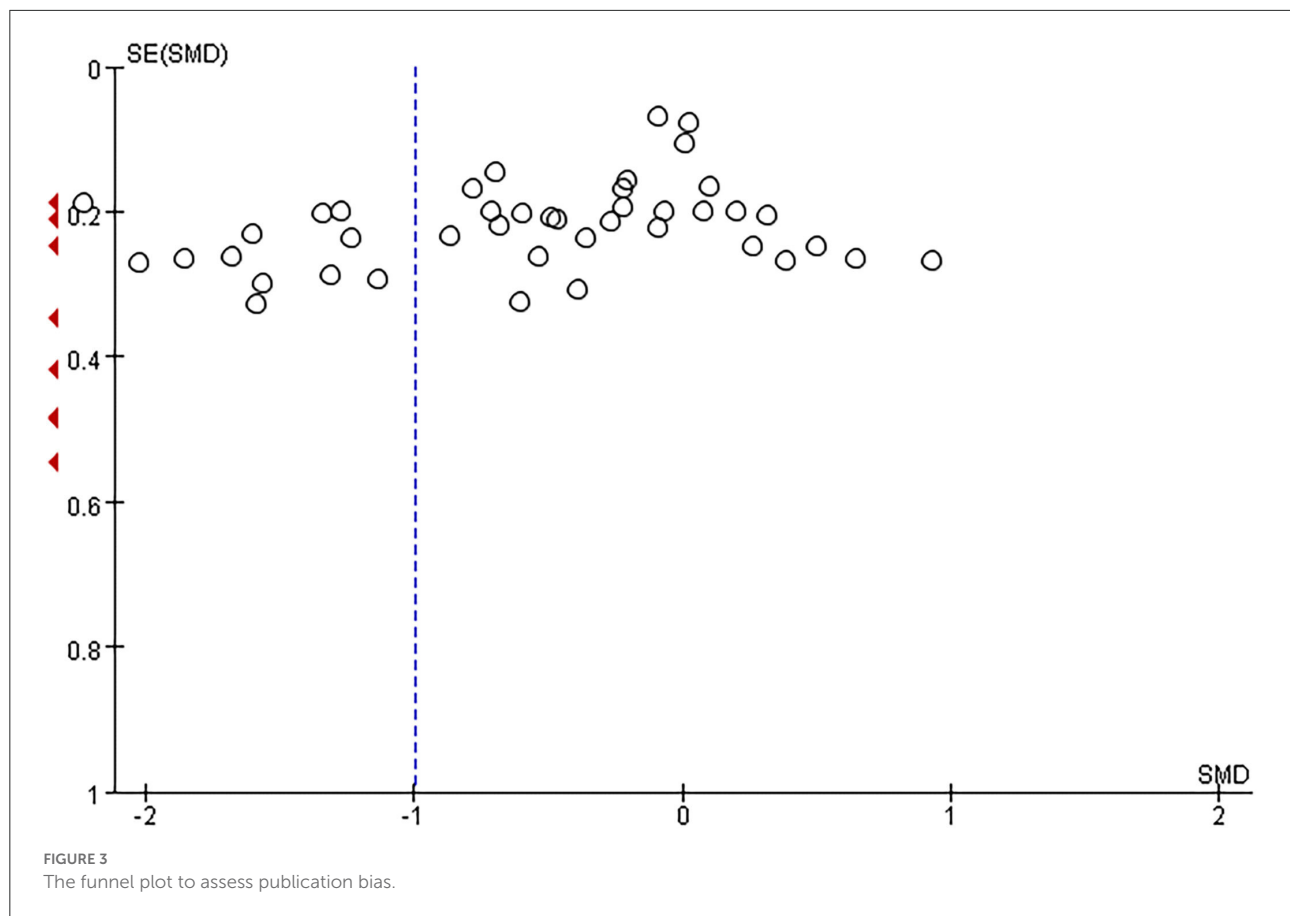
FIGURE 2

The forest plot for correlation between maternal serum Zn levels in preeclamptic women and healthy pregnant women.

Zn deficiency can impair macrophage, neutrophil functions, natural killer (NK) activity and complementary response (74). It also helps maintain skin integrity and delay age-related macular degeneration, and vision loss (75, 76). Zn level mainly depends on dietary intake as no specific Zn storage system has been identified (77). The Recommended Dietary Allowance (RDA) of Zn for pregnant women in the US is at least 11 mg/d. Oysters, red meat like beef, poultry, and beans are zinc-rich diet choices. A higher level of zinc-containing supplements may bring more benefits (4). Zn-containing supplements are additional resources

for Zn intake. The median level of Zn is 15 mg in prenatal supplements in the US markup-to-date evidence suggests zinc sulfate, zinc gluconate or zinc lactate may be beneficial, while zinc acetate should be avoided (4). Despite the maternal serum levels of Zn can be influenced by confounders such as stress and infections, Zn toxicity barely occurs in women with an average daily intake of zinc-containing supplements or food (16). The Tolerable Upper Limit (TUL) is 40 mg daily.

During pregnancy, Zn is essential in embryogenesis and fetal development. Animal studies have shown that Zn deficiency



could lead to abnormal placental morphogenesis, which is one of the presumptive etiology of preeclampsia (78, 79). Despite being minimal in serum, the Zn level is consistently lower in preeclamptic women. This indicates that Zn has a role to play in the pathogenesis of this pregnancy-specific disorder. There are studies suggest Zn as an antioxidant trace element, can relieve the oxidative stress in rats (78, 80). Oxidative stress is believed one of the key pathogenesis in the development of preeclampsia. A more recent study has identified that Zn may also participate in ferroptosis, a newly-discovered iron-dependent form of non-apoptotic cell death (81, 82). Furthermore, as there has been no solid evidence to demonstrate a preventive effect of Zn supplements to reduce the risk of preeclampsia, clinical research can be conducted to explore the possibility that Zn takes part in ferroptosis to mediate the development of preeclampsia (83).

Despite four meta-analysis having reached similar conclusions that maternal serum Zn levels were lower in preeclamptic women, there were reasons why we updated the evidence and added more information (17–20). (1) Three meta-analysis were conducted before 2016, but 20 articles we had included were after that (21–24, 32, 39, 41, 42, 46, 51, 54, 57, 60–65, 68, 72). This indicates there are still unclear or unreasonable phenomena and different conclusions may be drawn with

more evidence accumulated and careful analysis. (2) The other meta-analysis, conducted last year focused on the African population while we have a global perspective. By comparison, we give more information for researchers who also care about the rest of the world. (3) All the four meta-analysis did not include adequate articles even in their claimed scope. We found more than 30 studies reporting a relationship between maternal Zn levels and preeclampsia by 2016 using almost the same search strategy as Zhu et al. (17, 18, 20). We had involved one more African study compared to Tesfa et al. (61) (4) We have the most complete sub-group analysis. Zhu et al. had sub-group analysis in terms of study design and geographical locations, but they only include a total of 13 studies (20). Ma et al. revealed a sub-group analysis in terms of continent (Asia, Europe, Africa), sample type (plasma, serum), fasting status (yes or no), individual age match or gestational age match) (18). These results of sub-group analysis may be extrapolated cautiously as the number in each group was relatively small. He et al. did not involve sub-group analysis (17). In general, we have synthesized the most up-to-date evidence, applied sub-group analysis to identify more information to encourage further research.

However, our studies have several limitations. First, we claim to have a global view, but there was scanty evidence

from Latin America or some non-English speaking European countries. This is due to that we have not searched the non-English databases. However, we have involved all the possible database we can have, including a pure African database and two Chinese databases to complete a global view. The English language accounts for most world's existing research articles, and we did not preclude non-English literature in the major database (i.e., PubMed, WOS, Embase). Therefore, we believe that we are very near to all the related literature available in the world. Second, only six western countries were included in our studies. This is less convincing to draw a global conclusion without obtaining enough evidence from an important part of the world (23, 25, 37, 45, 62). This may also reflect that the micronutrients have not been a focus in developed countries anymore as there is scarce research currently conducted in conventional western countries. Third, the between-study heterogeneity was significant even though we had considered different definitions of preeclampsia, different methods of measurement, participants' fasting status, and various conditions for storage. However, it was also reflected in other similar meta-analysis covering the correlation between trace elements or vitamins and preeclampsia (84–86).

Conclusion

In summary, we have confirmed that maternal serum Zn levels are negatively associated with preeclampsia risk. This correlation is more prominent in Asian countries and low-income economies and is also inversely related to the severity of preeclampsia. Well-designed large cohort or interventional studies in the future may explore why and how maternal serum Zn levels affect the risk of preeclampsia.

Data availability statement

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

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Author contributions

SJ and YZ: conceptualization, methodology, software, investigation, resources, project administration, modification, and writing back to reviewers. SJ and CH: validation, formal, analysis, and data curation. SJ: writing original draft preparation. CH: writing review, editing, and supervision. YZ: visualization and funding acquisition. 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.2022.968045/full#supplementary-material>

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Ferritin, transferrin, and transferrin receptor in relation to metabolic obesity phenotypes: Findings from the China Health and Nutrition Survey

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Background: This study aimed to explore the relationship between iron markers and metabolic obesity phenotypes and the role of age.

Methods: Data were from the China Health and Nutrition Survey 2009. Metabolic obesity phenotypes included metabolically healthy with normal weight (MHNW), metabolically unhealthy with normal weight (MUNW), metabolically healthy with overweight/obesity (MHO), and metabolically unhealthy with overweight/obesity (MUO). Iron markers including ferritin, transferrin, and soluble transferrin receptor were calculated as Log and quartered. The linear regression and multinomial logistic regression were used to explore the association of iron markers with age and metabolic obesity phenotypes, respectively.

Results: Ferritin was linearly related with age, with β (95% confidence interval, CI) of 0.029 (0.027 to 0.032) and -0.005 (-0.007 to -0.002) for women and men. Transferrin was negatively associated with age in both men and women ($\beta < -0.011$). Furthermore, compared with participants in the quartile 1 ferritin group, those in the quartile 4 had increased odds of MUNW, MHO, and MUO, with odds ratio and 95% confidence interval (OR, 95% CI) of 3.06 (2.20 to 4.25), 1.66 (1.35 to 2.05), and 5.27 (4.17 to 6.66). Transferrin showed similar relationships with MUNW, MUO, and MHO; whereas transferrin receptor showed no significance. We also found joint associations of ferritin and transferrin with MUNW, MUO, and MHO. The interactive effect of ferritin and transferrin on MUO was significant ($P = 0.015$).

Conclusion: Increased ferritin and transferrin were associated with MUNW, MHO, and MUO. Age should be considered when investigating iron.

KEYWORDS

iron indicator, age, metabolic heterogeneity, overweight, obesity, sex-stratified

Introduction

Iron is a nutritionally essential trace element that is absorbed mostly through the gastrointestinal tract or released by senescent red blood cells (1), then bound by transferrin and transported to the cell surface, where it attaches to transferrin receptors and enters the cell (2). Iron is stored as ferritin, which is found largely in the liver and red blood cells (3). Iron is crucial for a variety of physiological processes, such as oxygen transport and protein repair (4, 5) and can also fluctuate due to factors such as inflammation levels and disease states (6). Three blood-based biomarkers are widely used to measure iron status: ferritin, transferrin, and transferrin receptor (7–9). These iron markers may change due to age-related physiological dysregulations (10). According to a previous study, serum ferritin grew with age in women but had an inverted U-shaped relationship in men (11). Since anemia and iron status are tightly associated (12), the impact of anemia should be taken into account while performing iron biomarker studies. However, sex-stratified associations of age with ferritin, transferrin, and transferrin receptors after excluding the potential effect of anemia remain unknown.

Obesity is a risk factor for a wide range of disorders, and its incidence has risen globally over the last 50 years (13). Given that the traditional obesity markers, such as the body mass index (BMI), cannot describe body fat distribution, metabolic abnormalities were used to further distinguish metabolic heterogeneity of obesity, with metabolically healthy obesity (MHO) being the healthier phenotype vs. metabolically unhealthy obesity (MUO) (14). Individuals with MHO, despite developing elevated BMI, possessed normal serum lipid, blood glucose, insulin sensitivity, and waist circumference (WC), and are less likely to develop cardiovascular illnesses than those with MUO (15–17). Currently, abnormal iron levels, such as iron overload and deficiency, have been found to be related to metabolic abnormalities like metabolic syndrome and type 2 diabetes mellitus (T2DM) (18, 19). A recent study also found that increased transferrin receptor levels were associated with lower risks of type 2 diabetes mellitus in non-obese subjects but increased risks in obese subjects (20), suggesting the importance of considering metabolic obesity phenotypes when studying markers. However, limited studies have assessed the association of iron markers with metabolic obesity phenotypes. Kim et al. (21) found that serum ferritin was positively associated with metabolically unhealthy but normal weight (MUNW) in the Korean population. Suárez-Ortegón et al. (22) also found that higher ferritin was associated with MUO in prepubertal children. However, the sex-stratified associations of ferritin, transferrin, and transferrin receptor with various metabolic obesity phenotypes in the general Chinese population remain unknown. Since the Chinese have a distinct dietary structure (23), it is of interest to explore the association between iron markers and metabolic obesity phenotypes in the Chinese population.

Therefore, we conducted this study to explore the relationship between age and the three iron markers (i.e., ferritin, transferrin, and transferrin receptor), as well as the associations of these iron markers with different metabolic obesity phenotypes in the general Chinese population after excluding the effect of anemia.

Materials and methods

Study population

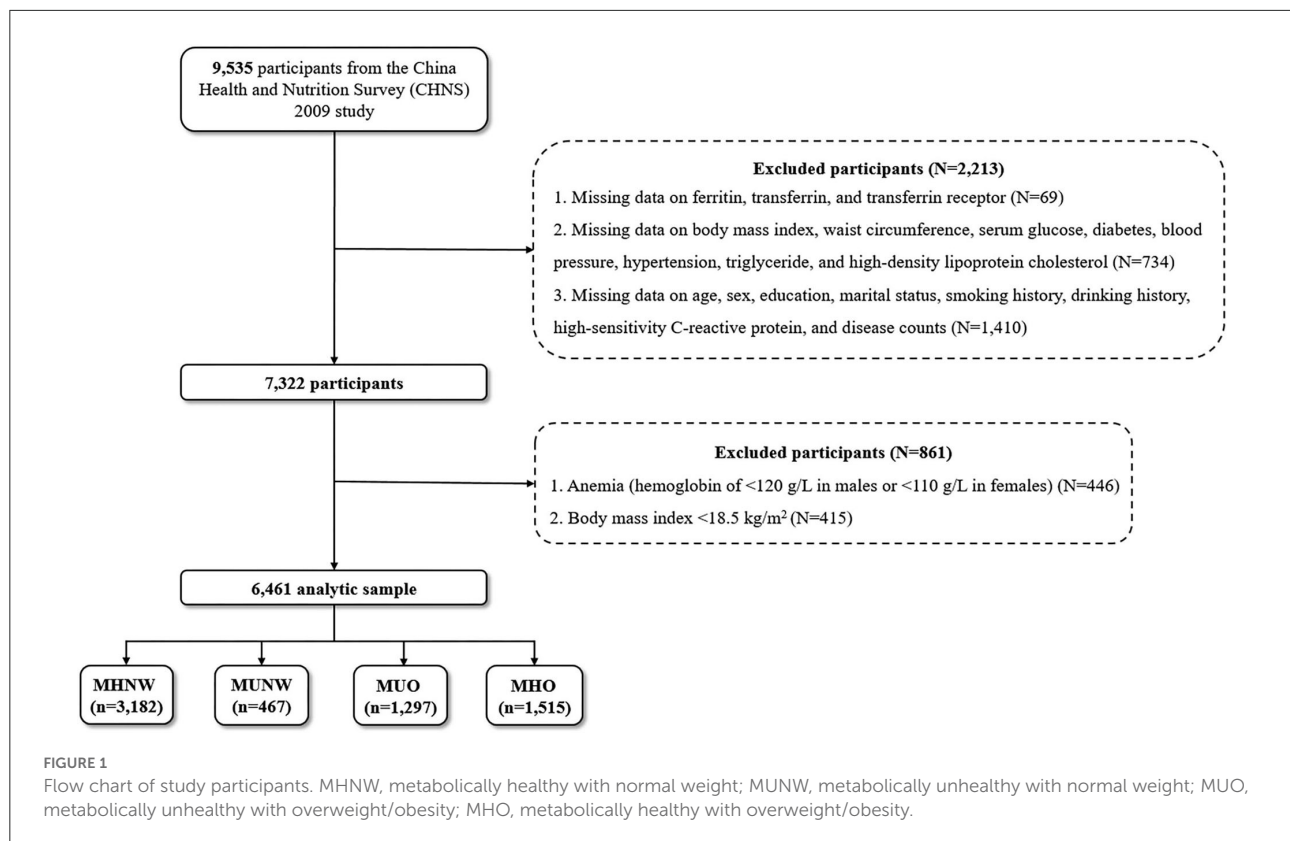
The China Health and Nutrition Survey (CHNS) is an ongoing and prospective household-based cohort study that began in 1989 and investigates a wide range of socio-economic, demographic, nutritional, and health-related information. A multistage random cluster sampling design was applied to select a sample of roughly 7,200 households from 15 provinces across China, with a total population of over 30,000 people. Detailed information on the CHNS was described elsewhere (24, 25). The study was approved by the Institutional Review Boards at the University of North Carolina at Chapel Hill and the National Institute of Nutrition and Food Safety, and all participants completed a written informed consent form.

Data from the CHNS 2009 (with blood-based biomarkers available) were utilized. Participants with missing data on ferritin, transferrin, and transferrin receptor ($N = 69$); with missing data on BMI, WC, serum glucose, diabetes, blood pressure, hypertension, triglyceride (TG), and high-density lipoprotein cholesterol (HDL-C) ($N = 734$); with missing data on age, sex, education, marital status, smoking history, drinking history, high-sensitivity C-reactive protein (hs-CRP), and disease counts ($N = 1410$); and who had anemia or with a BMI of $<18.5 \text{ kg/m}^2$ were excluded, leaving a final analytic sample of 6,461 Chinese participants of all age ranges (Figure 1).

Measurement of blood-based biomarkers

Biomarker levels were determined using an overnight fasting (12 h) blood sample (12 ml). All samples were centrifuged at $3,000 \times g$ at room temperature for 10 min before being separated into 9 aliquots. Other plasma and serum samples were kept in -80°C freezers for laboratory analysis, except those for field testing. The samples were subsequently assessed at the Beijing National Central Laboratory (Medical Laboratory Accreditation Certificate ISO 15189:2007) under stringent quality control (26, 27).

Radioimmunoassay was used to test serum ferritin on a gamma counter XH-6020 made by the North Institute of Bio-Tech in China. Serum transferrin and soluble transferrin receptor were measured using nephelometry on Siemens BNP made by Siemens, Germany. Since serum ferritin,



transferrin, and soluble transferrin receptor did not follow a normal distribution, they were calculated as log (ferritin), log (transferrin), and log (transferrin receptor) and allocated by quartiles.

Fasting blood glucose (FBG) measurements (enzymatic method) and regular blood tests were conducted at local hospitals. TG and HDL-C were measured using the enzymatic method and Glycerol phosphate oxiase-peroxidase anti peroxidase (GPO-PAP) on a Hitachi 7600 (Kyowa, Japan). Serum hs-CRP was measured using immunoturbidimetric on a Hitachi 7600 made by Denka Seiken, Japan.

Assessment and definition of metabolically unhealthy and overweight/obesity

Universally trained interviewers (physicians and nutritionists) conducted anthropometric measurements including weight, height, WC, systolic blood pressure (SBP), and diastolic blood pressure (DBP) in 2009 (28).

Metabolically unhealthy included three or more of the following components (29, 30):

1. Central Obesity: WC of ≥ 90 cm for men, of ≥ 85 cm for women.

2. Diabetes: FBG of ≥ 5.6 Mmol/L and/or Diagnosed as Diabetes or Diabetes Therapy.
3. Hypertension: Blood Pressure of $\geq 130/85$ MmHg and/or Diagnosed as Hypertension or Hypertension Therapy.
4. High TG: TG of ≥ 1.70 Mmol/L.
5. Low HDL-C: HDL-C of <1.00 Mmol/L for men and <1.30 Mmol/L for Women.

We calculated BMI as weight (kg) divided by the square of height (m^2). BMI was defined as obesity ($\geq 28 \text{ kg/m}^2$), overweight ($<28 \text{ kg/m}^2$ and $\geq 24 \text{ kg/m}^2$), and normal weight ($<24 \text{ kg/m}^2$ and $\geq 18.5 \text{ kg/m}^2$), which is widely used in the Chinese population (31, 32).

Metabolically healthy with normal weight was defined as MHNW; metabolically unhealthy with normal weight was defined as MUNW; metabolically unhealthy with overweight/obesity was defined as MUO; and metabolically healthy with overweight/obesity was defined as MHO.

Measurement of covariates

Information on age, sex, education, marital status, residence, smoking history, and drinking history was collected through questionnaires in 2009. Education was categorized into four levels (no schooling, primary school, middle school, and high

school or more). Marital status was dichotomized as currently married or others. The residence was defined by the community in which participants lived, and it was further divided into urban or rural. Smoking history was classified as nonsmokers or smokers (former or current smokers), respectively. Drinking history was also classified as nondrinkers or drinkers (former or current drinkers). Disease counts were the sum of hypertension, diabetes mellitus, myocardial infarction, stroke, hip fracture, asthma, and cancer, which were further classified as 0, 1, 2, or more.

Statistical analysis

The medians and interquartile ranges (IQRs) for non-normally distributed continuous variables, and No. (%) for categorical variables were employed to compare baseline characteristics of included participants by different metabolic obesity phenotypes.

First, to examine group comparisons for each variable, Wilcoxon rank sum tests for continuous variables and Chi-squared test for categorical variables were utilized. We also plotted sex-stratified trends of log (ferritin), log (transferrin), and log (transferrin receptor) with age. Since the values of three iron indicators after Log transform were not normally distributed, we further used the Blom transform to normalize them and generate their ranks. Multivariable linear regression was used to analyze the associations of age with the ranks of three iron markers. Model 1 was sex-adjusted. Model 2 further adjusted for smoking history, drinking history, education, residence, marital status, BMI, hs-CRP, and disease counts. In the sex-stratified analysis, the same models (except sex) were used.

Second, we utilized multinomial logistic regression models to examine the relationship between the three iron markers and metabolic obesity phenotypes with MHNW assigned as the reference for MUNW, MUO, and MHO. The test of the proportional odds assumption in PROC LOGISTIC was significant ($P < 0.05$). Meanwhile, the exposures (i.e., ferritin, transferrin, and transferrin receptor) were quartered, and quartile 1 was assigned as the reference for quartiles 2–4. Odds ratios (ORs) and 95% confidence intervals (95% CIs) were reported. Model 1 was sex-adjusted. Model 2 further adjusted for age based on Model 1. Model 3 further adjusted for smoking history, drinking history, education, residence, marital status, hs-CRP, and disease counts based on Model 2. These models were then used to conduct sex-stratified analysis (except sex). We further defined metabolically healthy more rigorously to assure the robustness of our findings in the sensitivity analysis. Metabolically healthy was defined as possessing none of the above five metabolic abnormalities, metabolically sub-healthy was defined as one or two abnormalities, and metabolically unhealthy was defined as three or more abnormalities. Hence, we redefined metabolically healthy with normal weight as

MHNW, metabolically sub-healthy with normal weight as MSHNW; metabolically unhealthy with normal weight as MUNW, metabolically healthy with overweight/obesity as MHO, metabolically sub-healthy with overweight/obesity as MSHO, and metabolically unhealthy with overweight/obesity as MUO, and explored their associations with iron markers.

Third, since ferritin and transferrin were significantly associated with metabolic obesity phenotypes, we divided the study population into high and low according to 50% of the population based on ferritin or transferrin respectively and combined them into four iron groups: low ferritin and low transferrin, low ferritin and high transferrin, high ferritin and low transferrin, and high ferritin and high transferrin. Multinomial logistic regression was used to determine the relationship between the four joint iron groups and metabolic obesity phenotypes. We used the same three models above (Model 1, sex-adjusted; Model 2, age- and sex-adjusted; Model 3, fully adjusted). Sex-stratified analysis was also conducted. We also explored the interactive effect of ferritin and transferrin on metabolic obesity phenotypes using multinomial logistic regression with Model 3 adjusted.

A two-sided $P < 0.05$ was considered statistically significant. All analyses were conducted using SAS statistical software (version 9.4; SAS Institute Inc., Cary, NC, USA).

Results

The baseline characteristics of included participants were summarized in [Table 1](#). Of the 6,461 individuals included, 3,182 (49.2%) were defined as MHNW, 467 (7.2%) as MUNW, 1,297 (20.1%) as MUO, and 1,515 (23.4%) were classified as MHO. Age, sex, education, marital status, residence, smoking history, drinking history, disease counts, BMI, WC, SBP, DBP, FBG, TG, HDL-C, hs-CRP, log (ferritin), and log (transferrin) all had statistically significant differences across the four groups (all P values < 0.05).

[Supplementary Figure 1](#) shows an S-shaped change in ferritin with age in women (decreasing until approximately 32 years of age and increasing rapidly and then slowly thereafter) but a linear decrease with age in men. Transferrin, on the contrary, decreases linearly with age in both men and women. However, we did not find a significant relationship between the transferrin receptor and age. According to [Supplementary Table 1](#), age was significantly related to the rank of log (ferritin) ($\beta = 0.013$, 95% CI: 0.011 to 0.015 in all participants; $\beta = 0.029$, 95% CI: 0.027 to 0.032 in women; and $\beta = -0.005$, 95% CI: -0.007 to -0.002 in men; all P values < 0.001). Meanwhile, age was negatively associated with the rank of log (transferrin) ($\beta = -0.013$, 95% CI: -0.014 to -0.011 in all participants; $\beta = -0.014$, 95% CI: -0.017 to -0.011 in women; and $\beta = -0.011$, 95% CI: -0.014 to -0.008 in men; all P values < 0.001). The relationship between age and the rank of log (transferrin receptor) was non-significant.

TABLE 1 Basic characteristics of study participants in the CHNS 2009 ($N = 6,461$).

| | MHNW | MUNW | MHO | MUO | P value |
|--|------------------------|-------------------------|-------------------------|-------------------------|---------|
| Participants | 3,182 (49.3) | 467 (7.2) | 1,515 (23.5) | 1,297 (20.1) | |
| Age, year | 48.00 (37.00–59.00) | 60.00 (50.00–68.00)* | 56.00 (45.00–63.00)* | 49.00 (39.00–59.00) | <0.001 |
| Sex | | | | | <0.001 |
| Female | 1,661 (52.2) | 294 (63.0)* | 806 (53.2) | 717 (55.3) | |
| Male | 1,521 (47.8) | 173 (37.0) | 709 (46.8) | 580 (44.7) | |
| Education | | | | | <0.001 |
| No schooling | 781 (24.5) | 168 (36.0)* | 350 (23.1) | 380 (29.3)* | |
| Primary school | 697 (21.9) | 110 (23.6) | 334 (22.1) | 303 (23.4) | |
| Middle school | 1,248 (39.2) | 130 (27.8) | 603 (39.8) | 463 (35.7) | |
| High school or more | 456 (14.3) | 59 (12.6) | 228 (15.1) | 151 (11.6) | |
| Marital status | | | | | <0.001 |
| Currently married | 2,674 (84.0) | 386 (82.7) | 1,352 (89.2)* | 1,119 (86.3) | |
| Others | 508 (16.0) | 81 (17.3) | 163 (10.8) | 178 (13.7) | |
| Residence | | | | | <0.001 |
| Urban | 806 (25.3) | 177 (37.9)* | 420 (27.7) | 439 (33.9)* | |
| Rural | 2376 (74.7) | 290 (62.1) | 1095 (72.3) | 858 (66.2) | |
| Smoking history | | | | | <0.001 |
| Nonsmoker | 2,208 (69.4) | 363 (77.7)* | 1,155 (76.2)* | 964 (74.3)* | |
| Smoker | 974 (30.6) | 104 (22.3) | 360 (23.8) | 333 (25.7) | |
| Drinking history | | | | | 0.001 |
| Nondrinker | 2,118 (66.6) | 362 (77.5)* | 1,005 (66.3) | 878 (67.7) | |
| Drinker | 1,064 (33.4) | 105 (22.5) | 510 (33.7) | 419 (32.3) | |
| Disease counts | | | | | <0.001 |
| 0 | 2,780 (87.4) | 306 (65.5)* | 1,253 (82.7)* | 788 (60.8)* | |
| 1 | 348 (10.9) | 110 (23.6) | 222 (14.7) | 376 (29.0) | |
| 2 or more | 54 (1.7) | 51 (10.9) | 40 (2.6) | 133 (10.3) | |
| Body mass index, kg/m ² | 21.47 (20.30–22.62) | 22.56 (21.41–23.37)* | 25.65 (24.69–27.02)* | 27.12 (25.49–29.00)* | <0.001 |
| Waist circumference, cm | 77.30 (72.80–82.00) | 85.00 (80.00–90.00)* | 87.5 (83.00–93.00)* | 94.00 (89.00–99.00)* | <0.001 |
| Systolic blood pressure, mmHg | 119.33 (110.00–128.67) | 132.00 (120.67–146.67)* | 120.67 (114.00–131.00)* | 133.33 (122.00–148.67)* | <0.001 |
| Diastolic blood pressure, mmHg | 78.67 (70.00–82.00) | 85.00 (79.33–90.33)* | 80.00 (75.33–86.00)* | 87.33 (80.00–92.67)* | <0.001 |
| Fasting blood glucose, mmol/L | 4.94 (4.60–5.34) | 5.83 (5.40–6.72)* | 5.04 (4.69–5.39)* | 5.73 (5.16–6.55)* | <0.001 |
| Triglycerides, mmol/L | 1.04 (0.75–1.46) | 2.25 (1.76–3.13)* | 1.22 (0.88–1.62)* | 2.33 (1.74–3.29)* | <0.001 |
| High density lipoprotein cholesterol, mmol/L | 1.49 (1.28–1.74) | 1.16 (0.99–1.35)* | 1.39 (1.19–1.49)* | 1.15 (0.98–1.36)* | <0.001 |
| High-sensitivity C-reactive protein, mg/L | 1.00 (0–2.00) | 2.00 (1.00–3.00)* | 1.00 (0–1.00)* | 1.00 (1.00–2.00)* | <0.001 |
| Log (ferritin), ng/mL | 4.28 (3.59–4.86) | 4.60 (4.04–5.23)* | 4.36 (3.71–4.99)* | 4.71 (4.14–5.36)* | <0.001 |
| Log (transferrin), mg/dL | 5.61 (5.51–5.74) | 5.66 (5.53–5.78)* | 5.66 (5.55–5.77)* | 5.68 (5.57–5.79)* | <0.001 |
| Log (transferrin receptor), mg/L | 0.28 (0.08–0.48) | 0.31 (0.07–0.49) | 0.29 (0.08–0.48) | 0.30 (0.08–0.51) | 0.496 |

CHNS, China Health and Nutrition Survey; MHNW, metabolically healthy with normal weight; MUNW, metabolically unhealthy with normal weight; MHO, metabolically healthy with overweight/obesity; MUO, metabolically unhealthy with overweight/obesity. Values are presented as median (interquartile range) or No. (%).

P-value was calculated by Chi-square test or Wilcoxon rank sum test.

*Means $P < 0.05$ from MUNW, MUO, and MHO compared to MHNW, respectively.

In the multinomial logistic analysis, when compared to those who were defined as MHNW, we found significant relationships of higher log (ferritin) with MUNW (OR = 2.95, 95% CI: 2.14 to 4.06 for quartile 4; P for trend <0.001), MHO (OR = 1.56, 95% CI: 1.27 to 1.91 for quartile 4; P for trend <0.001), and MUO (OR = 4.93, 95% CI: 3.93 to 6.17 for quartile 4;

P for trend <0.001) after adjusting for sex (not in the sex-stratified analysis) and age. In women, nevertheless, we found no significant relationships between higher log (ferritin) and MHO (OR = 1.11, 95% CI: 0.84 to 1.47 for the quartile 4). In Model 3, we adjusted for additional factors and found that the relationships remained similar. After Model 3 adjustment in

the total population, significant relationships of log(transferrin) with MUNW (OR = 2.54, 95% CI: 1.91 to 3.39 for the quartile 4; P for trend <0.001), MHO (OR = 1.82, 95% CI: 1.52 to 2.18 for the quartile 4; P for trend <0.001), and MUO (OR = 3.31, 95% CI: 2.70 to 4.07 for the quartile 4; P for trend <0.001) were found, which was also found in sex-stratified analyses (all P for trend <0.001). However, in all models, there was no evidence of a significant association between log (transferrin receptor) and metabolic obesity phenotypes (Tables 2, 3, and Supplementary Table 2). The results of the sensitivity analysis were similar to our main findings, but the relationship between ferritin and MHO became non-significant when compared with MHNW (Supplementary Table 3).

We also found significant associations of low ferritin and high transferrin (OR = 1.71, 95% CI: 1.25 to 2.35), high ferritin and low transferrin (OR = 1.80, 95% CI: 1.32 to 2.46), and high ferritin and high transferrin (OR = 4.26, 95% CI: 3.08 to 5.89) with MUNW when compared to low ferritin and low transferrin. Moreover, the relationships of MUO with low ferritin and high transferrin (OR = 1.83, 95% CI: 1.47 to 2.28), high ferritin and low transferrin (OR = 2.06, 95% CI: 1.65 to 2.56), and high ferritin and high transferrin (OR = 5.95, 95% CI: 4.73 to 7.48) were significant. Similar results were also found among the relationships of low ferritin and high transferrin (OR = 1.42, 95% CI: 1.19 to 1.69) and high ferritin and high transferrin (OR = 2.09, 95% CI: 1.71 to 2.56) with MHO when compared to low ferritin and low transferrin (Table 4). The interactive effect of ferritin and transferrin on MUO in the total population was also found (P for interaction = 0.015), as shown in Table 4.

Discussion

In this population-based cross-sectional study, we found a significant association of ferritin and transferrin with MUNW, MHO, and MUO after adjusting for a set of covariates including age, sex, smoking history, drinking history, education, residence, and marital status in both women and men (except sex in sex-stratified analysis). Transferrin receptors, on the contrary, did not exhibit this association. We also found that higher ferritin and transferrin levels were associated with MUNW, MHO, and MUO when compared to low ferritin and low transferrin. The findings suggest an important relationship between iron markers and metabolic obesity.

Our study demonstrated a significant linear relationship between age and serum ferritin, which is consistent with earlier research (11, 33). We also found significant linear relationships between age and serum transferrin in both men and women. People's hematological capacity and liver function deteriorated with age, which might lead to more ferritin release and transferrin inhibition (34). Cellular dystrophy, iron homeostasis dysregulation, and other factors due to aging may also play

an important role in elevated ferritin and decreased transferrin (35, 36).

In this study, higher ferritin or transferrin levels were found to be significantly related to MUNW, MHO, and MUO, which is consistent with previous studies (37, 38). Our findings suggest that iron-related biomarkers like ferritin and transferrin are significantly associated with separate obesity or separate metabolic abnormalities. Moreover, it seems that the strength of the associations of ferritin and transferrin with the coexistence of both metabolic abnormalities and obesity was stronger than that for obesity alone (i.e., the ORs of MHO were lower than those of MUO). Iron can block insulin's inhibitory impact on liver glucose synthesis and diminish insulin extraction in the liver, leading to peripheral hyperinsulinemia (39, 40). Insulin, in turn, can stimulate the redistribution of transferrin receptors to the cell surface, resulting in an increase in intracellular iron through ferritin deposition (18, 41). Thus, a vicious cycle between ferritin and insulin develops. Hyperinsulinemia also increases the risk of insulin resistance, as well as lipid and carbohydrate metabolism disorders (42), all of which are metabolic abnormalities and may lead to abnormal iron metabolism.

In particular, we found no significant association between transferrin receptors and metabolic obesity phenotypes. According to prior research, obesity and metabolic abnormalities are significantly associated with chronic inflammatory states (43). However, transferrin receptors are less affected by inflammation, which may partially explain our non-significant findings. In addition, a recent study found that children on a vegetarian diet had significantly higher serum transferrin receptor concentrations than omnivorous children (44), suggesting that dietary iron intake may influence serum iron marker levels. Given the limitations of our data, we cannot clarify the influence of dietary iron intake on our findings. Therefore, more research is needed to validate our findings.

Major findings in this study are in line with a prior study conducted by Han et al. (11). However, we further excluded participants with anemia and focused more on the health consequences of obesity, i.e., distinguishing the metabolic phenotypes of obesity that were not taken into account in Han's study. We also further estimated the relationship of age with transferrin and transferrin receptor, as well as the correlation of iron markers with metabolic obesity phenotypes. Findings from our study demonstrated that it was appropriate to consider age as a confounder when examining the association between iron markers and health outcomes.

To the best of our knowledge, this is the first comprehensive investigation to explore the associations of ferritin, transferrin, and transferrin receptor with age and various metabolic obesity phenotypes in the general population. We found significant linear relationships of age with ferritin and transferrin, which emphasize the importance of adjusting for age when studying iron markers and may contribute to better clinical criteria for iron overload and iron deficiency. Considering that obesity and

TABLE 2 Odds ratios and 95% CIs of different metabolic obesity phenotypes by ferritin levels in CHNS 2009.

| | MUNW | | | MHO | | | MUO | | |
|----------------------------|----------------------------|----------------------------|----------------------------|----------------------------|----------------------------|----------------------------|----------------------------|----------------------------|----------------------------|
| | Model 1 | Model 2 | Model 3 | Model 1 | Model 2 | Model 3 | Model 1 | Model 2 | Model 3 |
| Overall (N = 6,461) | | | | | | | | | |
| Quartile 1 | Reference | Reference | Reference | Reference | Reference | Reference | Reference | Reference | Reference |
| Quartile 2 | 1.92 (1.41 to 2.61) | 1.26 (0.92 to 1.73) | 1.28 (0.93 to 1.76) | 1.18 (0.99 to 1.40) | 1.16 (0.97 to 1.39) | 1.21 (1.01 to 1.45) | 2.20 (1.78 to 2.71) | 1.78 (1.43 to 2.20) | 1.83 (1.47 to 2.29) |
| Quartile 3 | 2.90 (2.13 to 3.93) | 1.71 (1.25 to 2.34) | 1.73 (1.26 to 2.38) | 1.17 (0.97 to 1.40) | 1.15 (0.95 to 1.39) | 1.22 (1.00 to 1.48) | 2.95 (2.38 to 3.65) | 2.26 (1.82 to 2.82) | 2.34 (1.86 to 2.94) |
| Quartile 4 | 4.94 (3.60 to 6.79) | 2.95 (2.14 to 4.06) | 3.06 (2.20 to 4.25) | 1.57 (1.29 to 1.92) | 1.56 (1.27 to 1.91) | 1.66 (1.35 to 2.05) | 6.33 (5.08 to 7.89) | 4.93 (3.93 to 6.17) | 5.27 (4.17 to 6.66) |
| P for trend | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 |
| Female (N = 3,478) | | | | | | | | | |
| Quartile 1 | Reference | Reference | Reference | Reference | Reference | Reference | Reference | Reference | Reference |
| Quartile 2 | 1.33 (0.87 to 2.03) | 0.86 (0.56 to 1.35) | 0.90 (0.57 to 1.40) | 1.08 (0.86 to 1.35) | 1.02 (0.81 to 1.28) | 1.06 (0.84 to 1.34) | 1.54 (1.15 to 2.05) | 1.21 (0.90 to 1.63) | 1.26 (0.93 to 1.72) |
| Quartile 3 | 2.96 (2.00 to 4.36) | 1.35 (0.89 to 2.05) | 1.32 (0.86 to 2.02) | 1.29 (1.02 to 1.63) | 1.14 (0.89 to 1.46) | 1.15 (0.89 to 1.48) | 2.87 (2.18 to 3.79) | 1.80 (1.34 to 2.42) | 1.74 (1.28 to 2.37) |
| Quartile 4 | 4.45 (3.03 to 6.52) | 1.56 (1.02 to 2.39) | 1.49 (0.96 to 2.30) | 1.33 (1.04 to 1.70) | 1.11 (0.84 to 1.47) | 1.10 (0.83 to 1.46) | 5.03 (3.83 to 6.59) | 2.66 (1.96 to 3.60) | 2.48 (1.81 to 3.41) |
| P for trend | <0.001 | 0.008 | 0.021 | 0.009 | 0.365 | 0.418 | <0.001 | <0.001 | <0.001 |
| Male (N = 2,983) | | | | | | | | | |
| Quartile 1 | Reference | Reference | Reference | Reference | Reference | Reference | Reference | Reference | Reference |
| Quartile 2 | 1.26 (0.76 to 2.07) | 1.37 (0.83 to 2.28) | 1.42 (0.85 to 2.36) | 1.13 (0.88 to 1.45) | 1.12 (0.88 to 1.43) | 1.12 (0.87 to 1.44) | 1.65 (1.21 to 2.25) | 1.71 (1.25 to 2.34) | 1.82 (1.32 to 2.52) |
| Quartile 3 | 1.66 (1.03 to 2.69) | 1.81 (1.12 to 2.95) | 2.00 (1.22 to 3.27) | 1.18 (0.92 to 1.52) | 1.17 (0.91 to 1.51) | 1.21 (0.94 to 1.56) | 2.14 (1.58 to 2.90) | 2.23 (1.64 to 3.02) | 2.48 (1.81 to 3.41) |
| Quartile 4 | 3.48 (2.21 to 5.48) | 4.07 (2.57 to 6.46) | 4.23 (2.64 to 6.78) | 1.69 (1.30 to 2.18) | 1.66 (1.29 to 2.15) | 1.69 (1.30 to 2.20) | 4.87 (3.63 to 6.54) | 5.19 (3.86 to 6.98) | 5.65 (4.14 to 7.71) |
| P for trend | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 |

CI, confidence interval; MHNW, metabolically healthy with normal weight; MUNW, metabolically unhealthy with normal weight; MHO, metabolically healthy with overweight/obesity; MUO, metabolically unhealthy with overweight/obesity.

The reference of multinomial logistic models was MHNW. Model 1 adjusted for sex. Model 2 further adjusted for age based on Model 1. Model 3 further adjusted for smoking history, drinking history, education, residence, marital status, high-sensitivity C-reactive protein, and disease counts based on Model 2.

The bold values indicate the statistically significant.

TABLE 3 Odds ratios and 95% CIs of different metabolic obesity phenotypes by transferrin levels in CHNS 2009.

| | MUNW | | | MHO | | | MUO | | |
|----------------------------|----------------------------|----------------------------|----------------------------|----------------------------|----------------------------|----------------------------|----------------------------|----------------------------|----------------------------|
| | Model 1 | Model 2 | Model 3 | Model 1 | Model 2 | Model 3 | Model 1 | Model 2 | Model 3 |
| Overall (N = 6,461) | | | | | | | | | |
| Quartile 1 | Reference | Reference | Reference | Reference | Reference | Reference | Reference | Reference | Reference |
| Quartile 2 | 1.07 (0.81 to 1.42) | 1.29 (0.97 to 1.71) | 1.37 (1.03 to 1.83) | 1.21 (1.01 to 1.44) | 1.23 (1.03 to 1.47) | 1.25 (1.05 to 1.49) | 1.51 (1.24 to 1.83) | 1.69 (1.39 to 2.06) | 1.78 (1.45 to 2.18) |
| Quartile 3 | 1.25 (0.95 to 1.65) | 1.73 (1.30 to 2.30) | 1.86 (1.39 to 2.49) | 1.49 (1.26 to 1.78) | 1.55 (1.30 to 1.84) | 1.58 (1.32 to 1.88) | 1.94 (1.60 to 2.35) | 2.39 (1.96 to 2.91) | 2.55 (2.08 to 3.12) |
| Quartile 4 | 1.52 (1.16 to 1.99) | 2.37 (1.78 to 3.15) | 2.54 (1.91 to 3.39) | 1.72 (1.45 to 2.05) | 1.81 (1.51 to 2.16) | 1.82 (1.52 to 2.18) | 2.38 (1.97 to 2.88) | 3.16 (2.59 to 3.84) | 3.31 (2.70 to 4.07) |
| P for trend | 0.001 | <0.001 | <0.001 | 0.001 | <0.001 | <0.001 | 0.001 | <0.001 | <0.001 |
| Female (N = 3,478) | | | | | | | | | |
| Quartile 1 | Reference | Reference | Reference | Reference | Reference | Reference | Reference | Reference | Reference |
| Quartile 2 | 1.13 (0.79 to 1.61) | 1.53 (1.06 to 2.23) | 1.52 (1.04 to 2.22) | 1.17 (0.92 to 1.49) | 1.24 (0.97 to 1.58) | 1.23 (0.96 to 1.58) | 1.29 (1.00 to 1.67) | 1.62 (1.24 to 2.12) | 1.63 (1.23 to 2.16) |
| Quartile 3 | 1.31 (0.92 to 1.86) | 2.12 (1.47 to 3.06) | 2.18 (1.50 to 3.16) | 1.28 (1.01 to 1.63) | 1.41 (1.11 to 1.80) | 1.41 (1.10 to 1.81) | 1.52 (1.18 to 1.95) | 2.17 (1.66 to 2.83) | 2.27 (1.71 to 3.00) |
| Quartile 4 | 1.36 (0.96 to 1.94) | 2.71 (1.86 to 3.96) | 2.75 (1.87 to 4.03) | 1.60 (1.27 to 2.03) | 1.84 (1.44 to 2.35) | 1.80 (1.41 to 2.31) | 1.64 (1.28 to 2.11) | 2.75 (2.10 to 3.61) | 2.84 (2.13 to 3.77) |
| P for trend | 0.056 | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 |
| Male (N = 2,983) | | | | | | | | | |
| Quartile 1 | Reference | Reference | Reference | Reference | Reference | Reference | Reference | Reference | Reference |
| Quartile 2 | 1.01 (0.65 to 1.58) | 1.15 (0.73 to 1.80) | 1.27 (0.80 to 2.02) | 1.18 (0.92 to 1.53) | 1.17 (0.91 to 1.52) | 1.18 (0.91 to 1.53) | 1.74 (1.28 to 2.37) | 1.85 (1.35 to 2.52) | 1.91 (1.38 to 2.64) |
| Quartile 3 | 1.10 (0.70 to 1.73) | 1.34 (0.85 to 2.13) | 1.55 (0.97 to 2.49) | 1.51 (1.18 to 1.95) | 1.49 (1.16 to 1.93) | 1.55 (1.19 to 2.02) | 2.35 (1.73 to 3.18) | 2.59 (1.90 to 3.52) | 2.87 (2.08 to 3.96) |
| Quartile 4 | 1.61 (1.04 to 2.48) | 1.99 (1.28 to 3.09) | 2.31 (1.46 to 3.63) | 1.79 (1.38 to 2.31) | 1.76 (1.36 to 2.28) | 1.86 (1.42 to 2.42) | 3.85 (2.87 to 5.17) | 4.27 (3.16 to 5.75) | 4.70 (3.43 to 6.44) |
| P for trend | 0.033 | 0.002 | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 |

CI, confidence interval; MHNW, metabolically healthy with normal weight; MUNW, metabolically unhealthy with normal weight; MHO, metabolically healthy with overweight/obesity; MUO, metabolically unhealthy with overweight/obesity.

P-value for trend across categories, based on category midpoint. The reference of multinomial logistic models was MHNW. Model 1 adjusted for sex. Model 2 further adjusted for age based on Model 1. Model 3 further adjusted for smoking history, drinking history, education, residence, marital status, high-sensitivity C-reactive protein, and disease counts based on Model 2.

The bold values indicate the statistically significant.

TABLE 4 Odds ratios and 95% CIs of different metabolic obesity phenotypes by four iron groups in CHNS 2009.

| | MUNW | MHO | MUO |
|------------------------------------|---------------------|---------------------|----------------------|
| | OR (95% CI) | | |
| Overall | | | |
| Low ferritin and low transferrin | Reference | Reference | Reference |
| Low ferritin and high transferrin | 1.71 (1.25 to 2.35) | 1.42 (1.19 to 1.69) | 1.83 (1.47 to 2.28) |
| High ferritin and low transferrin | 1.80 (1.32 to 2.46) | 1.20 (0.99 to 1.44) | 2.06 (1.65 to 2.56) |
| High ferritin and high transferrin | 4.26 (3.08 to 5.89) | 2.09 (1.71 to 2.56) | 5.95 (4.73 to 7.48) |
| P for interaction | 0.759 | 0.953 | 0.015 |
| Females | | | |
| Low ferritin and low transferrin | Reference | Reference | Reference |
| Low ferritin and high transferrin | 1.77 (1.23 to 2.53) | 1.47 (1.19 to 1.81) | 1.89 (1.46 to 2.45) |
| High ferritin and low transferrin | 1.22 (0.82 to 1.82) | 1.06 (0.80 to 1.39) | 1.51 (1.12 to 2.04) |
| High ferritin and high transferrin | 3.12 (2.06 to 4.73) | 1.51 (1.09 to 2.09) | 3.85 (2.79 to 5.31) |
| P for interaction | 0.550 | 0.200 | 0.576 |
| Males | | | |
| Low ferritin and low transferrin | Reference | Reference | Reference |
| Low ferritin and high transferrin | 1.91 (0.94 to 3.90) | 1.41 (1.01 to 1.96) | 2.13 (1.34 to 3.38) |
| High ferritin and low transferrin | 2.69 (1.51 to 4.82) | 1.18 (0.89 to 1.55) | 2.73 (1.85 to 4.01) |
| High ferritin and high transferrin | 5.11 (2.78 to 9.41) | 2.14 (1.60 to 2.86) | 7.90 (5.32 to 11.73) |
| P for interaction | 0.282 | 0.568 | 0.136 |

CI, confidence interval; MHNW, metabolically healthy with normal weight; MUNW, metabolically unhealthy with normal weight; MHO, metabolically healthy with overweight/obesity; MUO, metabolically unhealthy with overweight/obesity.

The reference of multinomial logistic models was MHNW. Odds ratios were adjusted for age, sex, smoking history, drinking history, education, residence, marital status, high-sensitivity C-reactive protein, and disease counts (sex was not included in sex stratified analysis).

The bold values indicate the statistically significant.

metabolic abnormalities often coexist, our findings demonstrate that the strength of association of ferritin and transferrin with different metabolic obesity phenotypes varies, which emphasizes the importance of taking full account of the metabolic status of obese subjects when treating their iron dysfunction. Moreover, since the definition of metabolically healthy is controversial (45), we defined metabolically healthy more rigorously (none of the metabolic abnormalities) to assure the robustness of our findings in the sensitivity analysis and found the association between ferritin and MHO became non-significant when compared to MHNW, which may help promote a better understanding of metabolically healthy.

On the contrary, our study remains limited by several shortcomings. First, since this study is cross-sectional in design, the causal relationships between iron markers and metabolic obesity phenotypes remain unclear. Moreover, though the CHNS has stringent technical procedures, our findings may be influenced by the birth cohort effect since our data were from the 2009 wave. Though we have adjusted for several covariates, there were still some potential confounders not measured in this study, such as dietary patterns, medications, and other inflammatory factors like interleukin-6. Finally, other iron indicators like transferrin

saturation were not included, which should be explored in future studies.

Conclusion

We found that there were significant associations of ferritin and transferrin with age and various metabolic obesity phenotypes. Our findings suggest the need to control for age when studying iron and provide population evidence for the clinical treatment of subjects with iron dysfunction of different ages. Moreover, this study emphasizes the importance of focusing on iron dysfunction and unhealthy metabolic status in obese subjects.

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 Institutional Review Board at the University of North Carolina at Chapel Hill, the China-Japan Friendship Hospital, Ministry of Health, and the National Institute for Nutrition and Health, Chinese Center for Disease Control and Prevention granted approval for the China Health and Nutrition Survey. The patients/participants provided their written informed consent to participate in this study.

Author contributions

ZL designed the study. ZR, XC, and CL managed and analyzed the data. ZR prepared the first draft. XC, CL, JZ, and XL reviewed and edited the manuscript, with comments from PS, YZ, and ZL. All authors were involved in revising the paper, had full access to the data, and gave final approval of the submitted versions.

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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.2022.922863/full#supplementary-material>

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Dietary copper intake and the prevalence of kidney stones among adult in the United States: A propensity score matching study

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Background: Trace metals are essential trace elements for the human body, but insufficient or excessive levels of metal ions can lead to the development of disease. The purpose of this study was to examine the relationship between dietary intake of copper and the prevalence of kidney stones in U.S. adult population.

Methods: We included data on dietary intake of trace metals from 28,623 adult participants in the National Health and Nutrition Examination Survey (NHANES) database between 2007 and 2018. Multivariate logistic regression and restricted cubic spline (RCS) dose-response curves were used to explore the association between trace metals and kidney stones, and 1:1 propensity score matching (PSM) was performed between the stone formers and non-stone formers to test the validity of the results.

Results: Dose-response curves showed a non-linear negative association between dietary copper intake and kidney stones, and an increase in copper intake reduced the risk of kidney stones. Multivariate logistic regression analysis showed that the odds ratio (95% confidence interval) for kidney stones in each quartile of copper intake compared to the lowest quartile were 0.905 (0.808–1.011, $p = 0.075$), 0.880 (0.785–0.987, $p = 0.028$) and 0.853 (0.756–0.959, $p = 0.009$). In addition, similar conclusions were reached after analysis of PSM in the stone formers and non-stone formers groups.

Conclusion: Dietary copper intake was negatively and non-linearly correlated with kidney stones, which is worthy of further research and application in clinical practice.

KEYWORDS

kidney stones, dietary copper intake, dose-response curves, propensity score matching, NHANES database

Introduction

Kidney stones are a common urological condition caused by the deposition of mineral crystals in the urine in the kidneys (1, 2). Severe kidney stones can cause symptoms such as severe back pain, nausea, hematuria, and difficulty in urination. Bilateral urinary tract obstruction can lead to uremia, and the atrophy of kidney tissue caused by stones severely affects kidney function and may lead to loss of function of the entire kidney (3, 4). Kidney stones are a complex disease caused by environmental, dietary and genetic factors, with an incidence of over 6–12% and a 5-year recurrence rate of up to 50%, seriously affecting human health (5).

Trace metals are indispensable trace elements for living organisms, but insufficient or excessive levels of metal ions can lead to cell death, which in turn cause abnormalities or diseases in human physiology (6, 7). Some studies have found a relationship between trace metals and the occurrence and development of urinary stones (8–10). However, the role of trace metals in the pathogenesis of kidney stones is unclear. Some studies have reported that dietary zinc intake may be associated with an increased risk of kidney stones; whereas manganese intake may be associated with a reduced risk of kidney stones (11, 12). Copper, a trace metal, is an important cofactor in living organisms, and its redox properties make it both beneficial and toxic to cells (13, 14). As an essential trace element, copper is vital for the growth and development of the brain, bones, and other organs (15). Copper can only be consumed from dietary sources, and nut foods, animal offal, vegetables, and legumes contain high amounts of copper (16). Recent studies have found that intracellular copper accumulation can lead to a novel type of programmed cell death (PCD) called cuproptosis (17). However, the relationship between dietary copper intake and the risk of kidney stones is unclear.

In the current study, we used a large US population survey database, the National Health and Nutrition Examination Survey (NHANES) 2007–2018, to explore the dose-response relationship between copper intake and kidney stone risk, and validated it by propensity score matching (PSM) analysis.

Materials and methods

Data sources and preparation

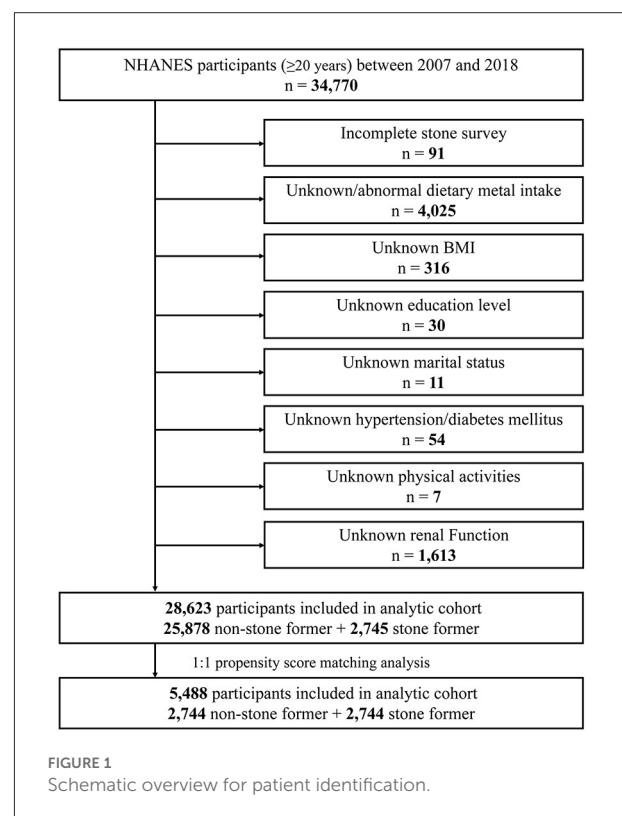
The population for this study was drawn from the NHANES database, a cross-sectional survey designed to assess the health and nutritional status of non-institutionalized civilians in all 50 states and the District of Columbia in the United States (18). Approximately 5,000 individuals were sampled each year through a multilevel stratified probability design, and all participants receive a structured questionnaire at home and a physical examination at a mobile testing center. Data from this

representative survey have been published online on a 2-year cycle since 1999. The data files published online are available for public use and can be found on the official website: <https://www.cdc.gov/nchs/nhanes/index.htm>.

In this study, we used publicly available data from NHANES for six cycles: 2007–2008, 2009–2010, 2011–2011, 2013–2014, 2015–2016, and 2017–2018. A total of 34,770 adults (≥ 20 years) participated in the survey during this 12-year period. We developed the following exclusion criteria (Figure 1): (a) incomplete stone survey ($n = 91$); (b) unknown/abnormal dietary trace metal intake ($n = 4,025$); (c) unknown body mass index (BMI) ($n = 316$); (d) unknown education level ($n = 30$); (e) unknown marital status ($n = 11$); (f) unknown hypertension/diabetes mellitus ($n = 54$); (g) unknown physical activities status ($n = 7$); (h) unknown renal function indicators (creatinine, urea nitrogen, and uric acid) ($n = 1,613$). A total of 6,147 individuals were excluded based on the above criteria, and a total of 28,623 study subjects were finally included in this study.

Study variables

In NHANES, dietary protein, moisture and trace metals (phosphorus, magnesium, iron, zinc, copper, selenium) intake was assessed by the 24 h dietary review method, in which participants were asked by a professional technician about the



types and amounts of foods and beverages consumed during 24 h and recorded in the NHANES computer-assisted dietary survey system. The intake of each food component was then estimated based on the University of Texas Food Intake Analysis System and the USDA Survey Nutrient Database.

In addition, we included other variables such as gender, age, race, marital status, family income, education level, BMI, hypertension, diabetes mellitus, smoking status, drinking, physical activities status (vigorous and moderate activities), blood urea nitrogen, creatinine, uric acid, and estimated glomerular filtration rate (eGFR). BMI was calculated as $\text{weight (kg)}/[\text{height (m)}^2 \times \text{height (m)}^2]$. Hypertension and diabetes mellitus were diagnosed by a physician or other health professional. The eGFR was calculated were followed as described previously (3, 19).

Kidney stones assessment

In the questionnaire, the participant was asked by a trained professional: “Have you ever had kidney stones”, and the participant was considered to have a history of kidney stones if he/she answered “Yes”.

Statistical analysis

All data and figures in this study were organized and analyzed using R software (version 3.5.3) and SPSS software (version 24.0). Sampling weights for interviews (WTSA2YR) and study design variables (SDMVPSU and SDMVSTRA) were used in the analysis of the data, and the weighting analysis was performed to consider the complex sampling design and to obtain the appropriate weights. Normally distributed continuous data were described by mean \pm standard deviation (SD), and categorical data were described by frequency (*n*) and percentage (%). Trace metals were quadratically divided and multivariate logistic regression models were used to assess the association between different dietary trace metal intakes and kidney stones, with results presented as adjusted odds ratios (aORs) and 95% confidence intervals (CIs) intervals. For multivariate logistic regression analysis, we used two models to assess the effect of trace metals on kidney stones: the single-metal model: adjusted for individual trace metals and all other covariates; the multi-metal model: adjusted for all trace metals and all other covariates. Restricted cubic spline (RCS) functions were used to visually describe the dose-response relationship between dietary trace metal intake and kidney stones.

A 1:1 propensity score matching (PSM) analysis was used to balance the differences between the stone forming and non-stone formers, adjusted for confounding variables including: gender, age, race, marital status, family income, education level, BMI, hypertension, diabetes mellitus, smoking

status, drinking, vigorous recreational activities and moderate recreational activities. The data after PSM were also reanalyzed to further test the correctness of the results. *P*-values were considered statistically significant when calculated at <0.05 .

Results

A total of 28,623 participants were included in the study between 2007 and 2018, of which 2,745 (9.6%) were stone formers and 25,878 (90.4%) were non-stone formers. The clinicopathological characteristics of all participants are shown in Table 1. The chi-square test showed significant differences between stone formers and non-stone formers on the variables of gender, age, race, marital status, BMI, hypertension, diabetes mellitus, and physical activities variables (all $P < 0.001$). In addition, stone formers had higher levels of blood urea nitrogen, creatinine, and uric acid and lower eGFR compared to non-stone formers. Moreover, the dietary intakes of phosphorus, magnesium, copper and selenium were lower in stone formers compared to non-stone formers (all $P < 0.05$). Spearman's rank correlation coefficient analysis showed a positive correlation between dietary intakes of the six trace metals (Supplementary Figure S1).

Dose-response curve analysis of the RCS was used to assess the relationship between dietary phosphorus, magnesium, copper, selenium intakes and kidney stones. The results showed that dietary phosphorus ($p = 0.038$), magnesium ($p < 0.001$), and copper ($p = 0.002$) intakes were negatively and non-linearly associated with kidney stone risk in all populations, and the risk of kidney stones decreased progressively with increasing phosphorus, magnesium, or copper intakes (Figure 2).

To better assess the relationship between dietary phosphorus, magnesium, copper, selenium intake and kidney stones, we quadrupled-classified phosphorus, magnesium, copper, and selenium. After adjusting for all confounding variables, multivariate single-metal model logistic regression analysis revealed that dietary phosphorus, magnesium or copper intake was an independent risk factor for kidney stones. Compared with the lowest quartile, the ORs of kidney stones for each quartile of phosphorus intake were 0.977 (0.868–1.086, $p = 0.605$), 0.899 (0.801–1.010, $p = 0.072$) and 0.821 (0.727–0.929, $p = 0.002$), respectively; 0.874 (0.782–0.977, $p = 0.018$), 0.819 (0.730–0.919, $p = 0.001$), and 0.770 (0.683–0.868, $p < 0.001$) for each quartile of magnesium intake, respectively; 0.905 (0.808–1.011, $p = 0.075$), 0.880 (0.785–0.987, $p = 0.028$), and 0.853 (0.756–0.959, $p = 0.009$) for each quartile of copper intake, respectively (Table 2). However, in multivariate multi-metal logistic regression, only magnesium intake was associated with the risk of kidney stones (Supplementary Table S1).

Dietary copper enters the body and is absorbed by the digestive system, synthesized by the liver into copper cyanobrotein and released into the blood, which is mainly

TABLE 1 Baseline characteristics of NHANES participants between 2007 and 2018.

| Characteristic | Total | None-stone formers | Stone formers | P-value |
|---|---------------|--------------------|---------------|---------|
| | No. (%) | No. (%) | No. (%) | |
| Total patients | 28,623 | 25,878 (90.4) | 2,745 (9.6) | |
| Gender | | | | <0.001 |
| Male | 13,924 (48.6) | 12,390 (47.9) | 1,534 (55.9) | |
| Female | 14,699 (51.4) | 13,488 (52.1) | 1,211 (44.1) | |
| Age | | | | <0.001 |
| <50 years | 14,402 (50.3) | 13,451 (52.0) | 951 (34.6) | |
| ≥50 years | 14,221 (49.7) | 12,427 (48.0) | 1,794 (65.4) | |
| Race | | | | <0.001 |
| Non-Hispanic white | 12,098 (42.3) | 10,580 (40.9) | 1,518 (55.3) | |
| Non-Hispanic black | 5,891 (20.6) | 5,539 (21.4) | 352 (12.8) | |
| Mexican American | 4,381 (15.3) | 4,027 (15.6) | 354 (12.9) | |
| Other Hispanic | 3,005 (10.5) | 2,701 (10.4) | 304 (11.1) | |
| Other | 3,248 (11.3) | 3,031 (11.7) | 217 (7.9) | |
| Education level | | | | 0.550 |
| Less than high school | 6,859 (24.0) | 6,178 (23.9) | 681 (24.8) | |
| High school or equivalent | 6,541 (22.9) | 5,922 (22.9) | 619 (22.6) | |
| College or above | 15,223 (53.2) | 13,778 (53.2) | 1,445 (52.6) | |
| Marital status | | | | <0.001 |
| Married | 14,740 (51.5) | 13,166 (50.9) | 1,574 (57.3) | |
| Unmarried | 13,883 (48.5) | 12,712 (49.1) | 1,171 (42.7) | |
| Family income | | | | 0.198 |
| <\$20,000 | 6,572 (23.0) | 5,922 (22.9) | 650 (23.7) | |
| ≥\$20,000 | 20,647 (72.1) | 18,669 (72.1) | 1,978 (72.1) | |
| Unknown | 1,404 (4.9) | 1,287 (5.0) | 117 (4.3) | |
| BMI (kg/m²) | | | | <0.001 |
| <25.0 | 8,048 (28.1) | 7,519 (29.1) | 529 (19.3) | |
| ≥25.0 | 20,575 (71.9) | 18,359 (70.9) | 2,216 (80.7) | |
| Hypertension | | | | <0.001 |
| Yes | 10,336 (36.1) | 8,943 (34.6) | 1,393 (50.7) | |
| No | 18,287 (63.9) | 16,935 (65.4) | 1,352 (49.3) | |
| Diabetes mellitus | | | | <0.001 |
| Yes | 3,716 (13.0) | 3,099 (12.0) | 617 (22.5) | |
| No | 24,236 (84.7) | 22,202 (85.8) | 2,034 (74.1) | |
| Borderline | 671 (2.3) | 577 (2.2) | 94 (3.4) | |
| Smoking status | | | | <0.001 |
| Never | 15,951 (55.7) | 14,608 (56.4) | 1,343 (48.9) | |
| Former | 6,900 (24.1) | 6,045 (23.4) | 855 (31.1) | |
| Current | 5,772 (20.2) | 5,225 (20.2) | 547 (19.9) | |
| Drinking | | | | 0.216 |
| Yes | 20,334 (71.0) | 18,356 (70.9) | 1,978 (72.1) | |
| No/Unknown | 8,299 (29.0) | 7,522 (29.1) | 767 (27.9) | |
| Vigorous recreational activities | | | | <0.001 |
| Yes | 6,306 (22.0) | 5,890 (22.8) | 416 (15.2) | |
| No | 22,317 (78.0) | 19,988 (77.2) | 2,329 (84.8) | |
| Moderate recreational activities | | | | <0.001 |
| Yes | 11,574 (40.4) | 10,590 (40.9) | 984 (35.8) | |

(Continued)

TABLE 1 (Continued)

| Characteristic | Total | None-stone formers | Stone formers | P-value |
|--------------------------------------|----------------|--------------------|----------------|---------|
| | No. (%) | No. (%) | No. (%) | |
| No | 17,049 (59.6) | 15,288 (59.1) | 1,761 (64.2) | |
| Blood urea nitrogen (mg/dL) | 13.76, 5.99 | 13.60, 5.81 | 15.29, 7.27 | <0.001 |
| Creatinine (mg/dL) | 0.90, 0.45 | 0.89, 0.41 | 0.98, 0.72 | <0.001 |
| Uric acid (mg/dL) | 5.45, 1.45 | 5.43, 1.44 | 5.65, 1.52 | <0.001 |
| eGFR [mL/(min·1.73 m ²)] | 83.71, 25.80 | 84.63, 25.65 | 75.04, 25.61 | <0.001 |
| Daily intake (M, SD) | | | | |
| Protein (mg) | 80.75, 42.16 | 81.01, 42.33 | 78.38, 40.49 | 0.002 |
| Moisture (g) | 2.88, 1.50 | 2.89, 1.50 | 2.83, 1.47 | 0.062 |
| Phosphorus (mg) | 1,340.9, 667.7 | 1,344.4, 670.2 | 1,307.5, 642.2 | 0.006 |
| Magnesium (mg) | 294.63, 146.58 | 295.88, 147.25 | 282.78, 139.54 | <0.001 |
| Iron (mg) | 14.42, 8.33 | 14.42, 8.33 | 14.41, 8.33 | 0.966 |
| Zinc (mg) | 11.04, 6.71 | 11.06, 6.74 | 10.87, 6.43 | 0.157 |
| Copper (mg) | 1.23, 0.74 | 1.24, 0.74 | 1.20, 0.69 | 0.007 |
| Selenium (mcg) | 112.36, 63.40 | 112.61, 63.50 | 110.03, 62.45 | 0.043 |

For categorical variables, *P*-values were analyzed by chi-square tests. For continuous variables, the *t*-test for slope was used in generalized linear models. NHANES, National Health and Nutrition Examination Survey; BMI, body mass index; eGFR, estimated glomerular filtration rate; SD, standard deviation.

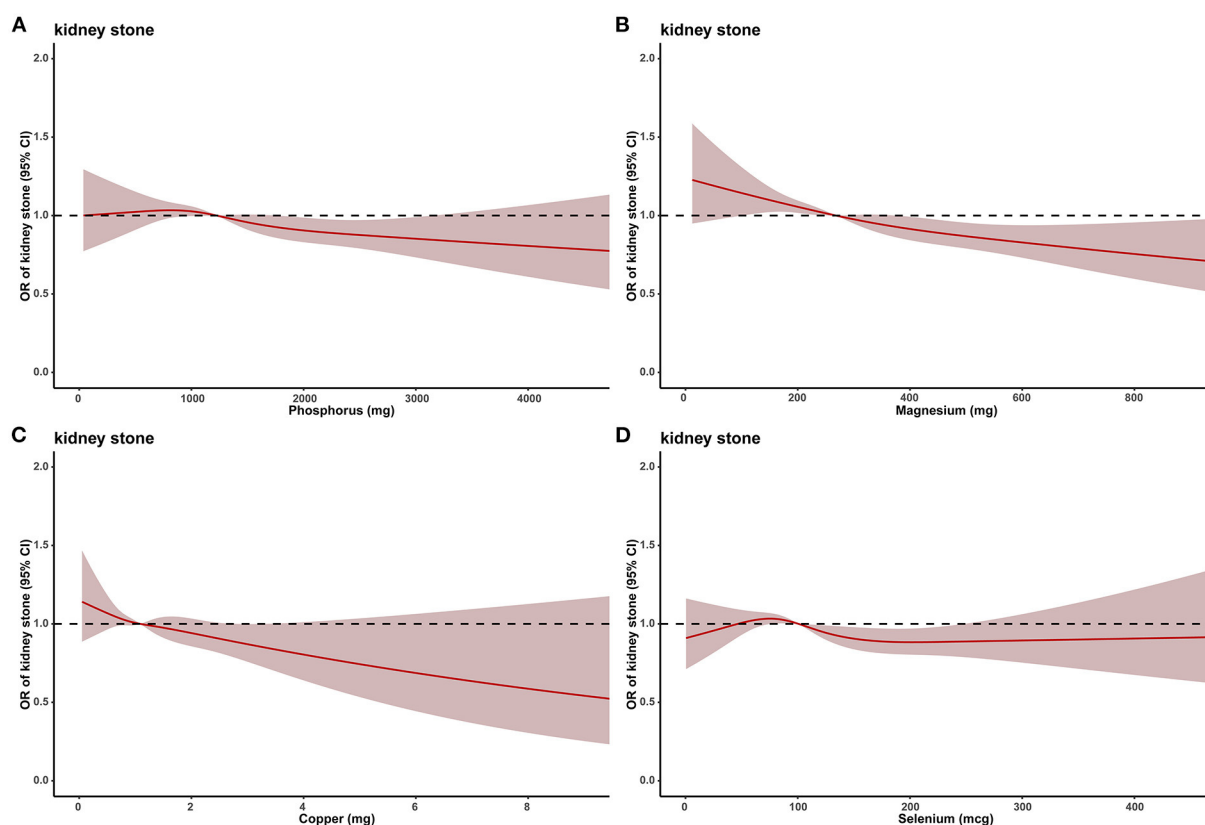


FIGURE 2

The dose-response analysis between dietary metal intake and presence of kidney stones before propensity score matching. (A) Phosphorus. (B) Magnesium. (C) Copper. (D) Selenium.

TABLE 2 Multivariate single-metal model logistic regression the relationship between the heavy metals and the presence of kidney stone.

| Metals | Phosphorus | | Magnesium | | Copper | | Selenium | |
|------------------------------|---------------------|-------|---------------------|--------|---------------------|-------|---------------------|-------|
| | aOR (95% CI) | P | aOR (95% CI) | P | aOR (95% CI) | P | aOR (95% CI) | P |
| Overall | | 0.008 | | <0.001 | | 0.041 | | 0.133 |
| Q1 | Reference | | Reference | | Reference | | Reference | |
| Q2 | 0.971 (0.868–1.086) | 0.605 | 0.874 (0.782–0.977) | 0.018 | 0.905 (0.808–1.011) | 0.075 | 0.983 (0.879–1.100) | 0.769 |
| Q3 | 0.899 (0.801–1.010) | 0.072 | 0.819 (0.730–0.919) | 0.001 | 0.880 (0.785–0.987) | 0.028 | 0.952 (0.849–1.068) | 0.399 |
| Q4 | 0.821 (0.727–0.929) | 0.002 | 0.770 (0.683–0.868) | <0.001 | 0.853 (0.756–0.959) | 0.009 | 0.872 (0.772–0.986) | 0.029 |
| Male | | 0.007 | | 0.001 | | 0.137 | | 0.054 |
| Q1 | Reference | | Reference | | Reference | | Reference | |
| Q2 | 1.026 (0.864–1.217) | 0.773 | 0.901 (0.763–1.065) | 0.222 | 0.937 (0.793–1.106) | 0.441 | 0.972 (0.820–1.153) | 0.747 |
| Q3 | 0.991 (0.838–1.171) | 0.911 | 0.925 (0.787–1.088) | 0.347 | 0.919 (0.781–1.082) | 0.310 | 0.951 (0.807–1.122) | 0.554 |
| Q4 | 0.810 (0.684–0.958) | 0.014 | 0.736 (0.625–0.867) | <0.001 | 0.829 (0.704–0.976) | 0.024 | 0.821 (0.696–0.968) | 0.019 |
| Female | | 0.063 | | 0.001 | | 0.343 | | 0.670 |
| Q1 | Reference | | Reference | | Reference | | Reference | |
| Q2 | 0.929 (0.799–1.080) | 0.340 | 0.868 (0.746–1.010) | 0.066 | 0.897 (0.768–1.048) | 0.170 | 0.963 (0.828–1.120) | 0.624 |
| Q3 | 0.798 (0.675–0.943) | 0.008 | 0.701 (0.591–0.831) | <0.001 | 0.875 (0.740–1.034) | 0.116 | 0.917 (0.778–1.080) | 0.299 |
| Q4 | 0.958 (0.793–1.157) | 0.653 | 0.932 (0.776–1.119) | 0.449 | 0.968 (0.810–1.158) | 0.726 | 1.024 (0.845–1.241) | 0.809 |
| BMI < 25.0 kg/m ² | | 0.412 | | 0.136 | | 0.623 | | 0.403 |
| Q1 | Reference | | Reference | | Reference | | Reference | |
| Q2 | 0.919 (0.715–1.181) | 0.509 | 0.897 (0.698–1.153) | 0.396 | 0.924 (0.715–1.195) | 0.546 | 1.112 (0.869–1.422) | 0.399 |
| Q3 | 0.949 (0.735–1.225) | 0.688 | 0.842 (0.649–1.091) | 0.193 | 0.934 (0.722–1.209) | 0.604 | 0.922 (0.709–1.198) | 0.544 |
| Q4 | 0.794 (0.602–1.049) | 0.105 | 0.728 (0.556–0.953) | 0.021 | 0.837 (0.642–1.092) | 0.190 | 0.913 (0.695–1.200) | 0.515 |
| BMI ≥ 25.0 kg/m ² | | 0.019 | | 0.001 | | 0.173 | | 0.199 |
| Q1 | Reference | | Reference | | Reference | | Reference | |
| Q2 | 0.983 (0.866–1.114) | 0.784 | 0.867 (0.766–0.982) | 0.025 | 0.908 (0.801–1.030) | 0.135 | 0.952 (0.839–1.080) | 0.447 |
| Q3 | 0.884 (0.776–1.007) | 0.063 | 0.813 (0.715–0.924) | 0.002 | 0.885 (0.778–1.007) | 0.063 | 0.958 (0.843–1.089) | 0.513 |
| Q4 | 0.828 (0.722–0.949) | 0.007 | 0.781 (0.683–0.893) | <0.001 | 0.874 (0.765–0.998) | 0.047 | 0.864 (0.753–0.991) | 0.036 |

CI, confidence interval; aOR, adjusted odds ratio.

Adjusted to: gender, age, race, marital status, education level, BMI, hypertension, diabetes mellitus, vigorous physical activities, moderate physical activities, blood urea nitrogen, creatinine, uric acid, and estimated glomerular filtration rate (eGFR).

involved in the physiological functions of the body in the form of plasma copper cyanobrotein (20). Most of the endogenous copper is excreted into the gastrointestinal tract along with unabsorbed copper, and a small amount of copper is excreted through other routes (21). One study found that increased exposure of serum copper was significantly associated with the risk of non-alcoholic fatty liver disease (NAFLD) and was particularly prominent in women, middle age and participants with improved insulin resistance status, with a 97% increased risk of NAFLD in the highest quartile of copper compared to the lowest quartile (22). Gao et al. (23) found that serum copper levels in the highest quartile were associated with urge incontinence (aOR = 1.74, 95% CI 1.11–2.74), especially in women over 50 years of age, serum copper levels in the highest quartile were associated with urge incontinence (aOR = 1.74, 95% CI 1.57–5.49), any urinary incontinence (aOR = 1.97, 95% CI 1.19–3.27), mixed incontinence (aOR = 2.43, 95% CI 1.19–4.97), and moderate/severe incontinence (aOR = 2.37, 95% CI

1.06–5.31). In addition, Bagheri et al. (24) found that serum copper levels were higher in patients with atherosclerosis and positively correlated with the severity of atherosclerosis ($p = 0.001$). The above findings suggest that excess serum copper levels can also adversely affect the organism and that excessive copper intake should be limited for normal individuals as well.

Due to the large difference in the number of stone formers and non-stone formers groups, we used a 1:1 PSM to correct for the difference between the two groups (Figure 3), 2,744 participants were included in both the stone formers and non-stone formers groups after the PSM. The clinicopathological characteristics of all participants after PSM are shown in Table 3. Subsequently, dose-response curves showed that only dietary copper ($p = 0.001$) intake was negatively and non-linearly associated with the risk of kidney stones (Figure 4). Multivariate single-metal model logistic regression results also demonstrated that dietary copper ($p = 0.001$) intake remained an independent risk factor for kidney stones, and the ORs for kidney stones in

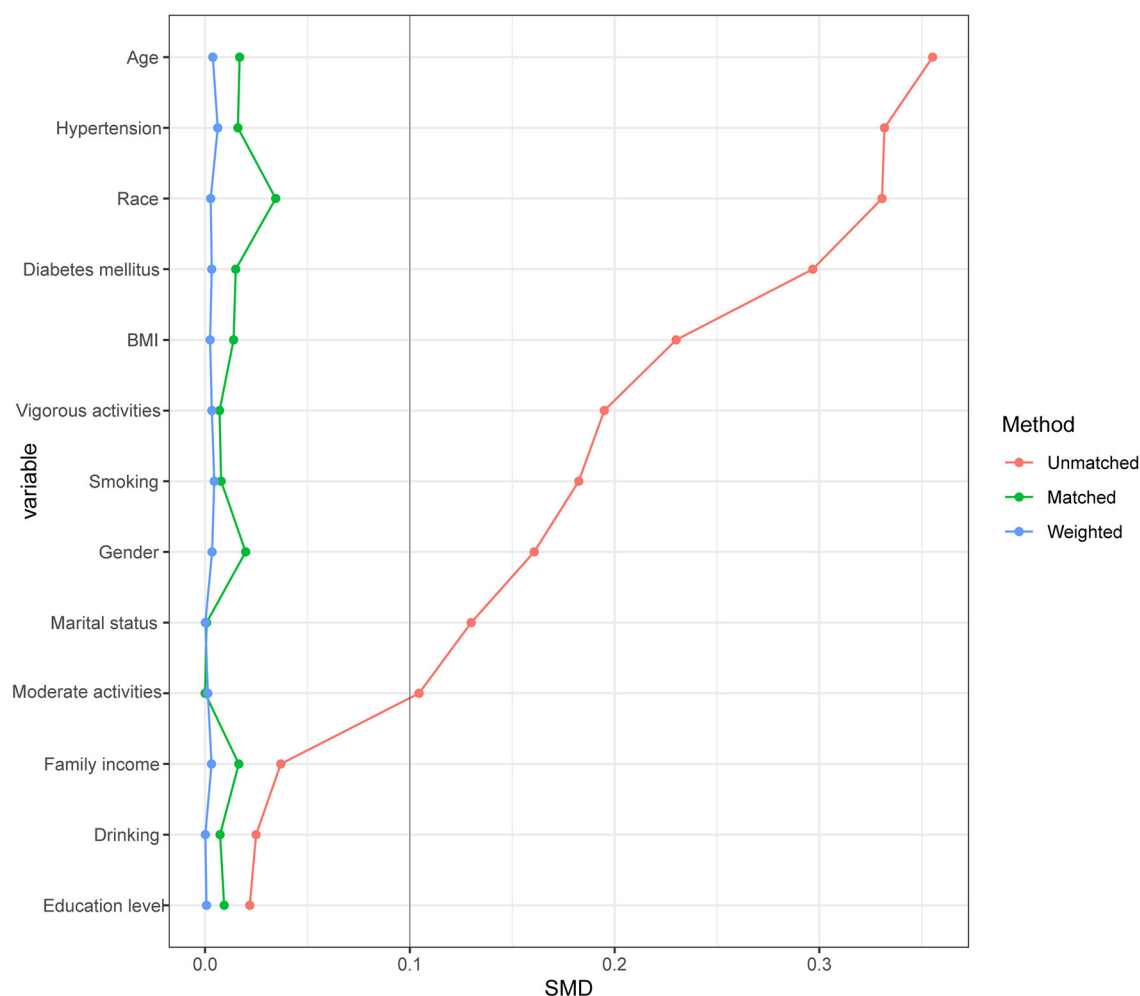


FIGURE 3
Propensity score matching analysis of the standardized mean difference results for the different variables.

each quartile of copper intake compared to the lowest quartile were 0.877 (0.754–1.021, $p = 0.092$), 0.876 (0.751–1.022, $p = 0.093$) and 0.732 (0.626–0.856, $p < 0.001$) (Table 4). Moreover, multivariate multi-metal logistic regression showed that only copper intake was associated with the risk of kidney stones (Table 5).

Discussion

As a common kidney disease, the high incidence and recurrence rate of kidney stones bring serious impact on patients' life, and cause huge economic burden. Therefore, it is of great clinical and social significance to explore the factors associated with kidney stones and to identify risk factors and protective factors for kidney stones in advance. In this study, we first investigated the dietary trace metals associated with kidney

stones, and found that increased dietary intake of phosphorus, magnesium, and copper were associated with lower odds of kidney stones, and this finding was confirmed by the dose-response curves. Subsequently, multivariate logistic regression also revealed that dietary phosphorus, magnesium, and copper were independent protective factors for kidney stones. To exclude as much as possible the influence of other confounding variables and to test the correctness of the results, we used PSM analysis to adjust for differences between the stone formers and non-stone formers groups. Subsequent dose-response curves as well as multivariate logistic regression confirmed that dietary copper intake may be a protective factor for kidney stones.

Copper, an essential trace element, is widely distributed in tissues and organs such as the liver, kidney and brain, and the body meets its requirement for copper mainly through daily dietary intake (25). Copper is involved in energy metabolism, immune function, antioxidant and energy metabolism in the

TABLE 3 Baseline characteristics of NHANES participants between 2007 and 2018 after PSM.

| Characteristic | Total | None-stone formers | Stone formers | P-value |
|---|--------------|--------------------|---------------|---------|
| | No. (%) | No. (%) | No. (%) | |
| Total patients | 5,488 | 2,744 (50.0) | 2,744 (50.0) | |
| Gender | | | | 0.786 |
| Male | 3,056 (55.7) | 1,523 (55.5) | 1,533 (55.9) | |
| Female | 2,432 (44.3) | 1,221 (44.5) | 1,211 (44.1) | |
| Age | | | | 0.459 |
| <50 years | 1,876 (34.2) | 925 (33.7) | 951 (34.7) | |
| ≥50 years | 3,612 (65.8) | 1,819 (66.3) | 1,793 (65.3) | |
| Race | | | | 0.783 |
| Non-Hispanic white | 3,059 (55.7) | 1,542 (56.2) | 1,517 (55.3) | |
| Non-Hispanic black | 711 (13.0) | 359 (13.1) | 352 (12.8) | |
| Mexican American | 697 (12.7) | 343 (12.5) | 354 (12.9) | |
| Other Hispanic | 610 (11.1) | 306 (11.2) | 304 (11.1) | |
| Other | 411 (7.5) | 194 (7.1) | 217 (7.9) | |
| Education level | | | | 0.991 |
| Less than high school | 1,363 (24.8) | 683 (24.9) | 680 (24.8) | |
| High school or equivalent | 1,240 (22.6) | 621 (22.6) | 619 (22.6) | |
| College or above | 2,885 (52.6) | 1,440 (52.5) | 1,445 (52.7) | |
| Marital status | | | | 0.155 |
| Married | 3,200 (58.3) | 1,626 (59.3) | 1,574 (57.4) | |
| Unmarried | 2,288 (41.7) | 1,118 (40.7) | 1,170 (42.6) | |
| Family income | | | | 0.786 |
| <\$20,000 | 1,324 (24.1) | 668 (24.3) | 656 (23.9) | |
| ≥\$20,000 | 3,945 (71.9) | 1,971 (71.8) | 1,974 (71.9) | |
| Unknown | 219 (4.0) | 105 (3.8) | 114 (4.2) | |
| BMI (kg/m²) | | | | 0.891 |
| <25.0 | 1,062 (19.4) | 533 (19.4) | 529 (19.3) | |
| ≥25.0 | 4,426 (80.6) | 2,211 (80.6) | 2,215 (80.7) | |
| Hypertension | | | | 0.666 |
| Yes | 2,768 (50.4) | 1,376 (50.1) | 1,392 (50.7) | |
| No | 2,720 (49.6) | 1,368 (49.9) | 1,352 (49.3) | |
| Diabetes mellitus | | | | 0.096 |
| Yes | 1,219 (22.2) | 603 (22.0) | 616 (22.4) | |
| No | 4,107 (74.8) | 2,073 (75.5) | 2,034 (74.1) | |
| Borderline | 162 (3.0) | 68 (2.5) | 94 (3.4) | |
| Smoking status | | | | 0.084 |
| Never | 2,684 (48.9) | 1,339 (48.8) | 1,345 (49.0) | |
| Former | 1,703 (31.0) | 882 (32.1) | 821 (29.9) | |
| Current | 1,101 (20.1) | 523 (19.1) | 578 (21.1) | |
| Drinking | | | | 0.768 |
| Yes | 3,856 (70.3) | 1,933 (70.4) | 1,923 (70.1) | |
| No/Unknown | 1,632 (29.7) | 811 (29.6) | 821 (29.9) | |
| Vigorous recreational activities | | | | 0.519 |
| Yes | 815 (14.9) | 399 (14.5) | 416 (15.2) | |
| No | 4,673 (85.1) | 2,345 (85.5) | 2,328 (84.8) | |
| Moderate recreational activities | | | | 0.693 |
| Yes | 1,954 (35.6) | 970 (35.3) | 984 (35.9) | |

(Continued)

TABLE 3 (Continued)

| Characteristic | Total | None-stone formers | Stone formers | P-value |
|--------------------------------------|----------------|--------------------|----------------|---------|
| | No. (%) | No. (%) | No. (%) | |
| No | 3,534 (64.4) | 1,774 (64.7) | 1,760 (64.1) | |
| Blood urea nitrogen (mg/dL) | 15.02, 6.96 | 14.74, 6.63 | 15.29, 7.27 | 0.003 |
| Creatinine (mg/dL) | 0.96, 0.60 | 0.95, 0.45 | 0.98, 0.72 | 0.057 |
| Uric acid (mg/dL) | 5.69, 1.50 | 5.73, 1.48 | 5.65, 1.52 | 0.042 |
| eGFR [mL/(min·1.73 m ²)] | 75.36, 25.24 | 75.68, 24.85 | 75.05, 25.61 | 0.352 |
| Daily intake (M, SD) | | | | |
| Protein (mg) | 79.46, 40.56 | 80.38, 41.18 | 78.53, 39.92 | 0.129 |
| Moisture (g) | 2.88, 1.48 | 2.88, 1.47 | 2.88, 1.50 | 0.959 |
| Phosphorus (mg) | 1,304.9, 642.0 | 1,301.9, 642.3 | 1,307.9, 641.9 | 0.727 |
| Magnesium (mg) | 285.38, 138.83 | 287.90, 138.12 | 282.86, 139.52 | 0.178 |
| Iron (mg) | 14.50, 8.26 | 14.58, 8.20 | 14.41, 8.33 | 0.461 |
| Zinc (mg) | 11.08, 6.68 | 11.30, 6.93 | 10.87, 6.43 | 0.018 |
| Copper (mg) | 1.23, 0.72 | 1.27, 0.75 | 1.20, 0.69 | <0.001 |
| Selenium (mcg) | 109.18, 61.61 | 108.30, 60.76 | 110.06, 62.44 | 0.290 |

For categorical variables, *P*-values were analyzed by chi-square tests. For continuous variables, the *t*-test for slope was used in generalized linear models.

NHANES, National Health and Nutrition Examination Survey; BMI, body mass index; eGFR, estimated glomerular filtration rate; SD, standard deviation; PSM, propensity score matching.

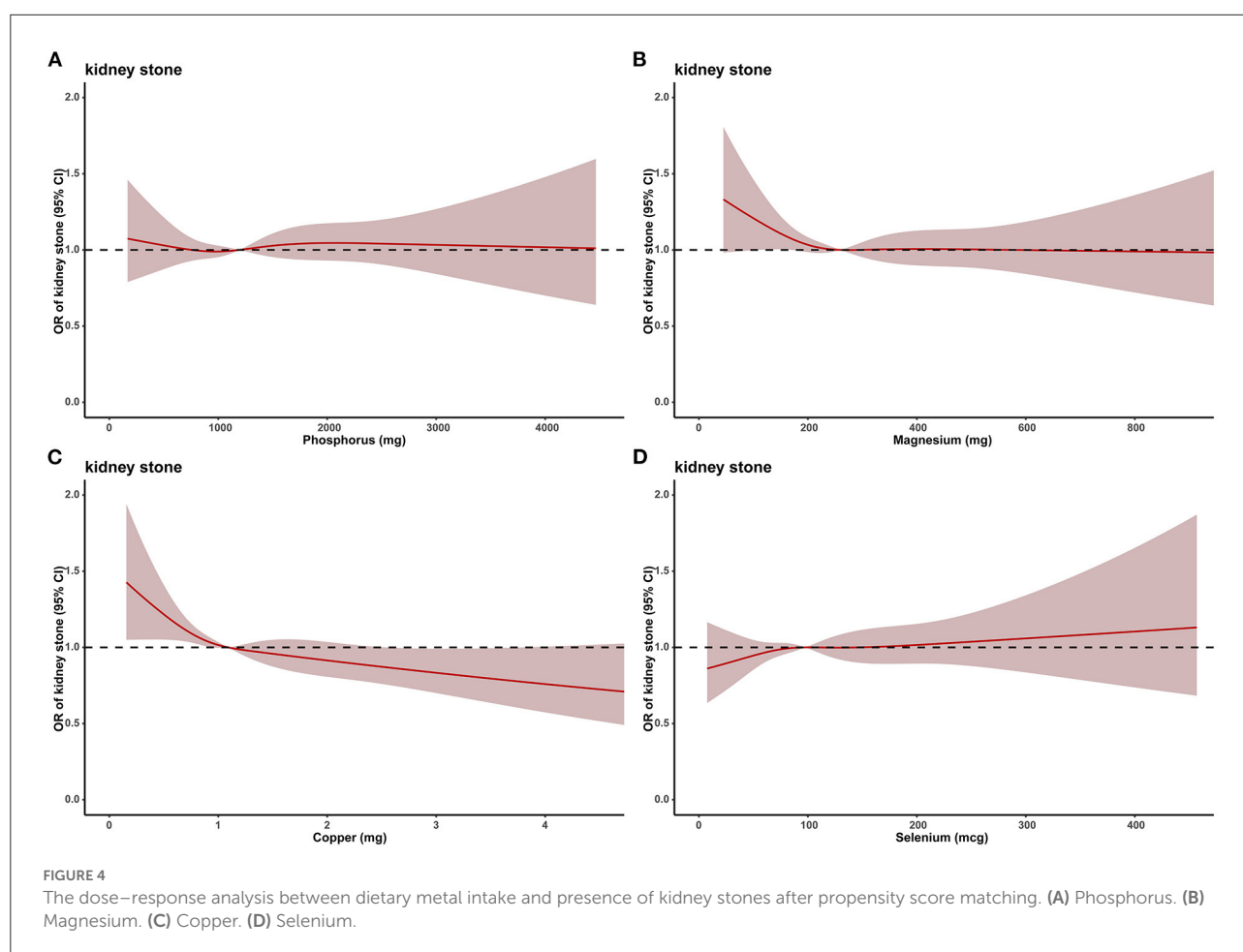


TABLE 4 Multivariate single-metal model logistic regression the relationship between the heavy metals and the presence of kidney stone after PSM.

| Metals | Phosphorus | | Magnesium | | Copper | | Selenium | |
|------------------------------|---------------------|-------|---------------------|-------|---------------------|--------|---------------------|-------|
| | aOR (95% CI) | P | aOR (95% CI) | P | aOR (95% CI) | P | aOR (95% CI) | P |
| Overall | | 0.929 | | 0.307 | | 0.001 | | 0.297 |
| Q1 | Reference | | Reference | | Reference | | Reference | |
| Q2 | 1.051 (0.906–1.219) | 0.512 | 0.903 (0.779–1.047) | 0.177 | 0.877 (0.754–1.021) | 0.092 | 1.134 (0.977–1.315) | 0.098 |
| Q3 | 1.023 (0.878–1.193) | 0.770 | 0.903 (0.775–1.052) | 0.191 | 0.876 (0.751–1.022) | 0.093 | 1.113 (0.957–1.295) | 0.165 |
| Q4 | 1.016 (0.865–1.193) | 0.849 | 0.865 (0.739–1.014) | 0.073 | 0.732 (0.626–0.856) | <0.001 | 1.034 (0.881–1.213) | 0.684 |
| Male | | 0.680 | | 0.129 | | <0.001 | | 0.306 |
| Q1 | Reference | | Reference | | Reference | | Reference | |
| Q2 | 1.032 (0.825–1.291) | 0.783 | 0.884 (0.710–1.101) | 0.270 | 0.792 (0.632–0.992) | 0.042 | 1.135 (0.908–1.418) | 0.265 |
| Q3 | 1.024 (0.823–1.275) | 0.830 | 0.930 (0.751–1.153) | 0.510 | 0.822 (0.659–1.025) | 0.082 | 1.127 (0.909–1.396) | 0.276 |
| Q4 | 0.926 (0.744–1.152) | 0.491 | 0.784 (0.632–0.972) | 0.026 | 0.618 (0.497–0.768) | <0.001 | 0.975 (0.787–1.207) | 0.814 |
| Female | | 0.265 | | 0.261 | | 0.827 | | 0.562 |
| Q1 | Reference | | Reference | | Reference | | Reference | |
| Q2 | 1.052 (0.860–1.287) | 0.621 | 0.925 (0.756–1.133) | 0.452 | 0.948 (0.769–1.169) | 0.619 | 1.108 (0.906–1.356) | 0.318 |
| Q3 | 0.972 (0.778–1.214) | 0.802 | 0.836 (0.667–1.047) | 0.119 | 0.903 (0.723–1.128) | 0.371 | 1.075 (0.864–1.338) | 0.517 |
| Q4 | 1.268 (0.976–1.647) | 0.075 | 1.074 (0.838–1.378) | 0.572 | 0.927 (0.731–1.176) | 0.535 | 1.192 (0.917–1.550) | 0.190 |
| BMI < 25.0 kg/m ² | | 0.888 | | 0.330 | | 0.291 | | 0.480 |
| Q1 | Reference | | Reference | | Reference | | Reference | |
| Q2 | 0.903 (0.642–1.269) | 0.556 | 1.097 (0.776–1.550) | 0.601 | 1.050 (0.737–1.496) | 0.788 | 1.244 (0.885–1.747) | 0.209 |
| Q3 | 0.892 (0.627–1.269) | 0.524 | 0.935 (0.654–1.336) | 0.711 | 1.024 (0.717–1.461) | 0.897 | 1.089 (0.762–1.554) | 0.641 |
| Q4 | 0.871 (0.592–1.280) | 0.481 | 0.784 (0.546–1.127) | 0.190 | 0.771 (0.538–1.104) | 0.155 | 0.961 (0.664–1.392) | 0.834 |
| BMI ≥ 25.0 kg/m ² | | 0.784 | | 0.356 | | 0.003 | | 0.474 |
| Q1 | Reference | | Reference | | Reference | | Reference | |
| Q2 | 1.091 (0.924–1.287) | 0.305 | 0.870 (0.738–1.025) | 0.097 | 0.843 (0.712–0.998) | 0.048 | 1.113 (0.943–1.313) | 0.207 |
| Q3 | 1.057 (0.891–1.254) | 0.526 | 0.898 (0.758–1.065) | 0.217 | 0.846 (0.712–1.005) | 0.057 | 1.125 (0.951–1.330) | 0.169 |
| Q4 | 1.051 (0.880–1.257) | 0.581 | 0.884 (0.741–1.055) | 0.172 | 0.716 (0.601–0.853) | <0.001 | 1.050 (0.879–1.255) | 0.593 |

CI, confidence interval; aOR, adjusted odds ratio; PSM, propensity score matching.

Adjusted to: gender, age, race, marital status, education level, BMI, hypertension, diabetes mellitus, vigorous physical activities, moderate physical activities, blood urea nitrogen, creatinine, uric acid, and estimated glomerular filtration rate (eGFR).

body (26). Numerous studies have shown that deficiencies in dietary copper intake are associated with cardiovascular disease, central nervous system diseases, digestive system diseases, and skeletal system diseases (13, 14, 27). Li et al. (28) included 14,834 NHANES participants from 2009 to 2014 and found that dietary intake of zinc, iron, copper, and selenium were negatively associated with depression, and similar study has found that dietary intake of zinc, copper, and selenium was associated with cognitive performance (29). Yang et al. (30) found a non-linear negative association between dietary copper intake and stroke by analyzing clinical information from 10,550 participants in the NHANES database from 2013 to 2018. The above results suggest that increased dietary copper intake appears to be a protective factor against disease progression.

Recently, Tsvetkov et al. (17) found that intracellular copper accumulation can cause direct binding of copper to lipid acylated components of the tricarboxylic acid (TCA) cycle,

which subsequently leads to aggregation of lipid acylated proteins and loss of Fe-S cluster proteins, ultimately leading to cell death. Tsvetkov's study has led researchers to pay increasing attention to the role of copper in living organisms. To date, there has been only one study of copper intake and risk of kidney stones in adults. Ferraro et al. (12) studied participants in the Health Professionals Follow-up Study (HPFS) and the Nurses' Health Study (NHS) I and II and found no association between dietary copper intake and kidney stone risk, but total copper intake was associated with a higher risk of kidney stones in the NHS I cohort (OR = 1.14, 95% CI 1.02–1.28). However, in our study, we found that dietary copper intake was associated with a lower risk of kidney stones, and the same results were also obtained after 1:1 PSM analysis. We speculate that the conflicting results may be due to the following reasons: first, copper has two aspects; it is both an antioxidant and a pro-oxidant. One study found that either excessive or insufficient

TABLE 5 Multivariate multi-metal model logistic regression of the relationship between heavy metals and kidney stone after PSM.

| Metals | Phosphorus | | Magnesium | | Copper | | Selenium | |
|---------|---------------------|-------|---------------------|-------|---------------------|--------|---------------------|-------|
| | aOR (95% CI) | P | aOR (95% CI) | P | aOR (95% CI) | P | aOR (95% CI) | P |
| Overall | | 0.504 | | 0.599 | | <0.001 | | 0.246 |
| Q1 | Reference | | Reference | | Reference | | Reference | |
| Q2 | 1.109 (0.931–1.321) | 0.247 | 0.923 (0.768–1.110) | 0.394 | 0.827 (0.693–0.986) | 0.034 | 1.163 (0.990–1.366) | 0.067 |
| Q3 | 1.150 (0.924–1.430) | 0.210 | 0.989 (0.786–1.245) | 0.927 | 0.760 (0.616–0.938) | 0.011 | 1.185 (0.983–1.429) | 0.076 |
| Q4 | 1.224 (0.936–1.600) | 0.139 | 1.059 (0.800–1.402) | 0.689 | 0.581 (0.454–0.744) | <0.001 | 1.149 (0.922–1.433) | 0.216 |

CI, confidence interval; aOR, adjusted odds ratio; PSM, propensity score matching.

Adjusted to: gender, age, race, marital status, education level, BMI, hypertension, diabetes mellitus, vigorous physical activities, moderate physical activities, blood urea nitrogen, creatinine, uric acid, estimated glomerular filtration rate (eGFR), phosphorus, magnesium, copper and selenium.

copper intake increased susceptibility to atherosclerosis in rabbits, reflecting the bidirectional relationship between copper and atherosclerosis (31). Second, the year of sample collection, sample size, and type of variables adjusted for differed for each study. Therefore, further large samples are needed to elucidate the relationship between dietary copper intake and the risk of kidney stones.

There are several strengths deserve to be noted in our study. First, the sample of this study was large enough that the participants were representative of the national population of the United States, and the findings warrant generalization. Second, we used multivariate logistic regression analysis, to adjust for other confounding variables in our statistical analysis. Finally, to verify the correctness of the results, we also conducted a 1:1 PSM analysis, which was used to offset possible effects caused by gaps in the sample data. However, our analysis has some potential limitations. First, our study is a cross-sectional retrospective analysis with its inherent limitations. Second, although adjustments were made for some confounding factors, the effects of other unknown factors could not be excluded. Finally, the type of kidney stones was not available, and different stone types may have different results.

Conclusion

In conclusion, this study suggests that dietary copper intake was significantly and negatively associated with kidney stones in NHANES participants and that dietary copper intake may play an important role in the prevention of kidney stones in future clinical practice.

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 study was reviewed and approved by the NHANES Committee. Written informed consent was obtained from all the patients/participants for their participation in this study and for the publication of any potentially identifiable images or data included in this article, following the Declaration of Helsinki.

Author contributions

SC, WM, YC, and MC designed the research. WZ, CW, JW, and WM performed the research and analyzed the results. WZ and CW wrote the paper. JW, SC, WM, YC, and MC edited the manuscript and provided the critical comments. 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.2022.973887/full#supplementary-material>

SUPPLEMENTARY FIGURE S1

Spearman's rank correlation coefficients of six trace elements.

SUPPLEMENTARY TABLE S1

Multivariate multi-metal model logistic regression the relationship between the heavy metals and the presence of kidney stone.

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Heavy metal contamination assessment and probabilistic health risks in soil and maize near coal mines

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Objective: Coal mining activities have continuously introduced heavy metals into the soil–crop system, causing increasing damage to crops. This study integrated the analysis of the heavy metal contamination status and human health risk in soil and maize near coal mines to help formulate control strategies for soil quality, maize production, and safe consumption.

Method: This study was carried out on maize agricultural land near a coal mining plant. Heavy metal contamination was assessed by the geo-accumulation index (I_{geo}), enrichment factor (EF), and bioaccumulation factor (BCF). The Monte Carlo simulation was used to estimate the probabilistic health risk of heavy metals exposure in soil and maize. The relationship between the concentration of heavy metal in the soil and that in maize was further visualized by correlation analysis and random forest analysis.

Results: The results revealed that the mean concentrations of soil Ni, Cu, As, Cd, Sn, Zn, Pb, and Hg were all above the local background level. Ni was the most severely polluted heavy metal in maize and had a concentration higher than the risk control standard for corn in China (NY 861-2004). The I_{geo} values of all heavy metals were low, and EF values showed enrichment in V, Cr, Ti, Ni, and As. The assessment of probabilistic health risk exposed by heavy metals in soil and maize indicated that 1.16 and 1.46% of residents exceeded the carcinogenic risk level due to heavy metal exposure from soil and maize, respectively. Children were the most sensitive to maize and soil heavy metal exposure in the contaminated area. Ingestion of heavy metals was associated with the highest health risk to residents, followed by dermal contact and inhalation. As and Cr in soil and Cr and Ni in maize had the greatest impact on human health risk. Furthermore, maize heavy metals were affected the most by soil Cr, Cd, and V.

Conclusion: These results may provide useful information for human carcinogenic risk associated with soil and maize heavy metal exposure due to coal mining activities.

KEYWORDS

probabilistic health risk, heavy metal, soil, maize, Monte Carlo simulation, coal mines

Introduction

Soil heavy metal pollution has become an increasing problem with the expansion of urbanization, industry, and agriculture (1). In 2020, the China Ecological Environment Status Bulletin identified heavy metals as the main pollutant of soil, stating that heavy metals, primarily Cd, adversely affect the environmental quality of agricultural land (2). Extensive human activities, such as application of pesticides, metal smelting, mining, sewage irrigation, and transportation, have released huge quantities of heavy metals into the soil ecosystem (3). Non-biodegradable heavy metals can store in the soil over time, eventually reaching concentrations that exceed safe limits and exhibiting adverse effects on plant physiological functions and human health (4, 5). Zhou (6) analyzed heavy metal pollution survey data from cultivated land in China between 2008 and 2018 and reported that most severe heavy metal pollution was caused by Cd, which had an average geo-accumulation index (I_{geo}) of 0.63, followed by Hg and Ni. In Hanzhong, China, Cd and As had higher mobility than other heavy metals, and their average concentrations in rice were mildly above than the acceptable threshold (7).

Humans can be exposed to heavy metals in various ways, such as consumption of food crops, direct soil ingestion, dust inhalation, and dermal contact (8). Soil heavy metals pose severe risks to soil function and human health through the food chain (9). In addition, heavy metals in crops cause severe and destructive impacts on human health owing to long-time intake (10). Some heavy metals, such as Cd, Pb, Cu, and Cr, may affect the liver, central nervous system, kidneys, and mental health (4). In maize harvested in northern Ningxia, the content of Pb and Cr surpassed the standards (NY 861-2004) and posed non-carcinogenic and carcinogenic risks to 0.62 and 8.23% of residents, respectively (11). In Zambia, the non-carcinogenic risk values for Pb and Cd were much higher than 1, suggesting that people who consume corn grains might be at high risk of exposure to toxic levels (12). Therefore, it is necessary to assess the heavy metal pollution of soil and crops and the associated human health risks to address the concerns of public health and environmental quality.

Previous studies have estimated health risks with health risk model developed by the United States Environmental Protection Agency (US EPA), which is recognized as the most holistic approach for manifesting environmental contaminant risks (3, 13–15). However, the model's deterministic assessment focuses on the total heavy metal concentrations and exposure parameters, neglecting the significant differences among individuals and the dynamics, variability, and randomness of the heavy metal exposure process (16). The Markov Chain Monte Carlo simulation is often used to provide accurate and practical assessment of complex environmental pollution, and it has a good ability to discern the greatest influence parameter on health risks (1). Therefore, it is necessary to utilize the

Monte Carlo simulation to control for the differences, lack of comparability, and inaccuracy of health risk assessment.

Heavy metals from polluted agricultural soil can be transmitted by crop root accumulation to grains, and a relationship between heavy metal concentrations of the soil and those of crops was identified (17). Research in the northern region of Malaysia demonstrated that Cu was more mobile than As, Cr, Pb, and Cd from the soil to the roots of paddy plants, and the bioavailability of Cd from the soil to the roots was poor because of poor mobility (18). The different degrees of heavy metal concentrations might be caused by the variation in crops' absorption capacity for different soil heavy metals (17). Liu et al. (11) found that in maize from northern Ningxia, China, Cd had the highest accumulation ability, and Pb had the lowest. According to Wei et al. (13), Zn, Cd, Cr, and Cu are easily enriched in maize, and Zn could be quickly translocated into the aerial part of maize and ultimately accumulated in the grains. Vegetables harvested from farms contaminated with heavy metals in western Nigeria had a greater capacity to absorb Cd and Pb than Cr, and Zn (15). Moreover, the average bioaccumulation capacity of Cd was greater than that of other heavy metals in grape pulp from a vineyard in Henan Province (3). Further research showed that Cd had a greater ability for translocation than Zn and Pb in maize cultivated in Zambia (12). The mutual influence between multiple soil heavy metals makes it necessary to explore the relationships between various heavy metals in the soil–crop system.

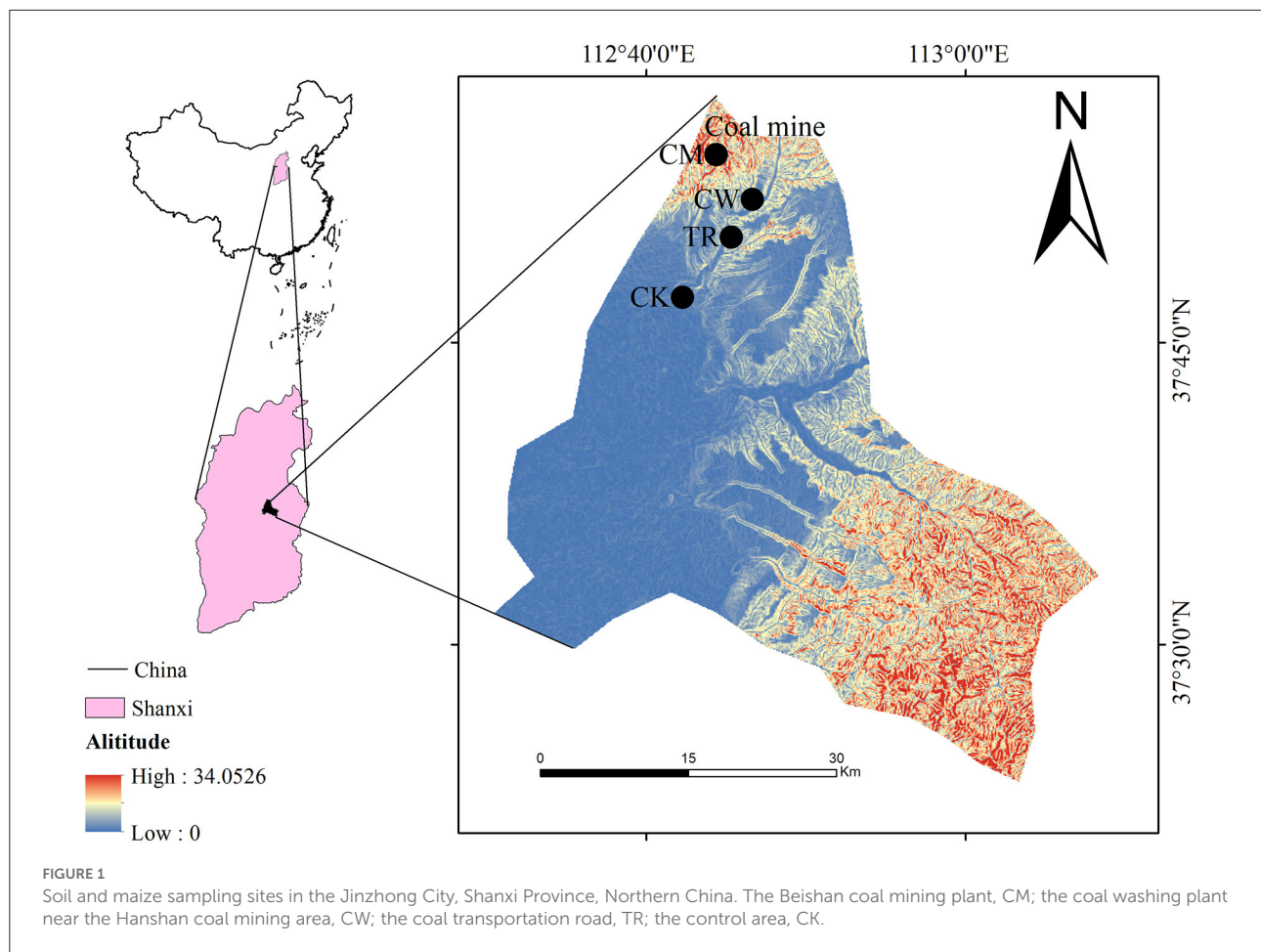
Shanxi Province is a representative region of prosperity and development in China, and it is an crucial industrial province because of its high coal production. However, in recent decades, rapid industrial development has led to environmental problems, causing major concern. Previous research reported serious pollution of cultivated land in Shanxi Province (6). However, rare studies have explored the heavy metal contamination of soil and plants in Shanxi Province. Therefore, it is necessary to access the ecological risks caused by heavy metals and the potential health risk to inhabitants of Shanxi Province.

This study aimed to (i) evaluate the pollution characteristics of heavy metals in agricultural soil and the harvested crops around a coal mining area; (ii) assess the probabilistic human health risk with heavy metal exposure in agricultural soil and its harvested crops; and (iii) evaluate the relationships between the concentrations of heavy metal in the soil and those in crops.

Materials and methods

Site description and soil sampling

We selected Jinzhong City (Shanxi Province, Northern China) as the investigation area. The study area is a warm, temperate, semiarid continental monsoon climate region,



which is characterized by hot, rainy summers and generally cold, dry winters. The city of Jinzhong ($36^{\circ}40' - 38^{\circ}06' \text{ N}$, $111^{\circ}25' - 114^{\circ}05' \text{ E}$), located in the hinterland of Shanxi Province, China, has moderate climate conditions with average temperatures between 4.2 and 14.2°C and a wide variety of soils, making it a suitable area for crop growth. Because of the unique climate and soil conditions, the local geonics is prosperous, which has led to huge inputs of fertilizers and pesticides. Additionally, the light industry is the dominant industry in this district, and other diversified industries, including machinery, metallurgy, electrical, chemical, coal, light textile, building materials, and food, are also present. However, the expansion of coal mining and increased industrial activity pose a growing severe threat to soil quality and crop safety because of heavy metal pollution.

The study was conducted in coal mining areas in Shanxi Province, namely the Beishan coal mining plant (CM), the coal washing plant near the Hanshan coal mining area (CW), and the coal transportation road (TR, [Figure 1](#)). The control area (CK) was located away from the coal mining areas and had no industrial influence. A random sampling method was used to select 36 paired samples of soil and maize from each study area,

and each of the 36 soil and maize samples was a well-mixed composite of 9 subsamples. The nine subsamples were sampled by the systematic grid method. Approximately 3 kg of topsoil subsamples (from a 0–20 cm depth) were sampled from each site by a stainless-steel shovel. Soil samples and their corresponding crop samples were collected at the same sites during the harvest season in October, 2020. We chose maize as the representative crop because it was the main crop in the investigated areas.

Analysis of heavy metal pollution status of soil and maize

The concentrations of nine heavy metals (Cr, V, Ni, Pb, As, Ti, Cd, Cu, and Zn) in the soil and crop samples were obtained. After sampling, the crop samples were dried at 105°C for 1 h and then dried to constant weight at 70°C . Subsequently, 300 g of crop samples were comminuted and ground, passed through a 100-mesh nylon sieve, and then packed in airtight polyethylene bags for further analysis and storage. The crop samples were microwave-digested, and the total concentrations

TABLE 1 Statistical characteristic of the heavy metal concentrations (mg/kg) in the soil and maize of the study area.

| Heavy metal | Means \pm SD (range) | CV | CK | Background | Screening value |
|--------------|-------------------------------------|--------|--------|------------|-----------------|
| Soil | | | | | |
| V | 45.13 \pm 2.51 (37.14–40.79) | 0.06 | 35.67 | 63.4 | – |
| Cr | 46.74 \pm 6.7 (28.72–36.37) | 0.18 | 29.94 | 55.3 | 250.00 |
| Ti | 562.5 \pm 46.8 (405.9–487.99) | 0.10 | 371.58 | 4000 | – |
| Ni | 30.76 \pm 1.66 (25.29–27.56) | 0.06 | 23.08 | 29.9 | 190.00 |
| Cu | 24.32 \pm 1.24 (19.52–21.19) | 0.06 | 23.16 | 22.9 | 100.00 |
| Zn | 88.7 \pm 7.22 (62.78–72.17) | 0.10 | 77.26 | 63.5 | 300.00 |
| As | 14.23 \pm 0.75 (11.64–12.58) | 0.06 | 11.45 | 9.1 | 25.00 |
| Cd | 0.39 \pm 0.06 (0.2–0.26) | 0.23 | 0.26 | 0.1 | 0.60 |
| Pb | 19.65 \pm 1.34 (15.5–17.49) | 0.08 | 21.09 | 14.7 | 170.00 |
| Mn | 564 \pm 13.37 (517.2–542.21) | 0.02 | 504.83 | 532 | – |
| Maize | | | | | |
| V | 0.0138 \pm 0.0068 (0.004–0.022) | 0.4906 | 0.0307 | – | – |
| Cr | 0.56 \pm 0.67 (0.09–1.5) | 1.19 | 0.28 | – | 1.00 |
| Ti | 6.76 \pm 2.28 (3.62–9.15) | 0.34 | 6.56 | – | – |
| Ni | 0.61 \pm 0.19 (0.35–0.82) | 0.31 | 0.31 | – | 0.40 |
| Cu | 2.86 \pm 1.46 (1.53–5.33) | 0.51 | 1.92 | – | 10.00 |
| Zn | 24.73 \pm 3.58 (20.61–30.37) | 0.14 | 20.40 | – | 50.00 |
| As | 0.0167 \pm 0.0149 (0.002–0.039) | 0.8955 | 0.0220 | – | 0.70 |
| Cd | 0.0026 \pm 0.0011 (0.0005–0.0038) | 0.4077 | 0.0021 | – | 0.05 |
| Pb | 0.0664 \pm 0.0676 (0.018–0.193) | 1.0170 | 0.0540 | – | 0.20 |
| Mn | 6.72 \pm 1.79 (4.63–9.09) | 0.27 | 5.27 | – | – |

of heavy metals were determined by inductively coupled plasma mass spectrometry (ICP-MS) using an Agilent 8800 (Agilent Technologies, Tokyo, Japan) (19). The soil samples were air-dried to a constant weight, ground, and passed through a 100-mesh nylon sieve. Subsequently, 0.2 g of dry soil samples were microwave-digested, and the heavy metal concentrations were determined by ICP-MS (19). Recovery for the analyzed heavy metals ranged from 90 to 115%, and the accuracy of the standard deviation for duplicate samples was within 10%. The entire process complied with the quality requirements of the Chinese National Standard HJ/T 166-2004, Soil Environmental Monitoring Technical Specifications.

The I_{geo} and enrichment factor (EF) were applied to evaluate the contamination status of heavy metals in the soil (1). The I_{geo} is widely used to evaluate the contamination degree of heavy metals; it compares the measured concentration and background value in the following equation:

$$I_{geo} = \log_2 \left(\frac{C_i}{1.5 \times B_i} \right) \quad (1)$$

where C_i and B_i are the concentrations of each element in the sample and the background soil, respectively. The heavy metal concentrations in the CK area were regarded as background values.

The EF can be used to comprehensively compute contamination due to multiple heavy metals, and it can be evaluated by the following equation (20):

$$EF = \left(\frac{C_i}{C_{ref}} \right) / \left(\frac{B_i}{B_{ref}} \right) \quad (2)$$

where C_{ref} and B_{ref} are the concentrations of a reference element in the sample and background soil, respectively. Mn was used as the reference element because of its stability in the soil and the lower coefficient of variation (CV) (Table 1).

The bioaccumulation factor (BCF) served as an evaluation index to assess the extent of heavy metal contamination in maize (13). It was calculated by the following formula:

$$BCF = \frac{C_{maize}}{C_{soil}} \quad (3)$$

where C_{maize} and C_{soil} are the concentrations of heavy metals in the maize and the soil, respectively.

Probabilistic health risk assessment of soil and maize

Health risk assessment was used to evaluate and predict the probability of the occurrence of adverse effects in humans,

including non-carcinogenic and carcinogenic risks (21). Heavy metals can impact the human body after dermal contact with soil (der), expiratory inhalation of soil particles (inh), and oral ingestion of soil dust (ing) (19). In this study, the human exposure risk assessment method was used to evaluate the health risk of heavy metals. The model of exposure and parameters referred to the US EPA Exposure Factors Handbook: 2011 Edition, and the exposure factors of skin and average body weight were modified according to the technical guidelines for risk assessment of contaminated sites (HJ25.3-2014), the technical guidelines for deriving soil environmental criteria for human health (draft for comment) (2018), and related studies (3, 8, 21). The probabilistic health risk was calculated by the following formulas:

$$ADD_{ing} = \frac{C_i \times IngR \times CF \times EF \times ED}{BW \times AT} \quad (4)$$

$$ADD_{der} = \frac{C_i \times CF \times SA \times AF \times ABS \times EF \times ED}{BW \times AT} \quad (5)$$

$$ADD_{inh} = \frac{C_i \times IngR \times EF \times ED}{PEF \times BW \times AT} \quad (6)$$

$$HQ_i = \frac{ADD_i}{RfD_i} \quad (7)$$

$$HI = \sum_{i=1}^n HQ_i \quad (8)$$

$$CR_i = ADD_i \times SF_i \quad (9)$$

where ADD_{ing} , ADD_{der} , and ADD_{inh} represent the average daily doses of heavy metals in the soil and maize in $mg/(kg \cdot d)$; HQ_i is the hazard quotient used for estimating the non-carcinogenic effects of the i th heavy metal on a specific exposure pathway; HI is the hazard index calculated by estimating the sum of the HQ_i s; CR_i is the carcinogenic risk of the i th element attributed to all the pathways (19). The interpretation and values of the exposure parameters are shown in [Supplementary Tables S1, S2](#). A value of HQ or HI of <1 suggests that detrimental health effects are not possible in the exposed population; when HQ or HI is close to or over 1, the adverse health effects should receive more attention (22, 23). Cancer index values of heavy metals in the range of 10^{-6} – 10^{-4} are acceptable and do not pose a significant carcinogenic risk (3).

Probabilistic estimation was adopted to estimate the non-determinacy and variation in the risk assessment by the Monte Carlo simulation. The distribution of parameters (the concentrations of heavy metals, daily maize intake, exposure frequency, body weight, etc.) was determined according to the US EPA guidelines and the Chinese Exposure Parameters Guidebook. The process was performed with Oracle[®] Crystal Ball software. The Monte Carlo simulation was run for 10,000 iterations with 95% confidence level by randomly sampling values from the distribution of the exposure parameter. The population distribution of health risks was derived. A sensitivity analysis was performed by Crystal Ball (Oracle, Redwood City,

CA, USA) to verify the contribution of each variable to the health risk model.

Data analysis

Descriptive statistical analysis was performed to calculate the characteristics of the heavy metal contamination in soil and maize in Microsoft Excel 2016 (Microsoft Corporation, Redmond, USA); data are presented as means \pm standard deviations. To compare the heavy metal pollution of soil and maize in the control and polluted areas, comparisons of two means were analyzed by a t -test, and multiple comparisons were analyzed by one-way analysis of variance (ANOVA) followed by the Student–Newman–Keuls (SNK) test with SPSS 22.0 (IBM Corp, Armonk, USA). The level of significance was set at 5%. Additionally, correlation analysis and random forest analysis were performed to describe the relationship between two or more heavy metal variables using R Studio (Integrated Development for R. RStudio, MA, USA). The determination of the best-fitting distribution, Monte Carlo simulation, and sensitivity analysis were carried out in Crystal Ball software. P -values < 0.05 were regarded as significantly different, and all P -values and 95% confidence intervals were two-tailed. All figures were constructed with Origin 2018 (Origin Lab, MA, USA).

Results

Basic characteristics of heavy metals in soil and maize

Nine heavy metals (Cr, V, Ni, Pb, As, Ti, Cd, Cu, and Zn) were detected in all samples. The descriptive statistics of the studied heavy metals in soil and maize are summarized in [Figure 2](#) and [Table 1](#). In the soil samples, the mean concentrations of all heavy metals except V, Cr, and Ti were greater than their corresponding background levels ([Table 1](#)). According to the risk control standard of agricultural soil in China (GB15618-2018), none of the soil samples had heavy metals concentrations exceeding the screening value. The concentrations of soil heavy metal in the polluted areas were all greater than those in the control areas, except for the concentration of Hg.

A total of 77.78% of maize samples had Ni concentrations higher than the standard levels for corn in China (NY 861-2004). In addition, the mean concentrations of maize Ni were greater than the standard levels (NY 861-2004). The concentrations of all heavy metals in this study except V were greater than those in the CK area. This could indicate that most heavy metals (except V) accumulate in maize.

The analysis results indicate that heavy metals induced varying degrees of contamination in soil and maize in our study

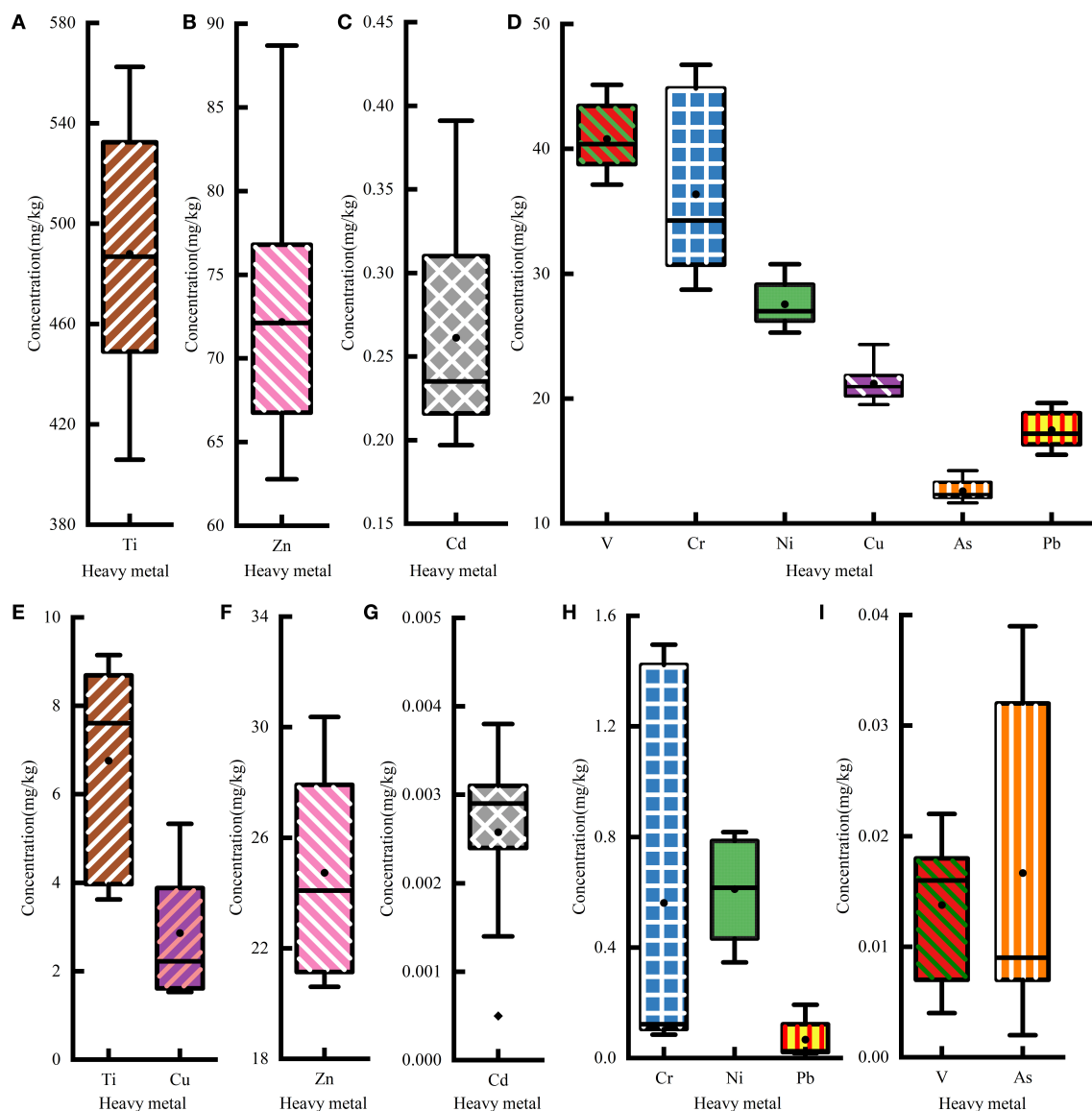


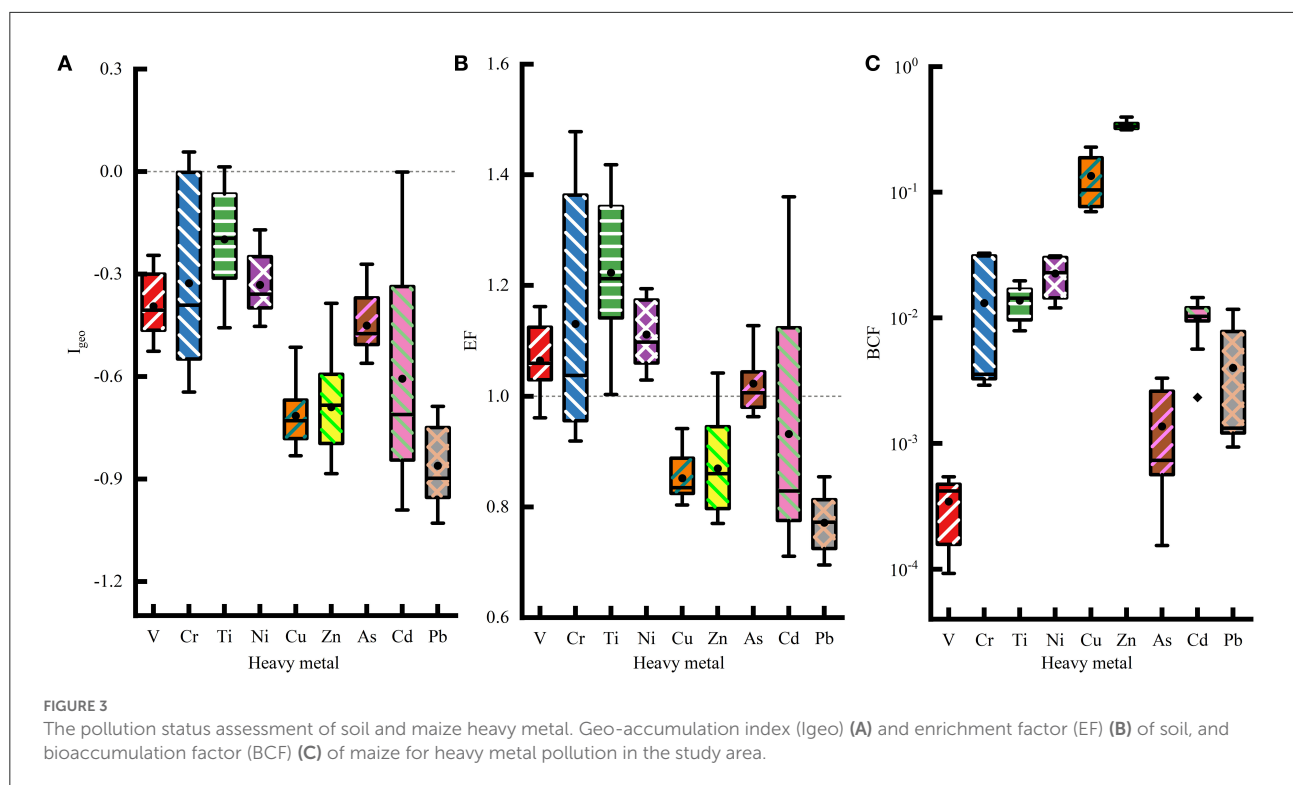
FIGURE 2
Statistics of the heavy metal concentration in agricultural soil and maize. The heavy metal concentrations of (A–D) soil and (E–I) maize.

area; Cd was the major soil pollutant, and Ni was the main maize pollutant.

Heavy metal pollution assessment of soil and maize

The I_{geo} , EF, and BCF were used to assess the pollution level of the soil and maize heavy metal, and the results are shown in Figure 3. The I_{geo} and EF were evaluated to assess soil heavy metal contamination status. The mean I_{geo} values of all heavy metals in the sampling areas were smaller than 0, indicating unpolluted levels (Figure 3A). The mean I_{geo} of

Cr in the TR area was higher than zero, indicating a slightly polluted level (Supplementary Figure S1A). The mean EF values of all heavy metals except for Zn, Cu, Cr, and Pb were higher than 1 (Figure 3B). Soil Pb had the lowest EF value (0.77) among the nine heavy metals. Soil V, Ti, and Ni were slightly enriched in all sampling areas. Soil Cr and Cd were slightly enriched in the TR area, and the soil Cr and As were slightly enriched in the CW area (Supplementary Figure S1B). Furthermore, soil V, Cr, Ti, and Ni had higher EF values than soil Cu, Zn, and Pb. Soil Ti was the most prevalent heavy metal in the study area and had the highest EF, followed by Cr. The EF values of Ti and Ni were higher than 1 in all samples.



The BCFs of all heavy metals in maize were < 1 (Figure 3C; Supplementary Figure S1C), which indicates that the physiological need for these elements was rather limited. In addition, the heavy metal pollution level was low. Zn had the highest BCF value, which indicates that it had the highest mobility in maize. Zn had the highest mean BCF values, followed by Ni, Cu, Ti, Cr, Mn, Cd, Pb, As, and V.

Health risk assessment of soil heavy metal

The probabilistic health risk assessment indexes related with the three direct soil exposure pathways (dermal absorption, ingestion, and inhalation) were assessed by the hazard quotient (HQ), hazard index (HI), carcinogenic risk (CR), and total carcinogenic risk (TCR) with the Monte Carlo simulation (Table 2).

The non-carcinogenic health risks were only estimated for Cr, V, Ni, Pb, As, Cd, Cu, and Zn because Ti lacked a reference exposure dose (Table 2). In children and adults, the mean HQ values of the eight heavy metals evaluated were lower than the risk threshold of 1. Particularly, the HQ value of As was at least an order of magnitude greater than values of the other heavy metals. The mean HI values were both lower than 1 (3.24×10^{-1} and 4.41×10^{-2} for children and adults, respectively; Figure 4A). These results indicate a low probability

of the occurrence of adverse health effects caused by the soil. Furthermore, the non-carcinogenic health risk to children was 7.35 times more severe than the risk to adults, indicating that children had a far higher chance of non-carcinogenic health consequences due to heavy metal exposure. For children and adults, soil ingestion posed the greatest health risk, followed by dermal absorption and inhalation (Table 2). As posed the highest total non-carcinogenic health risk (THQ) for adults and children, followed by Cr, Pb, V, Ni, Cu, Cd, and Zn. Specifically, As and Cr accounted for 73.92 and 13.27% of the HI for adults and 72.84 and 13.80% of the HI for children, respectively. Approximately 0.02% of all children had HI values > 1 , and none of the adults had an HI > 1 , suggesting a low non-carcinogenic health risk.

The CR was only estimated for Cr, Ni, As, Cd, and Pb because Zn, Ti, Cu, and V lacked carcinogenic slope factors (Table 2). The mean CR values of the five heavy metals evaluated were below the acceptable level of 1×10^{-4} . CR value of Ni contributed most to TCR, accounting for nearly 54.17 and 54.50% for children and adults, respectively. Ingestion was associated with the highest CR, followed by dermal contact and inhalation. The total CR of children was 7.77 times higher than that of adults. Notably, the CR value for heavy metal exposure *via* respiratory inhalation in children was approximately 106 times higher than that in adults. The mean TCR values were 1.14×10^{-5} and 2.19×10^{-5} in children and adults, respectively,

TABLE 2 Estimation of non-carcinogenic (Hazard quotient, HQ; Hazard index, HI) and carcinogenic risks (CR) posed by heavy metal in soil *via* Monte Carlo simulation.

| Population | V | Cr | Ni | Cu | Zn | As | Cd | Pb | Total |
|---------------------------|-----------------------|------------------------|-----------------------|-----------------------|-----------------------|------------------------|------------------------|------------------------|-----------------------|
| HQ-soil ingestion | | | | | | | | | |
| Adults | 2.09×10^{-3} | 5.72×10^{-3} | 6.36×10^{-4} | 2.45×10^{-4} | 1.11×10^{-4} | 1.94×10^{-2} | 1.22×10^{-4} | 2.32×10^{-3} | 3.06×10^{-2} |
| Children | 1.60×10^{-2} | 4.39×10^{-2} | 4.88×10^{-3} | 1.87×10^{-3} | 8.52×10^{-4} | 1.49×10^{-1} | 9.39×10^{-4} | 1.78×10^{-2} | 2.35×10^{-1} |
| HQ-dermal contact | | | | | | | | | |
| Adults | 4.73×10^{-5} | 1.29×10^{-4} | 1.44×10^{-5} | 5.54×10^{-6} | 2.52×10^{-6} | 1.32×10^{-2} | 2.77×10^{-6} | 5.25×10^{-5} | 1.34×10^{-2} |
| Children | 3.13×10^{-4} | 8.56×10^{-4} | 9.53×10^{-5} | 3.66×10^{-5} | 1.66×10^{-5} | 8.71×10^{-2} | 1.83×10^{-5} | 3.47×10^{-4} | 8.88×10^{-2} |
| HQ-soil inhalation | | | | | | | | | |
| Adults | 4.50×10^{-7} | 1.23×10^{-6} | 1.37×10^{-7} | 5.26×10^{-8} | 2.39×10^{-8} | 4.18×10^{-6} | 2.63×10^{-8} | 4.99×10^{-7} | 6.59×10^{-6} |
| Children | 8.98×10^{-7} | 2.46×10^{-6} | 2.73×10^{-7} | 1.05×10^{-7} | 4.78×10^{-8} | 8.33×10^{-6} | 5.26×10^{-8} | 9.96×10^{-7} | 1.32×10^{-5} |
| HI | | | | | | | | | |
| Adults | 2.14×10^{-3} | 5.85×10^{-3} | 6.51×10^{-4} | 2.50×10^{-4} | 1.14×10^{-4} | 3.26×10^{-2} | 1.25×10^{-4} | 2.37×10^{-3} | 4.41×10^{-2} |
| Children | 1.63×10^{-2} | 4.47×10^{-2} | 4.97×10^{-3} | 1.91×10^{-3} | 8.69×10^{-4} | 2.36×10^{-1} | 9.57×10^{-4} | 1.81×10^{-2} | 3.24×10^{-1} |
| CR-soil ingestion | | | | | | | | | |
| Adults | – | 2.41×10^{-6} | 6.05×10^{-6} | – | – | 2.45×10^{-6} | 2.14×10^{-7} | 1.93×10^{-8} | 1.11×10^{-5} |
| Children | – | 4.60×10^{-6} | 1.16×10^{-5} | – | – | 4.66×10^{-6} | 4.08×10^{-7} | 3.68×10^{-8} | 2.13×10^{-5} |
| CR-dermal contact | | | | | | | | | |
| Adults | – | 5.49×10^{-8} | 1.38×10^{-7} | – | – | 5.57×10^{-8} | 4.88×10^{-9} | 4.39×10^{-10} | 2.54×10^{-7} |
| Children | – | 9.09×10^{-8} | 2.29×10^{-7} | – | – | 9.23×10^{-8} | 8.08×10^{-9} | 7.28×10^{-10} | 4.21×10^{-7} |
| CR-soil inhalation | | | | | | | | | |
| Adults | – | 5.20×10^{-10} | 1.30×10^{-9} | – | – | 5.27×10^{-10} | 4.61×10^{-11} | 4.15×10^{-12} | 2.40×10^{-9} |
| Children | – | 5.49×10^{-8} | 1.38×10^{-7} | – | – | 5.57×10^{-8} | 4.88×10^{-9} | 4.39×10^{-10} | 2.54×10^{-7} |
| TCR | | | | | | | | | |
| Adults | – | 2.46×10^{-6} | 6.19×10^{-6} | – | – | 2.50×10^{-6} | 2.19×10^{-7} | 1.97×10^{-8} | 1.14×10^{-5} |
| Children | – | 4.74×10^{-6} | 1.19×10^{-5} | – | – | 4.81×10^{-6} | 4.21×10^{-7} | 3.80×10^{-8} | 2.19×10^{-5} |

within the acceptable range of 1×10^{-6} to 1×10^{-4} (Figure 4B). Approximately 0.02% of all adults and 1.14% of children had HI values $> 10^{-4}$. Therefore, the CR differed between age groups. In general, the risks of soil heavy metals to human health were tolerable, and might be close to the acceptable limit.

The sensitivity analyses to TCR and HI showed of the exposure parameters, oral ingestion rate of soil dust contributed the most to the HI of adults and children with correlation coefficients of 0.75 and 0.69, respectively (Figure 4C). The content of soil As contributed the most to the HI among these heavy metals (correlation coefficients of 0.12 in adults and 0.09 in children). Of the exposure parameters, the oral ingestion rate of soil dust contributed the most to CR in adults, followed by exposure duration; the correlation coefficients for soil ingestion rate and exposure duration were 0.58 and 0.50, respectively (Figure 4D). For children, the order was the opposite: exposure duration had the greatest contribution, followed by the soil ingestion rate. The Cr content in the soil contributed the most to the TCR among all heavy metals. Body weight and average exposure time showed a negative impact on the HI and TCR of inhabitants.

Health risk assessment of maize heavy metal

A comparison of non-carcinogenic human health risks between soil and maize exposure is provided in Table 3. In children and adults, the mean HQ values of the eight heavy metals evaluated for maize were lower than 1; Cr had the greatest HQ value, followed by Cu, Zn, As, Ni, Pb, Cd, and V. The mean HI values of children and adults *via* maize consumption were 2.51×10^{-4} and 1.95×10^{-3} , respectively (Figure 4E). Adults and children exhibited a lower risk from maize consumption than from soil exposure. Children were 7.77 times more likely to face serious risk from maize heavy metal pollution than adults. None of the HI for all inhabitants were more than 1, suggesting no non-carcinogenic health risk.

The health risk results of maize consumption revealed no carcinogenic effects of Cr, Ni, As, Cd, or Pb. The cancer risk of Ni was the greatest among these heavy metals *via* maize ingestion within an acceptable range for all inhabitants. The CR of Ni contributed the most to the TCR, with a contribution of

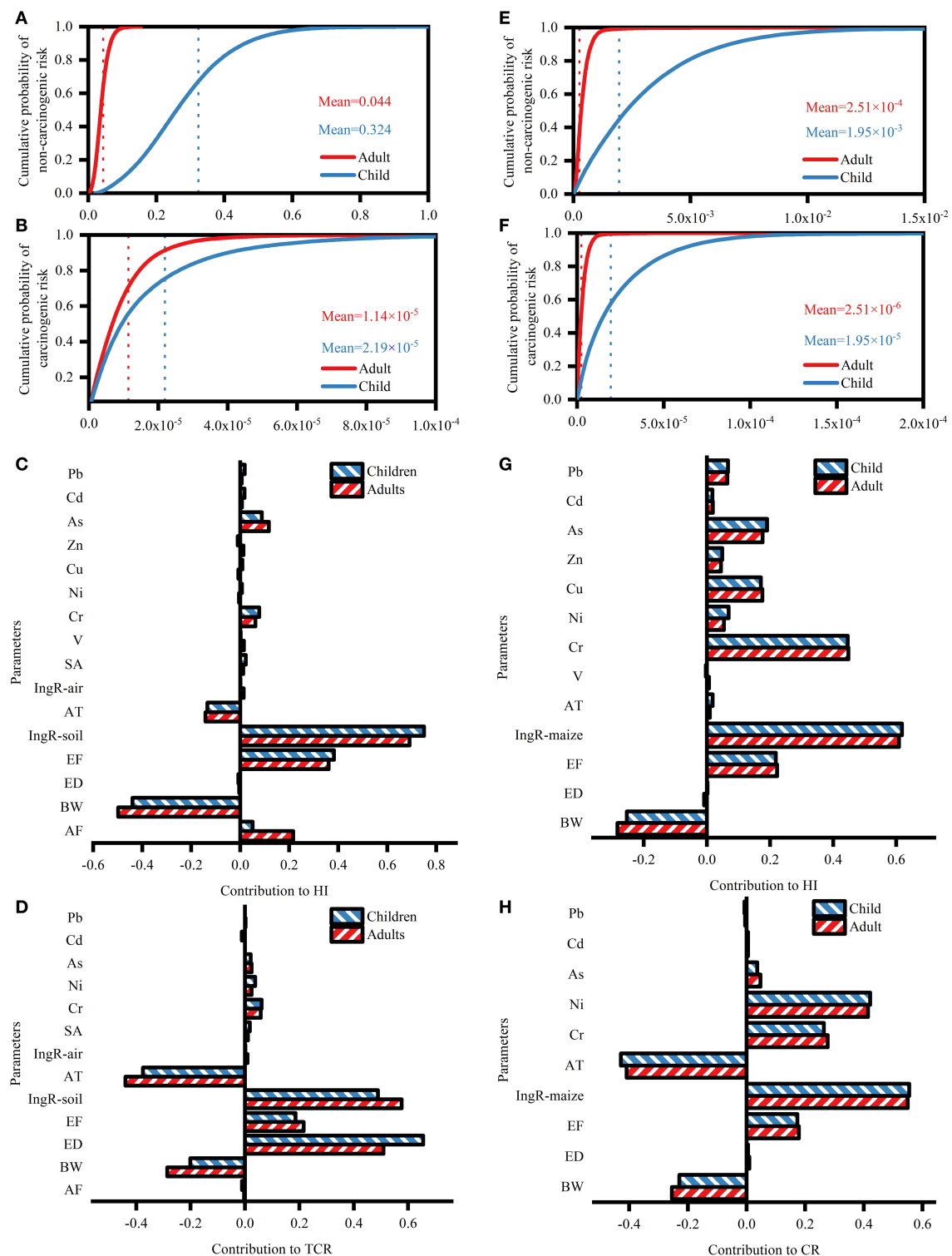


FIGURE 4

The health risk assessment of heavy metal. (A) Probability distribution of hazard index (HI) and (B) total cancer risk (CR) in soil. (the blue or red dashed vertical lines presented the mean values for adults and children); (C) Contribution of different exposure parameters to hazard index (HI) and (D) total cancer risk (TCR) in soil; (E) Probability distribution of hazard index (HI) and (F) total cancer risk (CR) in maize. (The blue or red dashed vertical lines presented the mean values for adults and children); (G) Contribution of different exposure parameters to hazard index (HI) and (H) total cancer risk (TCR) in maize.

69.32% for adults and 68.21% for children, respectively. The mean TCR values and *via* maize consumption were 2.51×10^{-6} for adults and 1.95×10^{-5} for children, respectively (Figure 4F). Approximately 0.02% of adults and 1.44% of children had HI values $> 10^{-4}$. Thus, the CR was greater for children than for adults. The overall risk from soil exposure was higher than that from maize consumption (4.54 times for adults and 1.12 times for children).

The results of the sensitivity analysis revealed that the consumption of maize was the most sensitive parameter for human health risk, with a contribution of 60.85% for adults and 61.82% for children to non-carcinogenic health

risk (Figure 4G) and a contribution of 54.95% for adults and 55.46% for children to CR (Figure 4H). The second most sensitive parameter in the non-carcinogenic health risk assessment was the maize Cr concentration (44.86% contribution for adults and 44.62% contribution for children). The second most sensitive parameter in the carcinogenic risk assessment was the concentration of Ni in maize (41.42% contribution for adults and 42.15% contribution for children). Body weight showed a negative impact on the HI and TCR in all inhabitants. Furthermore, the average exposure time negatively affected the TCR, with a higher contribution than body weight.

TABLE 3 Estimation of non-carcinogenic (Hazard quotient, HQ; Hazard index, HI) and carcinogenic risks (CR) posed by heavy metal in maize *via* Monte Carlo simulation.

| Population | V | Cr | Ni | Cu | Zn | As | Cd | Pb | Total |
|------------|-----------------------|-----------------------|-----------------------|-----------------------|-----------------------|-----------------------|-----------------------|-----------------------|-----------------------|
| HQ | | | | | | | | | |
| Adults | 8.96×10^{-7} | 1.20×10^{-4} | 1.33×10^{-5} | 3.98×10^{-5} | 3.65×10^{-5} | 3.02×10^{-5} | 1.12×10^{-6} | 1.01×10^{-5} | 2.51×10^{-4} |
| Children | 6.88×10^{-6} | 9.29×10^{-4} | 1.03×10^{-4} | 3.12×10^{-4} | 2.81×10^{-4} | 2.33×10^{-4} | 8.58×10^{-6} | 7.83×10^{-5} | 1.95×10^{-3} |
| CR | | | | | | | | | |
| Adults | – | 6.81×10^{-7} | 1.74×10^{-6} | – | – | 5.17×10^{-8} | 2.70×10^{-8} | 1.19×10^{-9} | 2.51×10^{-6} |
| Children | – | 5.59×10^{-6} | 1.33×10^{-5} | – | – | 3.96×10^{-7} | 2.08×10^{-7} | 8.95×10^{-9} | 1.95×10^{-5} |

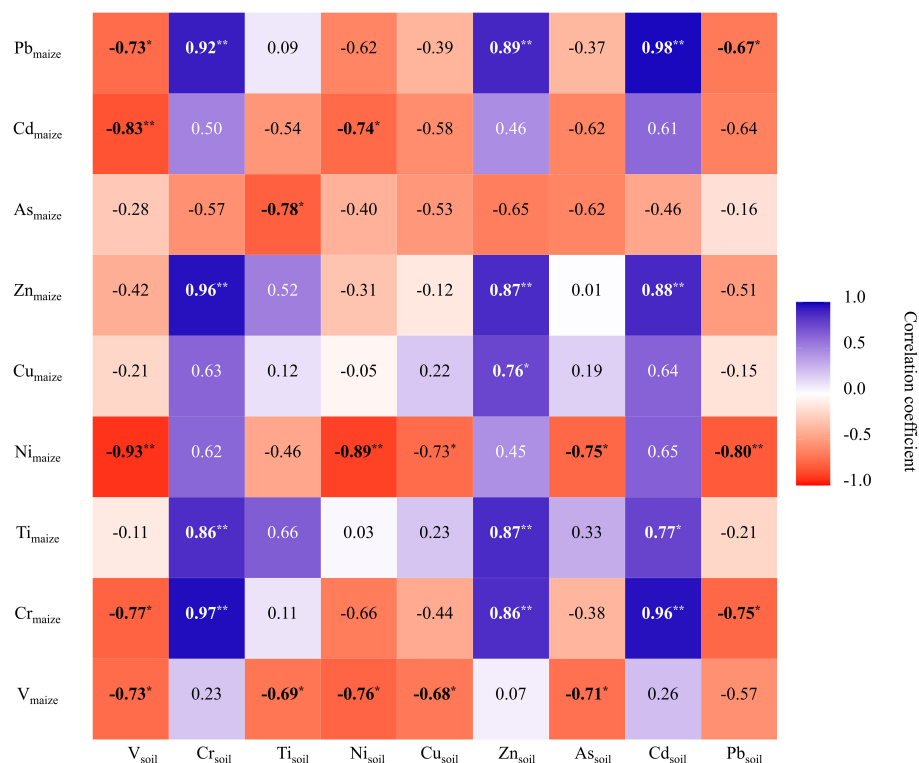


FIGURE 5 Correlations between heavy metal concentrations in maizes and soil.

Relationship between heavy metal concentrations in the soil and maize

Strong correlations were identified between the heavy metal concentrations of soil and maize. The Pearson correlation analysis results are shown in Figure 5. Overall, maize Ni, Pb, and Cr were affected the most by soil heavy metals, followed by V and Ti. Specifically, the maize Zn, Pb, and Cr concentrations showed a very significant positive correlation with Cr, Zn, and Cd concentrations in soil. Furthermore, maize Pb and Cr concentrations were significantly negatively correlated with soil V and Pb concentrations, and the maize Ni concentration showed a very significant negative correlation with soil Pb, Ni, and V. Moreover, the concentration of maize Ni showed a very significant negative correlation with soil Ni. By contrast, maize Cd showed a very significant negative correlation with soil V. The concentration of maize Ti showed a very significant positive correlation with soil Zn and Cr.

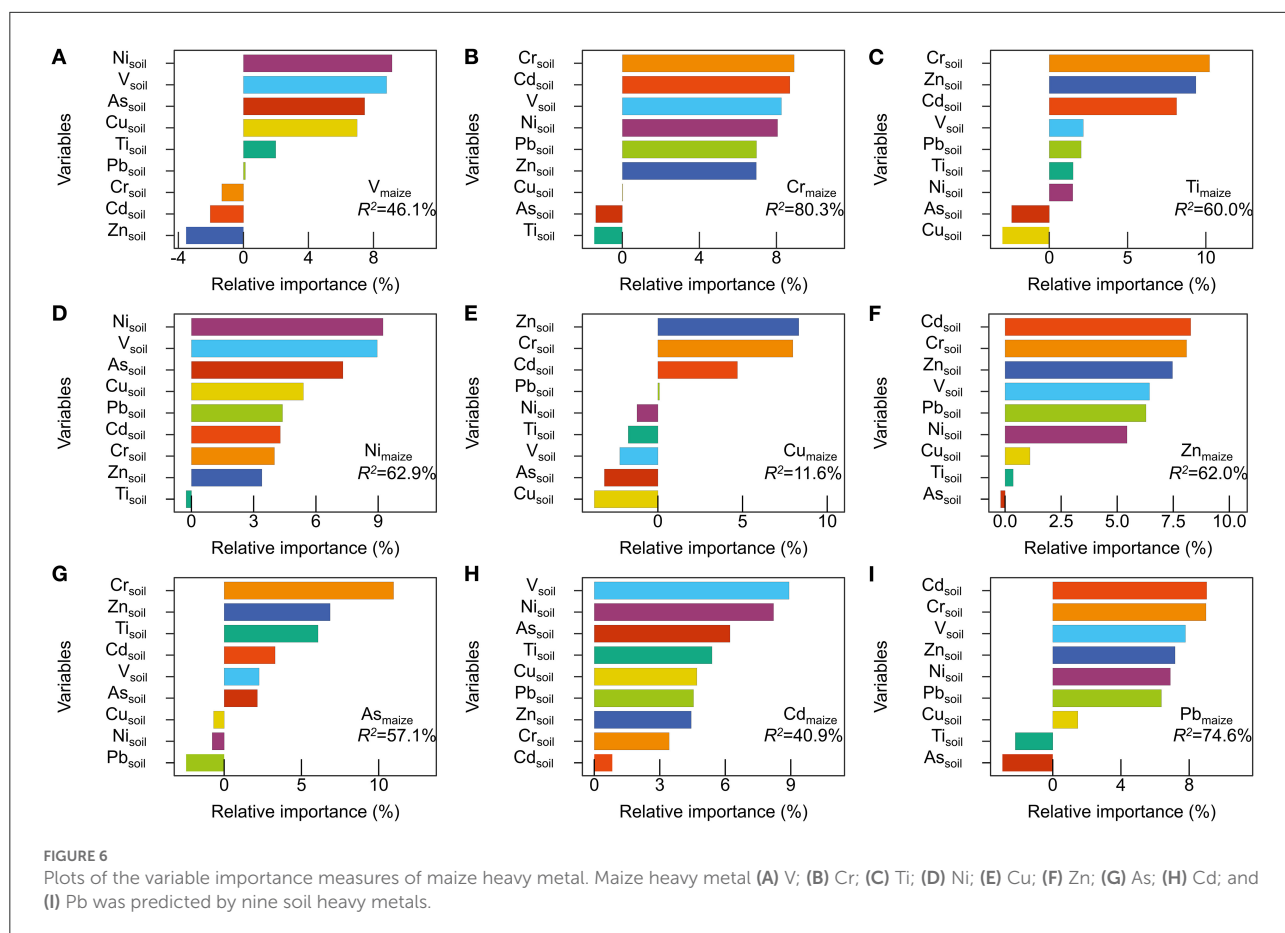
The relative importance of the nine soil heavy metals to the nine maize heavy metals was determined by random forest models (Figure 6). The concentrations of soil Cr, Cd, and V were the factors with the highest influence on the concentration of the nine maize heavy metals. Compared with other heavy metals in

maize, soil Cr contributed more to maize Cr, Cu, Ti, As, Pb and Zn; soil Cd contributed more to Cu, Cr, Ti, Pb, and Zn in maize; soil V contributed more to Cr, V, Ni, Cd, and Pb in maize. The three most crucial factors explaining the maize V, Ni, and Cd contents were soil Ni, As, and V; the three most crucial factors explaining the maize Cr and Pb contents were soil Cr, Cd, and V; and the three most crucial factors explaining the maize Ti, Zn, and Cu contents were soil Cr, Zn, and Cd. Finally, the three most crucial factors explaining the maize As content were soil Ti, Zn, and Cr.

Discussion

Assessment of heavy metal pollution in soil and maize

This study demonstrated that the pollution of soil and maize heavy metals, especially Ni, differed in the coal mining area. The mean concentrations of soil Ni, Ti, Cd, Cu, Pb, As, Sn, Hg, and Zn were all greater than the local background levels in Shanxi Province, but the values did not exceed the screening values (GB15618-2018). Notably, only the Ni concentration of maize



was higher than the screening value (NY 861-2004), which might be due to the higher capacity of maize to attract soil Ni (17). Furthermore, some researchers have reported that atmospheric deposition, such as coal dust, and polluted irrigation water, such as wastewater from coal washing plants, may also increase the maize Ni (24, 25). Coal dust is generated in the process of coal mining and transportation and can readily diffuse into the atmosphere and be deposited onto the surface of corn (26). Wastewater from coal mine processing around the investigated areas was positively correlated with the accumulation of maize Ni (27). Additionally, because Ni is a key soil heavy metal contaminant in the agricultural soil, higher Ni mobility might cause abnormal growth and development in the maize system (28). It is necessary to reverse Ni pollution in the soil to eliminate its risks to the ecosystem and human health.

The I_{geo} , EF, and BCF indices were evaluated to the contamination of the heavy metals in soil and maize. The mean I_{geo} of all heavy metals in the sampling areas was < 0 , which indicated low geochemical contribution to these heavy metals in the cultivated soil (20). The mean EF values of V, Cr, Ti, Ni, and As were higher than 1, suggesting the enrichment of these heavy metals. Specifically, the EFs of Cr and Cd in the TR area fell into the significantly enriched range, which suggests that this region had substantial Cr and Cd inputs (1). On a large farm in Ghana, the enrichment of Cr and Ni was greater than that of Zn, Hg, Cd, and Fe (20). In our study, V, Cr, Ti, and Ni had high enrichment (EFs > 1), consistent with the results of Affum et al. (20). The BCFs of all heavy metals in maize were < 1 , demonstrating that maize's physiological need for these heavy metals was rather limited (29). A higher BCF indicates a stronger ability of maize to attract heavy metals from the soil and an inferior capability to retain heavy metals (15). In our study, Zn and Cu had the highest BCFs, which implies that maize can store Zn and Cu more easily than other heavy metals (30).

Probabilistic health risk assessment of soil heavy metal

The hazard index values of all heavy metals in the soil of the study area were lower than 1, indicating no significant non-carcinogenic health risk (31). The hazard quotient of As was more than an order of magnitude greater than those of other heavy metals. In Panzhihua City, the non-carcinogenic health risk of soil As was the greatest among all heavy metals, with an $HQ > 1$ (32). Although As had a lower concentration in crops than other heavy metals in Hamadan and a lower reference dose, the As concentration was far above the tolerable limit (8). The total non-carcinogenic health risk of children was 7.35 times more severe than that of adults, which indicates that children were more impressionable to soil heavy metal contamination, possibly because of their frequent hand-to-mouth behavior and higher respiration rate per unit of body weight (19).

The total carcinogenic risk values for all inhabitants fell within the acceptable range of carcinogenic risk; similar results were found in a study by Chen et al. (19). The concentration of soil Ni had the greatest average CR value. Along the South China coast, soil Ni also had the highest CR value compared with As, Cd, and Cr (33). Cancer risk was largely attributed to Ni, which accounted for 54.17 and 54.50% of TCR (33). In addition, long-term environmental exposure to Ni was reported to be correlated with an growing risk of gastrointestinal cancer (34). The high CR in children might be related to frequent hand-to-mouth behavior; the relationship between their exposure and body size; their developing body; or their poor ability to metabolize and excrete toxins (8). In our study, the pathway of oral ingestion contributed most to human health risks. The results of research from Sialkot in Punjab, Pakistan, showed that the ingestion was a major contributor to TCR, followed by dermal and inhalation pathways (31).

The sensitivity analysis of health risk assessment showed that the contributions of soil As and Cr concentrations were significantly greater than those of other heavy metals; As and Cr had a strong impact on the potential non-carcinogenic health risk and carcinogenic health risk in all populations, respectively. However, the most influential factors of soil health risk were soil ingestion rate and exposure duration, whereas body weight and average exposure time had a negative influence. Consistent with our results, Kharazi et al. (8) supported that the soil ingestion rate and exposure duration most easily affected the risk assessment of different populations, and body weight was a sensitive parameter for CR with a negative correlation. In probabilistic health risk research conducted by Wen'ling, body weight showed a negative impact on the HI and TCR of heavy metal exposure (19).

In conclusion, the non-carcinogenic and carcinogenic risks were all within the acceptable range, but the exposure risk of soil As and Ni among all heavy metals contributed most to the non-carcinogenic and carcinogenic risks, respectively. Therefore, the monitoring of As and Ni in soils needs to be strengthened to prevent health risks. Soil ingestion was identified as the most crucial exposure pathway for soil heavy metals (31). The reduction of health risk by soil ingestion could be bought about by reducing human interaction with the soil and farming time; this could be accomplished with the automation of farming practices, such as wireless communications, machine learning, artificial intelligence, and deep learning (35).

Probabilistic health risk assessment of maize heavy metal

The potential non-carcinogenic health risk and carcinogenic risk of maize heavy metals fell within the acceptable range; these results were consistent with those of Liu et al. (11). The concentrations of maize Cr and Ni were the major

contributors to non-carcinogenic health risk and carcinogenic risk, respectively. Taiwo et al. (14) reported that Cr had the largest HQ of all metals, with an $HQ > 1$ in the maize samples, and the carcinogenic evaluation of Ni and Cr in crops showed CR values above the acceptable threshold of 1.0×10^{-4} . Children were the most sensitive population in terms of the non-carcinogenic health risk and carcinogenic risk of consuming maize in the contaminated area. Because maize is the residents' major food, it might contribute to the accumulation of heavy metals and heavy metal poisoning, especially in pregnant people and children (13).

Regarding the sensitivity analysis of health risk assessment, the consumption of maize was the most sensitive parameter for human health risk. These results are similar to those from research on heavy metals in agricultural soil and food crops in Hamadan, Iran (8). The contribution of maize Cr and Ni concentrations was significantly higher than that of other heavy metals in the assessment of non-carcinogenic health risk and carcinogenic risk, respectively. These results imply that controlling the consumption of maize and monitoring maize heavy metals, especially Cr, As, and Ni, could effectively reduce the health risk for residents.

Relationship between heavy metal concentrations in the soil and maize

In this study, correlation analysis and random forest analysis demonstrated that the concentrations of soil Cr, V, and Cd could contribute more to the absorption of heavy metals by maize. Soil Cr increased the absorption of Cr, Ti, Cu, Zn, and Pb by maize and decreased the absorption of maize As, suggesting that soil Cr could accelerate the crop assimilation of Ti, Cr, Cu, Zn, and Pb. Xiang et al. found that the assimilation of Zn by crops was affected by the synergistic effect of Cr in soil (17), which supports the findings of the present study. Huang et al. (30) reported that the concentrations of Cr and Ni in dryland soil were positively correlated with the concentrations of heavy metals in corresponding crops in Hunan Province, China. Soil Cd was positively correlated with maize Cd, but the correlation was not significant. A study by Wang et al. (36) found that different Cd sources, such as irrigation, fertilization, manure fertilizer, and atmospheric deposition, had a strong influence on the uptake of Cd by rice. Additionally, the assessment of heavy metal contamination of maize should consider the heavy metal availability instead of only relying on the total soil heavy metal concentrations (7). A change in the soil's physical and chemical properties could be one of the reasons for the lower mobility and bioavailability of heavy metals, which could lead to higher heavy metal concentrations (37). Soil Ni contributed negatively to the absorption of V, Ni, and Cd by maize. Using Pearson correlation analysis, Huang and Gui

observed a negative correlation between Ni in soil and Cd in maize grain parts (38). The concentrations of soil V and Ni had a very significant negative contribution to the absorption of corresponding heavy metals by maize, indicating that the pollution of soil V and Ni might not be the main source in maize and there might be other pollution sources (38). The establishment of random forest models would be helpful to preliminarily predict the concentrations of these heavy metals and contribute to quantitatively and comprehensively assessing the ecological and health risks of heavy metals in maize.

Conclusion

Soil heavy metals posed severe risks to human health through the food chain. This study intended to investigate the heavy metal content of soil and maize, assess health risks and explore the relationships between heavy metals in the soil and maize, to provide support for early warning of human health risks. Although the average soil heavy metal concentrations did not exceed the national standards, the average concentrations of maize Ni exceeded the standards for food. The health risks for nearly all maize and soil heavy metals were low. The soil As and Ni concentrations contributed the most to non-carcinogenic and carcinogenic risks, respectively, and maize Cr and Ni contributed the most to non-carcinogenic and carcinogenic risks, respectively. Furthermore, the results showed that maize heavy metals were influenced the most by Cr, Cd, and V in the soil. Further studies are recommended to explore the transfer mechanism of Ni between soil and maize and regulate the Ni content in maize within an appropriate range. The significance of limiting Ni concentrations in the maize to 0.40 mg/kg is highlighted. This study shed light on heavy metal pollution and build on previous by providing detailed information on maize.

The Monte Carlo method was employed for probabilistic estimation of health risks, taking into account the variability of exposure parameters and the uncertainty of heavy metal concentrations, which made the results more reliable. Whereas, the health risk of crop heavy metal in this study was assessed only for maize as the representative crop in the investigated areas, but did not consider any other crops, such as rice, wheat and vegetables. To comprehensively estimating human health risks in the future research, detailed consumption lists should be developed to quantify sources of heavy metals. The random forest model was used for assessing the inner relationship of pollution risk in soil-crop system, revealing the complex relationships between soil and maize heavy metals. However, the random forest model could not reveal the dynamic changes in heavy metal concentrations between soil and maize. Therefore, it is necessary to explore the dynamic process of heavy metal transfer from soil to crops.

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

XY and BC: conceptualization, methodology, software, validation, formal analysis, data curation, and visualization. XY, YG, and HZ: investigation. HZ and LL: resources and supervision. BC: writing—original draft preparation. XY and LL: writing—review and editing. LL: project administration. XY: funding acquisition. 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.2022.1004579/full#supplementary-material>

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Esophagus cancer and essential trace elements

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Numerous epidemiological and laboratory studies on essential trace elements have reported protective associations in developing various cancer types, including esophagus cancer (EC). However, the results are not always consistent. Some essential trace elements could play a vital role in preventing esophagus cancer. Some showed no association with esophageal cancer risk, while others harmed individuals. This article reviews the association between the intake or supplementation of essential trace elements (especially zinc, copper, iron, and selenium) and the risk of esophageal cancer. Generally, zinc intake may decrease the risk of esophageal cancer (EC), especially in high esophageal squamous cell carcinoma (ESCC) prevalence regions. The association between copper supplementation and EC remains uncertain. Total iron consumption is thought to be associated with lower EC risk, while heme iron intake may be associated with higher EC risk. Selenium intake showed a protective effect against EC, especially for those individuals with a low baseline selenium level. This review also prospects the research direction of the association between EC and essential trace elements.

KEYWORDS

esophagus cancer, essential trace elements, minerals, zinc, copper, iron, selenium

Introduction

Esophageal cancer (EC) is the seventh leading cause of cancer death worldwide (1) and the fourth leading cause of cancer death in China (2). The 5-year survival rate for EC remains one of the lowest in all cancer types, with a 5-year survival of 20% in the United States (1) and 40.1% in China (3). More than 90% of EC patients in China are diagnosed with esophageal squamous cell carcinoma (ESCC) (3, 4), the world's most common type of EC. In Western nations, alcohol drinking and smoking are the primary risk factors for ESCC (5), whereas, in so-called "esophageal cancer belts" such as South Africa, France, Iran, and China, these behaviors are less prevalent (6). Environmental and dietary factors have also been reported to affect ESCC, and essential trace elements may be dose-dependent on the risk of ESCC. Essential trace elements of the human body account for roughly 0.00001% to 0.01% of the total body weight, including Fe, Zn, Cu, Mo, F, V, Ni, Co, Se, Cr, I, and Mn. They play a significant role in maintaining normal biological function, acting as active centers of enzymes or tracing bioactive substances. Numerous studies have attempted to illustrate the meaning of balancing essential trace elements in EC protection or treatment. Yet, the findings of these studies are not always the same. Here, we focus on the connection of esophagus cancer with essential trace elements, especially zinc, copper, iron, and selenium.

Zinc

Zinc is one of the most abundant trace elements found in almost all organs and tissues of the body. It plays a critical role in stabilizing the structures of many proteins, especially those participating in DNA synthesis and RNA transcription, thus regulating cell growth, development, and differentiation, maintaining an immune response, and mediating oxidative stress and apoptosis (7–9). Among all the biological functions, it should be highlighted that zinc could inhibit chronic or acute oxidative stress, which is one of the mechanisms of cancer development. Zinc deficiency impairs the antioxidant activity and renders the organism more susceptible to injury induced by various oxidative stressors (10). Therefore, an adequate level of zinc is important for individuals. Some laboratory studies have established an association between zinc deficiency and cancer (11–16). Zinc homeostasis could influence T-cell activation as well as the polarization of T helper (Th) cells into their different subpopulations [Th1, Th2, Th17, regulatory T cells (Treg)], thus regulating cancer immune microenvironment (15). Choi et al. found that zinc could inhibit cell proliferation of EC cells through Orai1-mediated intracellular Ca^{2+} oscillations and revealed a possible molecular basis for zinc-induced cancer prevention and the Orai1-SOCE signaling pathway in cancer cells (16). Several studies have already shown the relationship between dietary zinc deficiency and ESCC (17, 18). ESCC in Linxian is a high-incidence area in China. A study from Linxian showed that the zinc concentration in biopsy samples was negatively correlated with the venture of EC development, exhibiting strong proof for the connection of dietary zinc deficiency with the higher risk of EC in humans (17).

Zinc consumption was significantly associated with a lower risk of EC and gastric cancer in Asia but not in the United States or Europe (19). The results of a meta-analysis also indicated that increasing zinc supplementation by 5 mg/day was associated with a 15% decrease in EC risk (20). It was reported that the mechanisms between zinc deficiency and the development of EC lie in that zinc deficiency could result in the upregulation of multiple genes related to DNA damage, oxidative stress, immune response, cell proliferation, and apoptosis, thus inducing the development of EC (18). Animal studies showed that ESCC cells proliferated more rapidly in mice fed zinc-deficient diets *via* inducing overexpression of COX-2, P38, PCNA, and NF- κ B (18). ESCC can also be promoted by zinc deficiency through inflammatory gene expression (21, 22) and oncogenic microRNA expression, including upregulation of oncogenic miR-31 and other miRNAs (21, 23). Deeper molecular mechanisms induced by nutrient bioavailability or dietary interventions have recently been studied using integrative genomics methods (24). Fong et al. (25) demonstrated that zinc intake reduced COX-2 mRNA by 80%, an enzyme involved in inflammation, thus bringing prevention or therapeutic possibilities of zinc supplementation for EC. A

recent study found that zinc supplementation could protect Barrett's epithelia from transforming into esophageal cancer cells (26), further revealing zinc supplementation's protective effects. The role of zinc in EC diseases is not fully answered and needs further investigation. It seems that zinc intake may decrease EC risk, especially in high ESCC prevalence regions.

Copper

Copper is an essential trace element that the body requires and is vital in many biological functions, including maintaining DNA integrity, synthesizing essential metabolites, transporting oxygen to the mitochondrial respiratory chain, and involving redox reactions as an active site metabolic cofactor. Emerging laboratory studies showed that copper could act as a dynamic signaling metal and metalloallosteric regulator, participating in cell growth and proliferation, autophagy, and antioxidant defense, thus regulating cancer development, as tumor growth and metastasis have a high requirement for this metal nutrient (27). Such properties make the dual copper effect beneficial and toxic to the cells. An intake of 900 μg per day is recommended for this essential trace element, while a level of 10 mg per day is the maximum permissible (28). Excess copper may promote radical damage and decrease the activity of proteins or enzymes, thus causing cellular injury *via* over-activated oxidative stress, lipid peroxidation, inflammation, and DNA damage, finally helping in the angiogenesis of tumors (29). It remains controversial whether copper intake contributes to EC prevention, despite many studies focusing on the relationship between copper intake and the disease. Chen et al. (30) claimed that copper intake was negatively correlated with EC mortality in Shanxi, China, *via* estimating average copper intake in 21 Chinese communes, where EC mortality rates were much higher than average (30). Similarly, according to Sohrabi et al. (31) the copper levels were obviously lowered in EC tissues compared to non-cancerous tissues (31). Recently, Zhuang et al. (32) even explored the therapeutic effect of copper nanoparticles in EC treatment *via* their antioxidant activities (32). However, in the Kashmir valley, an area at high risk for EC in India, as reported by Mir et al. (33) plasma levels of copper in patients with EC were significantly higher than in controls, indicating an imbalance in plasma levels of copper may be responsible for the development of the disease (33). Copper supplementation and ESCC risk were evaluated by Hashemian et al. (34) and a nonlinear association was found between copper intake and ESCC risk, but the supplementation trend related to ESCC was not evident (34). In addition, a meta-analysis found that copper supplementation at 1 mg/day did not reduce the risk of EC (20). Altogether, the relationship between copper and EC remains uncertain. Copper supplementation recommendations still require additional research.

Iron

Iron is an important component of heme, iron-sulfur proteins, and enzymes and participates in many biological processes, including oxygen transport, ferroptosis, immune response, cellular energy metabolism, and many other enzymatic reactions. Physiological processes have evolved for iron acquisition to meet metabolic needs while avoiding the toxicity of free radicals generated from iron. Iron excess and iron deficiency are related to pathological states (35). Recent studies have focused on ferroptosis, a newly discovered iron-dependent mode of cell death that plays an important role in the biological behavior of cancer cells. Zhu et al. (36) and Qiao et al. (37) found that ferroptosis-related noncoding RNAs correlate with the prognosis, tumor microenvironment, and therapeutic sensitivity of ESCC, indicating new therapeutic approaches for noncoding RNAs targeting ferroptosis in ESCC (36, 37). The role of iron in EC development has been under investigation. However, the results are still conflicting regarding the association of iron levels with the risk of EC. Sohrabi et al. (31) evaluated the iron concentrations between cancerous and non-cancerous tissues in EC. The results showed that the iron level in cancerous tissues was higher. However, no significant differences were revealed in EC (31). Several basic research indicated that the excess levels of iron enhanced EC (38–40), and the mechanisms possibly were related to the over-expression of iron import proteins, DNA damage, ferroptosis, and oxidative stress (41, 42).

Furthermore, it was found that total iron intake and heme iron intake were different from the risk of EC. Total iron supplementation was significantly inversely correlated with the risk of EC, especially among the Asian population and ESCC subgroup. A dose-response analysis indicated that each 5 mg/day increase in total iron supplementation was related to a 15% reduction in EC risk. However, heme iron intake was positively related to the risk of EC, especially in the United States. Each 1 mg/day increase in heme iron supplementation increased the risk of EC by 21% (20).

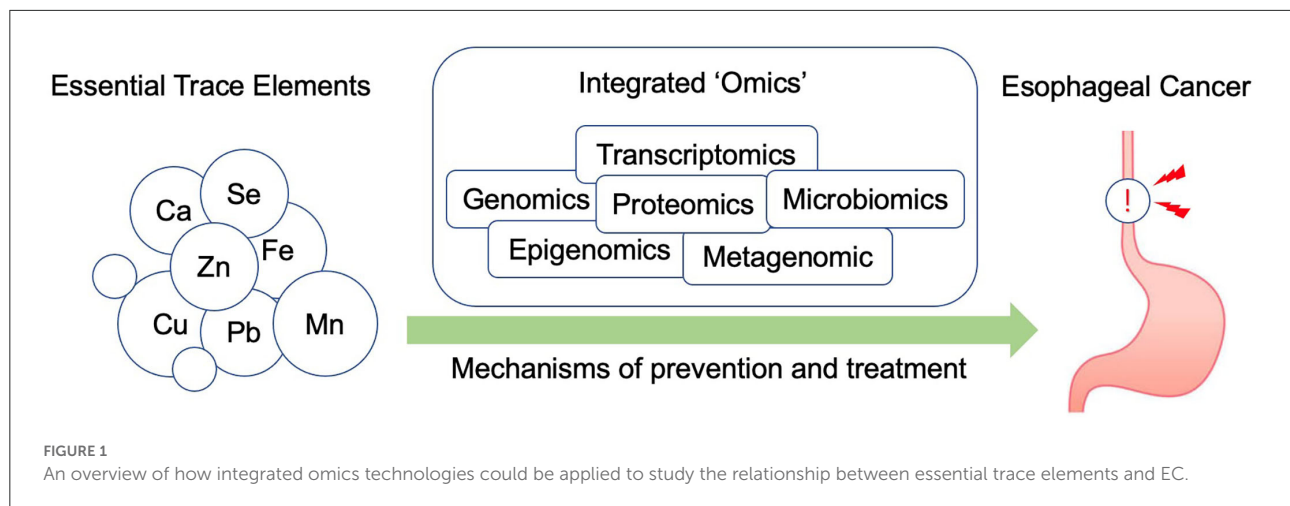
Similarly, a large European cohort study found that higher intakes of processed meat and heme iron may be related to an increased risk of developing EC, especially esophageal adenocarcinoma (43). Cross et al. (42) also found that heme iron supplementation may be associated with a risk for esophageal adenocarcinoma (EAC). They observed a positive association between red meat intake and ESCC but no association with adenocarcinoma (42). Overall, total iron intake may be related to reduced EC risk, while heme iron intake may be related to increased EC risk. More evidence is required to clarify the relationship between imbalanced iron levels (iron deficiency or overload) and the risk of EC.

Selenium

Selenium is a naturally occurring element to which humans are mostly exposed through food intake, air, drinking water, and dietary supplements. A moderate amount of selenium is vital for maintaining biological functions, but a slightly higher amount of selenium may have potential toxicity. Basic research showed an anti-tumor effect of selenium, including inhibiting cancer cell proliferation (44), preventing tumor formation in cell populations already exposed to carcinogens (45), reducing carcinogen-induced DNA mutations, and antioxidant and anti-inflammatory effects (46).

Numerous studies have shown selenium to be related to the risk of EC. It was reported that selenium could slow down the development of ESCC by decreasing the expression of Ki-67, inducing apoptosis, and lowering inflammation and oxidative DNA damage, thus exerting an important chemopreventive effect on ESCC by reducing high-grade dysplasia to low-grade dysplasia (47, 48). Zhang et al. (49) found that β -catenin/TCF pathway played a vital role in selenium induced-growth inhibition and apoptosis in ESCC cells (49). Liu et al. (50) also revealed that methylseleninic acid acted as a chemopreventive agent *via* the regulation of KLF4/miR-200a/Keap1/Nrf2 axis in ESCC cells (50). Several studies measured serum selenium levels between patients with EC and controls. Steevens et al. detected selenium levels in toenails. They found that selenium concentrations were inversely related to the risk of ESCC, while an inverse association was only found in esophageal adenocarcinoma (EAC) in women and non-smokers (51). Mark et al. (52) found a significant inverse relationship between serum selenium concentrations and the incidence of EC (52).

Further, they also observed significant inverse associations between the baseline concentration of serum selenium and death from ESCC (53), indicating that selenium intake may help reduce the incidence of EC and death from EC. A randomized controlled trial showed evidence that selenium played a preventive role in subjects with preexisting esophageal squamous dysplasia, which was reported as the precursor lesion of ESCC (54). In a follow-up of the Linxian General Population Nutrition Intervention Trial, selenium intake with vitamin E and β -carotene helped reduce the risk of EC, and the beneficial effects on mortality from EC were still evident up to 10 years after the cessation of supplementation and were consistently greater in younger patients (55). However, some meta-analyses showed different results regarding the relationship between selenium exposure and the risk of EC. Cai et al. (56) showed that high selenium exposure might decrease the risk of EC (56), while Hong et al. (57) observed that a higher selenium concentration was not significantly related to a decreased risk of EC (57). The Golestan Cohort Study also mentioned that the association between dietary selenium supplementation and the risk of ESCC was nonlinear but probably U-shaped, which suggests that the



risk of ESCC may increase with excessive selenium intakes (34). Generally, for those individuals with a low baseline selenium level, selenium intake could have a protective effect against EC; for general populations, the effect of daily supplementation of selenium remains unclear.

Other essential trace elements

In a cohort study, calcium (Ca) was reported to be related to a decreased risk of ESCC in men in the United States (58). Similarly, Hashemian et al. (34) observed a significant linear inverse association between calcium supplementation and the risk of ESCC (34). The possible molecular mechanisms may lie in the fact that calcium could inhibit the proliferation and invasion of cancer cells and promote apoptosis (59). The relationship between magnesium (Mg) in drinking water and the risk of EC was also reported. The result showed a significant trend toward decreasing EC risk with increasing magnesium concentration in drinking water (60). It was also reported that compared with healthy tissues, the levels of chromium (Cr), manganese (Mn), aluminum (Al), tin (Sn), and lead (Pb) were higher in cancerous tissues. However, no significant differences were revealed in EC (31). There are only limited studies focused on the relationship between the aforementioned essential trace elements and the risk of EC. Cr was reported to increase the risk of various cancer infections under environmental and occupational exposures (61) *via* ROS production, DNA damage, angiogenesis, and other molecular process (62, 63); however, its association with EC remains unclear. Mn has been widely studied in neurodegenerative diseases, and it mainly participates in biological roles in the form of manganese superoxide dismutase (MnSOD) by neutralizing the radical superoxide. A few studies have reported that MnSOD may play a role in EC protection (64, 65). In a mouse model, high levels of MnSOD expression promoted ESCC cell growth,

whereas moderate MnSOD expression suppressed tumor cell growth (64), indicating the dual effects of MnSOD on ESCC cell proliferation. No evidence showed a clear relationship between Al, Sn, Pb, and EC. Further study may reveal the underlying association.

Discussion

It was found that EC had a close relationship with environmental and dietary factors, in which essential trace elements played significant roles. Studies on the association between essential trace elements and EC were critical and have provided potential direction for the prevention of EC. In this review, we analyzed the studies of some main essential trace elements in EC. Zinc, iron, and selenium supplementation seem to be related to a reduced risk of EC, while copper showed an equivocal effect on the prevention of EC. Despite ample evidence and general consistency in the relationship between essential trace elements and EC, the public or health professionals often view the effect of essential trace elements with skepticism. One explanation could be that the physiologic systems affected by essential trace elements are so complicated that the effects of supplementing with only one or two elements are not effective enough or even sometimes harmful. Essential trace elements belong to the whole diet pattern. Many studies focused on only one essential trace element, which could not reflect the reality that diet as a whole is more important than the sum of its parts (66). In-depth studies on essential trace elements are required. Genome, epigenome, transcriptome, metabolome, proteome, and microbiome have long been used in cancer studies, which significantly improve our understanding of the mechanisms of cancer development or new therapy application of cancers. With the development of such technologies, a molecular biological approach that integrates data of “omics,” including metagenomics, transcriptomics, proteomics, metabolomics,

genetics, and other molecular technology, is expected to help us enhance our understanding of the association between essential trace elements and risk of EC incidence or development (66) (Figure 1). Moreover, a clearer connection between essential trace elements and the molecular alteration and variation of patients is gradually becoming identifiable and quantifiable, thereby renewing the old general view associating specific phenotypical changes with the differential intake of essential trace elements (67). Further research is needed and is expected to clarify how essential trace elements act in the development of EC and whether essential trace element supplementation could protect against EC.

Author contributions

XY carried out the primary literature search, drafted, and revised the manuscript. JL and ZT contributed to drafting and revising of the manuscript. JJ and JL helped modify the manuscript. ZT and JJ carried out the literature analysis and

revised the manuscript. All authors read and approved the final manuscript.

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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A global perspective of correlation between maternal blood lead levels and risks of preeclampsia: An updated systematic review and meta-analysis

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Background: Preeclampsia (PE) is a specific hypertensive disorder in pregnancy. Lead (Pb) is a heavy metal that affects women's reproductive health. However, it is unclear whether lead exposure during pregnancy can predispose maternal risk of developing preeclampsia. This systematic review and meta-analysis study aimed to explore the association.

Methods: We searched studies from three databases (PubMed, Web of Science, Embase). Only case-control, cross-sectional, and cohort studies reporting maternal blood lead levels (BLL) and PE were included from database inception to 31st July 2022. Pregnant women with blood lead levels measured were eligible. Those healthy pregnant women who did not develop preeclampsia were assessed as comparators. Letters, comments, case reports, and reviews were excluded. Newcastle-Ottawa Scale (NOS) and its adaptive form were applied for assessment. The random-effects method (REM) was applied to calculate the standardized mean difference (SMD) with a 95% confidence interval (CI). Stata 16.0 and RevMan 5.3 were the software used for data extraction and analysis.

Results: 25 studies out of 1,808 articles made the finalist for systematic reviews, of which 21 underwent further quantity analysis. A total of 1,533 preeclamptic women and 10,998 healthy pregnant controls were included in the meta-analysis. The overall result revealed that maternal lead exposure was significantly higher in women with preeclampsia (SMD: 1.06, 95% CI 0.69, 1.43); ($I^2 = 96.40\%$; $P = 0.000$).

Conclusion: This study demonstrates that maternal lead exposure is associated with preeclampsia during pregnancy. The association is present

even in low blood lead levels. The conclusion should be taken seriously and women should avoid unexpected exposure to a lead-containing environment as much as possible.

Systematic review registration: https://www.crd.york.ac.uk/prospero/display_record.php?RecordID=347220, identifier: CRD42022347220.

KEYWORDS

lead, Pb, heavy metals, hypertensive disorder complicating pregnancy, preeclampsia (PE), systematic review

1. Introduction

Preeclampsia (PE) is a pregnancy-specific disorder that can affect multi-systems. It features the late-onset hypertension, proteinuria, deranged liver enzymes, blurred vision, headache, etc. Globally, the incidence of this hypertensive disorder complicating pregnancy is around 5% (1–3). It remains one of the leading causes of maternal death in most countries, particularly in developing countries. Despite progress made in early screening and prevention in PE, the management is mainly unchanged. The most effective approach to stop the disease progression is still the termination of pregnancy. This may lead to iatrogenic preterm deliveries, causing heavy economic burden for the family and the society. Moreover, as the etiology of PE remains poorly understood, some researchers proposed that heavy metals may play a role (4–9).

Lead (Pb) is one of the most toxic heavy metals in the environment (10, 11). Environmental lead exposure can be inadvertent as it is contained in batteries, cosmetics, paints, metallic pipes, and some cooking pots (10). It can affect the biological function of major organs and systems, such as the central nervous and cardiovascular systems (12). Several studies have reported an association between occupational and environmental lead exposure and hypertension (12). Exposure to lead could affect the central nervous system, causing biological functioning of enzymes, behavioral disorders and brain damage (13). The association between lead exposure and reproductive health has also been studied across countries in recent decades (14–17). Male workers exposed to lead manifest higher blood lead levels (BLL), lower sperm count, and poor sperm motility compared to those without occupational lead exposure (18). By contrast, lead-exposed women are at higher risk of developing PCOS (19).

Many studies have reported an association between heavy metals and preeclampsia during pregnancy, but the results are inconsistent (16, 20–22). Several reasons, such as different ethnic backgrounds, geographical locations, and measurement methods, may explain the disparity. Therefore, we conducted this systematic review and meta-analysis to include all eligible studies to discuss: (1) whether there is an association between

maternal lead exposure and preeclampsia; (2) how maternal lead exposure may affect the risk of PE in pregnancy.

2. Methods

2.1. Protocol and registration

This study followed the Preferred Reporting Item for Systematic Reviews and Meta-analysis (PRISMA) Statement. We registered at the National Institution for Health Research with the registration identifier: CRD42022347220. https://www.crd.york.ac.uk/prospero/display_record.php?RecordID=347220.

2.2. Search strategy

We searched three electronic databases, PubMed, Web of Science, and Embase, from the inception to 31st Jul 2022. Two independent researchers (Z. X. Z. and Q. M. Y.) used a combination of Medical Subject Headings (MeSH) terms and free text words, e.g., “preeclampsia or pre-eclampsia or (hypertensive disorder complicating pregnancy) or (hypertensive disorder during pregnancy) or (Pregnancy-Induced Hypertension) or (gestational hypertension)” and “lead or Pb.” We have manually checked all included studies and references to complement our study. Studies were limited to humans, but there were no restrictions on language or places of study. The detailed search strategies can be accessed in [Supplementary material](#).

2.3. Eligibility criteria and the study selection process

Two researchers (Z. X. Z. and Q. M. Y.) independently included studies that were: Observational studies that measure maternal blood lead levels in preeclamptic women and healthy pregnant controls.

Studies were excluded if they were conference papers, editorials, letters, reviews or systematic reviews. Z. X. Z. and Q.

M. Y. screened the studies, and a third reviewer, F. F. Z., was to resolve any disagreement between the two.

2.4. Data extraction and quality assessment

Two independent investigators (Z. X. Z. and Q. M. Y.) extracted data *via* Microsoft Excel 2018. The title of studies, name of the authors, year of publication, study types, country of study, number of participants, blood lead levels (BLL), and average maternal age with standard deviation (SD) in each group were extracted from each study. F. F. Z. was to resolve any disagreement during data extraction between the two.

The Newcastle-Ottawa Scale (NOS) was adopted to evaluate case-control, cohort, and cross-sectional studies. A nine-star rating system was applied for quality assessment in case-control and cohort studies. A score between seven and nine indicates good quality, while four to six was considered moderate quality. Poor quality was defined if the score was three or less. For cross-sectional studies, a modified form of NOS was used. The scores rated from zero to ten. Scores of seven or more represent good quality, while three or fewer represent poor quality. Fair quality was defined as scores in between (23).

2.5. Sub-group analysis and meta-regression

Sub-group analysis and meta-regression were conducted to assess whether geographical locations, study or sample types, or measurement methods affected maternal exposure to lead, and how this correlated with the likelihood of preeclampsia. We divided all studies into five groups according to the original locations of the study population: Asian studies were from China and India. African studies consisted of reports from DR Congo, Egypt, and Nigeria. Middle-East studies cover reports from Iran, Saudi Arabia, and Turkey. European studies contain reports from Bulgaria, Finland, France, Malta, Poland, Portugal, and the UK. Other studies from the USA and Australia were allocated to Group 5 (Others).

We also applied meta-regression to determine whether the geographical locations, the study types, the methods of measurement, or the blood samples (the whole blood, plasma, serum) were the contributing factors to the overall results and heterogeneity.

2.6. Statistical analysis

We calculated results and performed data analysis *via* Review Manager 5.4.1 (The Nordic Cochrane Center, Copenhagen, Denmark) and Stata version 16.0 (StataCorp.,

College Station, TX, USA). The maternal blood lead exposure levels were pooled by standardized mean difference (SMD) with 95% confidence intervals (CI). The I^2 was used to test the heterogeneity ($I^2 \geq 50\%$ indicates significant heterogeneity). The forest plot was used to visualize the overall results, with the random-effect model (REM) being adopted for calculation as the heterogeneity was considered significant. A sensitivity analysis was performed with the removal of each study once to assess whether any single study could affect the overall outcome. Publication bias was visualized *via* funnel plot with Begg's test and tested with Egger's linear regression.

3. Results

3.1. Study selection

We searched three databases (PubMed, Web of Science, and Embase) and collected 1,801 articles. Seven additional studies were identified after checking all the references from full-text articles. Of the 1,808 studies, 28 were removed for duplication. One thousand seven hundred and eighty records were further screened, and 1,744 were removed after reading their title and abstract. There were 36 studies investigated for full-text assessment. Eleven of them were excluded for reasons: Letonoff et al. study was included in a recent meta-analysis, but we excluded it from our finalist as we had checked the measurement in this study and failed to identify the type of measurement used in the article and references (24). Moreover, the diagnostic criteria were significantly changed over the eight decades (24, 25). Three studies were excluded for not reporting blood samples of lead (6, 26, 27). Three articles were excluded as they overlapped the study population with the finalist articles (28–30). The other four articles failed to make it into the finalist as they studied the association between lead exposure and obstetric outcomes during pregnancy but did not involve preeclampsia (31–34). Of the rest 25 articles, 21 were included in systematic review and meta-analysis, while four studies were only assessed in the qualitative synthesis (14–17, 20–22, 35–48). Details of the study selection process can be seen in Figure 1.

3.2. Basic characteristics of included studies

There are 25 studies originated from 17 countries over four decades. There are contrasting differences between the size of the study population with the number of preeclamptic participants in any single study ranging from six to 427 (14, 47). Significant differences were also found in blood samples (whole blood, plasma, serum or red blood cells), and methods of measurement, such as atomic absorption spectrophotometer

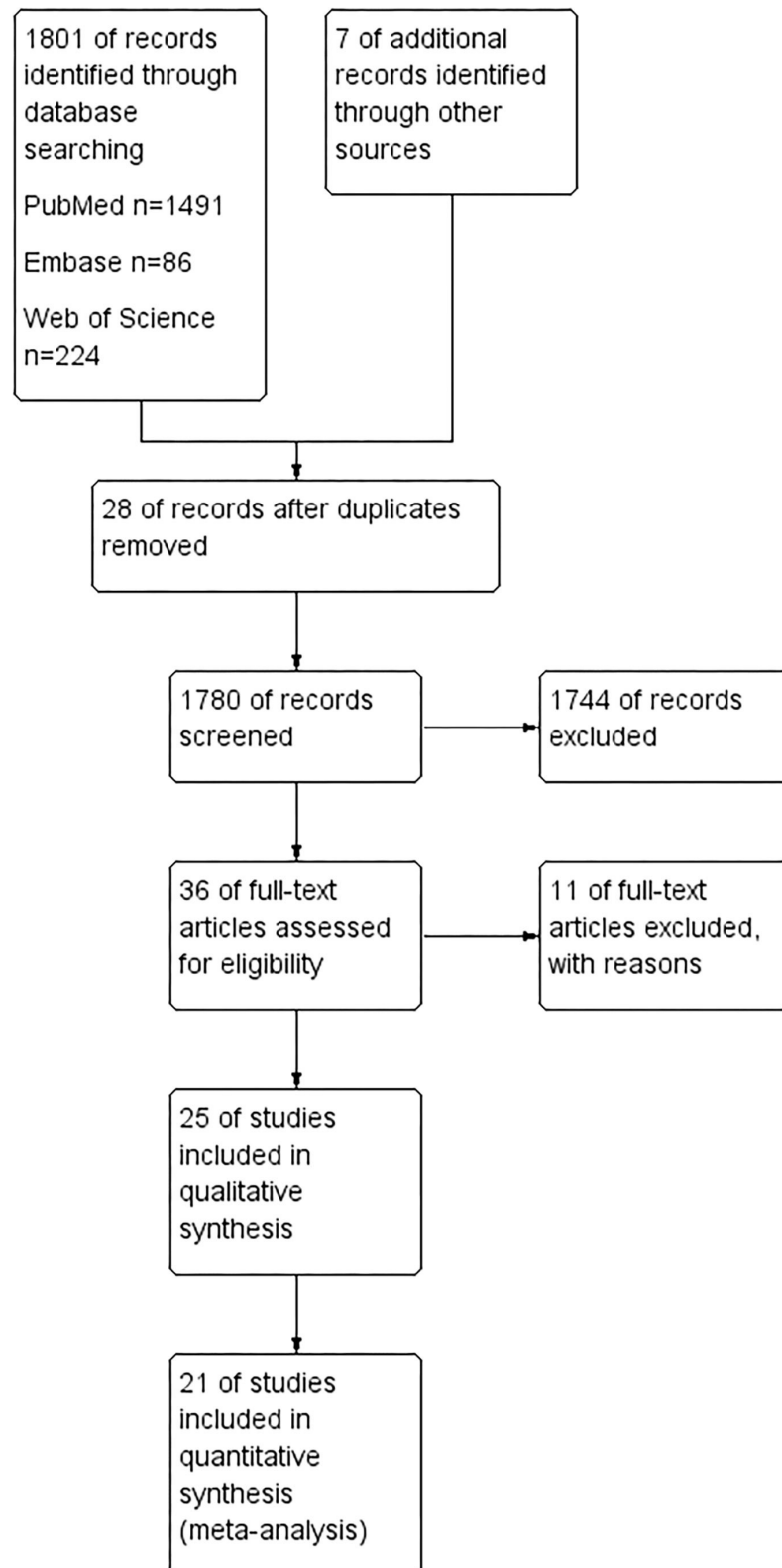


FIGURE 1
PRISMA flow diagram for study selection process.

TABLE 1 Characteristics of included studies.

| Reference | Country | Study type | Age of preeclampsia | | Age of healthy control | | BLL in preeclampsia | BLL in healthy control | Unit | Measurement | Diagnostic criteria |
|---------------------------|-----------------|------------|---------------------|-----|------------------------|-------|---------------------|------------------------|--------|----------------|----------------------------|
| | | | (Mean \pm SD) | N | (Mean \pm SD) | N | (Mean \pm SD) | (Mean \pm SD) | | | |
| Bayat et al. (51) | Iran | CC | 29.67 \pm 6.37 | NS | 27.37 \pm 6.1 | NS | 8.04 \pm 3.40 | 6.24 \pm 1.74 | ug/dL | Potentiometric | ACOG |
| Dawson et al. (35) | USA | Cohort | 22.00 \pm 4.00 | 19 | 25.00 \pm 6.00 | 20 | 1.73 \pm 0.32 | 1.35 \pm 0.27 | umol/L | AAS | NS |
| Disha et al. (36) | India | CS | 27.34 \pm 6.40 | 44 | 24.54 \pm 3.60 | 23 | 3.42 \pm 2.18 | 2.38 \pm 2.43 | ug/dL | AAS | ACOG |
| Gajewska et al. (22) | Poland-Portugal | CC | 26.80 \pm 3.29 | 66 | 32.70 \pm 6.18 | 40 | 3.36 \pm 1.23 | 2.04 \pm 1.30 | ug/dL | ICP-MS | ACOG |
| Hyvonen-Dabek et al. (14) | Finland | Cohort | NS | 6 | NS | 21 | 0.014 \pm 0.004 | 0.014 \pm 0.0058 | ppm | PIXE | NS |
| Ikechukwu et al. (37) | Nigeria | Cohort | 27.30 \pm 3.20 | 59 | 26.70 \pm 3.60 | 150 | 60.2 \pm 12.8 | 26.30 \pm 8.00 | ug/dL | AAS | ACOG |
| Jameil et al. (38) | KSA | CC | 31.55 \pm 6.14 | 40 | 31.20 \pm 5.84 | 40 | 27.18 \pm 2.13 | 18.23 \pm 2.34 | ug/dL | ICP-OES | ACOG |
| Kaul et al. (39) | India | CS | NS | 16 | NS | 84 | 18.40 \pm 1.40 | 6.20 \pm 2.00 | ug/dL | AAS | ACOG |
| Liu et al. (40) | USA | PC | 29.12 \pm 6.17 | 115 | 27.99 \pm 6.31 | 1,159 | 2.53 \pm 1.20 | 2.61 \pm 1.48 | ug/dL | ICP-MS | ACOG |
| Ma et al. (20) | China | NCC | NS | 146 | NS | 292 | 8.32 \pm 3.73 | 7.25 \pm 3.81 | ug/L | ICP-MS | NICE |
| Magri et al. (15) | Malta | CS | 30.00 \pm 6.00 | 30 | 27.00 \pm 6.00 | 93 | 9.60 \pm 6.00 | 5.80 \pm 3.00 | ug/dL | AAS | Mounier-Vehier et al. (67) |
| McKeating et al. (21) | Australia | NC | 31.55 \pm 3.80 | 38 | 32.24 \pm 3.91 | 193 | 0.29 \pm 0.44 | 0.44 \pm 1.86 | ug/L | ICP-MS | Kaitu'u-Lino et al. (68) |
| Mokhlesi et al. (41) | Iran | Cohort | NS | 20 | NS | 1,013 | 7.87 \pm 4.61 | 4.63 \pm 4.80 | ug/dL | NS | ACOG |
| Motawei et al. (42) | Egypt | CS | NS | 115 | NS | 25 | 37.68 \pm 9.17 | 14.5 \pm 3.18 | ug/dL | AAS | ACOG |
| Obadia et al. (43) | DR Congo | CC | 30.60 \pm 6.40 | 40 | 31.40 \pm 4.70 | 39 | 6.58 \pm 2.14 | 5.23 \pm 1.56 | ug/dL | ICP-MS | ACOG |
| Ovayolu et al. (44) | Turkey | CC | 30.61 \pm 7.74 | 46 | 28.00 \pm 6.59 | 46 | 39.27 \pm 33.67 | 28.48 \pm 13.06 | ug/L | ICP-MS | ACOG |
| Rothenberg et al. (63) | USA | PC | NS | NS | NS | NS | NS | NS | ug/dL | AAS | NS |
| Sowers et al. (64) | USA | PC | NS | NS | NS | NS | NS | NS | ug/dL | AAS | NS |
| Tabacova et al. (45) | Bulgaria | Cohort | 24.60 \pm 6.10 | 19 | 22.70 \pm 5.16 | 22 | 6.50 \pm 2.18 | 5.20 \pm 0.94 | ug/dL | AAS | NS |
| Taylor et al. (16) | UK | PC | NS | 91 | NS | 3976 | 3.63 \pm 1.22 | 3.67 \pm 1.47 | ug/dL | ICP-MS | ACOG |
| Ugwuja et al. (65) | Nigeria | PC | NS | NS | NS | NS | NS | NS | ug/dL | AAS | ACOG |
| Vigeh et al. (46) | Iran | CC | 26.00 \pm 4.00 | 31 | 26.90 \pm 5.70 | 365 | 5.09 \pm 2.01 | 4.82 \pm 2.22 | ug/dL | ICP-MS | ACOG |
| Wang et al. (47) | China | CC | NS | 427 | NS | 427 | 3.10 \pm 1.26 | 2.94 \pm 1.09 | ug/dL | ICP-MS | (69) |
| Wu et al. (17) | China | RC | NS | 59 | NS | 2,115 | 4.33 \pm 1.94 | 3.74 \pm 1.11 | ug/dL | AAS | ACOG |
| Yazbeck et al. (48) | France | PC | NS | 106 | NS | 865 | 2.20 \pm 1.40 | 1.90 \pm 1.20 | ug/dL | AAS | (48) |

AAS, atomic absorption spectrophotometer; CC, case-control; CS, cross-sectional; ICP-MS, inductively coupled plasma; NCC, nested case-control; NICE, National Institution of Care Excellence; NS, not stated; PC, prospective cohort; PIXE

ometry; ICP-OES, inductively coupled plasma optical emission spectrometry; KSA, Saudi Arabia; NC, nested cohort; X-ray emission; RBC, red blood cell; RC, retrospective cohort.

(AAS), inductively coupled plasma mass spectrometry (ICP-MS) or inductively coupled plasma optical emission spectrometry (ICP-OES), etc. (37, 38, 40). Most studies adopted ACOG's diagnostic criteria of preeclampsia for patient inclusion. More information can be seen in [Table 1](#).

3.3. Results of systematic review

There were 13 cohort studies, eight case-control studies, and four cross-sectional studies. All 25 studies were further assessed *via* the Newcastle-Ottawa Scale (NOS) quality assessment, of which four cross-sectional studies were evaluated with modified NOS. Overall, 19 reports were rated high quality, and six were rated moderate. Detailed scores can be accessed in [Supplementary Tables 1.1–1.3](#).

3.4. Results of meta-analysis

The total number of participants involved in the meta-analysis was 12,531 from 21 reports. The number of healthy pregnant controls was much more compared to the case group. Preeclamptic women accounted for 1,533, while non-preeclamptic pregnant women were more than 7-fold more (1,533 vs. 10,998). The single largest study with 3,976 participants was extracted from a prospective birth cohort in the US in 2015 (16). The overall result showed that maternal lead exposure in preeclamptic women was significantly higher than that of healthy pregnant control (SMD: 1.06, 95% CI 0.69, 1.43); ($I^2 = 96.4\%$; $P = 0.000$), see [Figure 2](#). The funnel plot indicated significant publication bias which can be seen in [Figure 3](#). Begg's test and Egger's test were applied to quantitatively assess the publication bias ($z = 3.47$, $p = 0.001$; $t = 3.87$, $p = 0.001$; see [Supplementary Figure 1](#), [Supplementary Table 2](#)). The leave-one-out sensitivity analysis ([Supplementary Figure 2](#)) showed that Wang et al. and Ikechukwu et al. reports reversely contributed to the pooled result (37, 47).

3.5. Results of meta-regression

Meta-regression was performed as a result of significant heterogeneity between studies. Different geographical locations, measurement methods, types of study design, and blood samples of lead were further tested for potential causes of heterogeneity. However, the results showed that none of them was the major contributor (P -value of location: 0.07; P -value of different measurements: 0.37; P -value of study types: 0.65; P -value of sample types: 0.73). Detailed information can be seen in [Supplementary Figure 3](#).

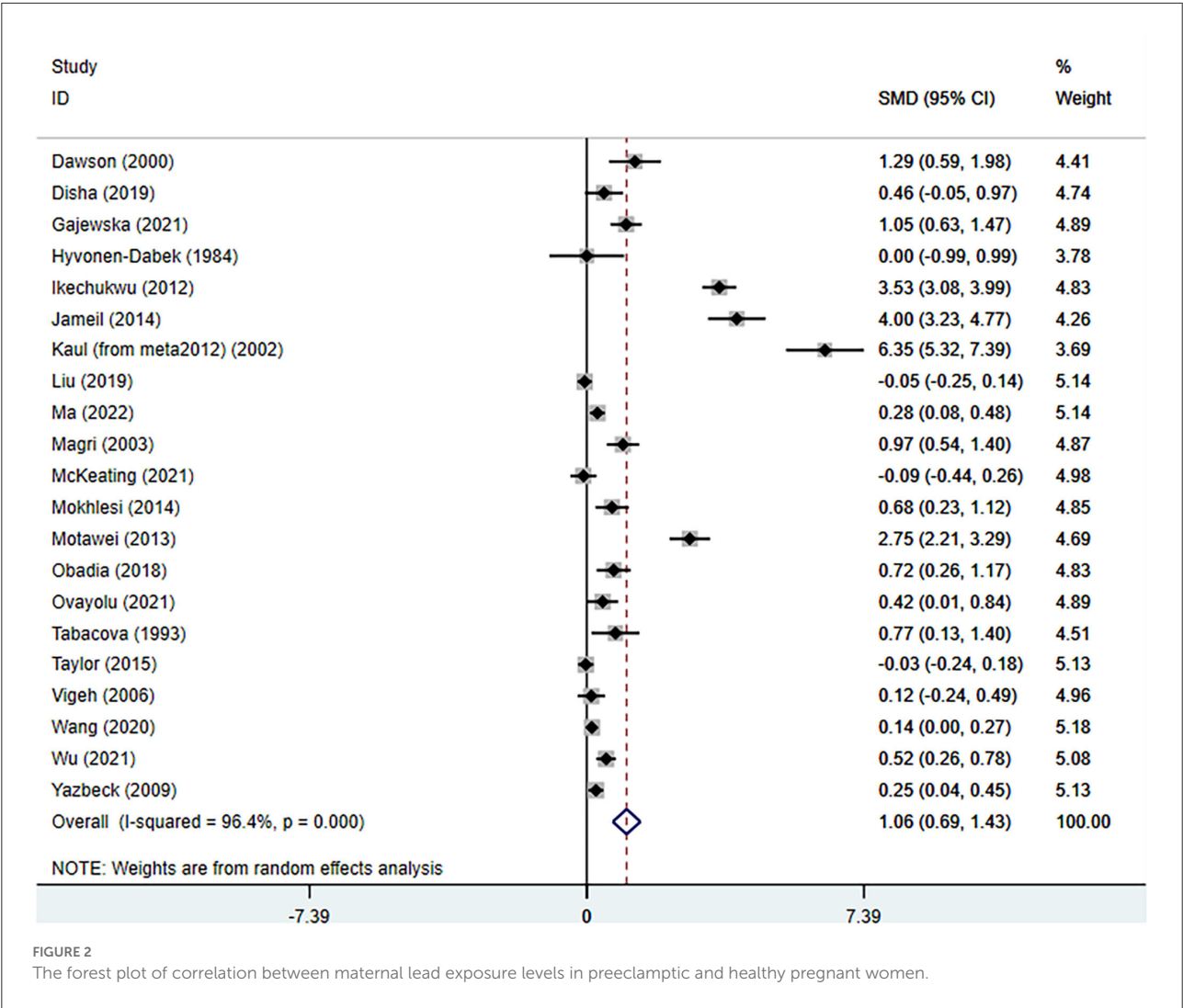
3.6. Results of sub-group analysis from a geographic perspective

Despite having evaluated the effect of geographical backgrounds on the general result, we further made a sub-group analysis to see how different the results were in different regions and continents. European studies made up the largest part of the whole study, with 5,325 participants (318 preeclamptic women vs. 5,007 healthy pregnant control). By contrast, African studies only contributed a minor part (428 participants evenly divided), but the effect size was disproportionally large (SMD 2.32; 95% CI 0.60, 4.05); ($I^2 = 97.00\%$; $P = 0.000$). The Asian studies were the most recent and had the lowest heterogeneity with $I^2 = 60\%$; $P = 0.06$; (SMD 0.30; 95% CI 0.12, 0.49). The fifth group consisted of studies from USA and Australia, and there was a significant between-study heterogeneity (SMD 1.74; 95% CI 0.25, 3.24; $I^2 = 98.00\%$; $P = 0.000$). More information is available in [Supplementary Figure 4](#).

We further applied the cut-off value of maternal blood lead level at 5 $\mu\text{g}/\text{dL}$ as the Center for Disease Control and Prevention (CDC) of the USA has recommended as the safe range (49). Eleven studies with both the case and control groups whose mean values lower than 5 $\mu\text{g}/\text{dL}$ were included for further analysis (14, 16, 17, 20–22, 36, 40, 44, 47, 48). The pooled result showed that the SMD: 0.25, 95% CI 0.09, 0.40; $I^2 = 75.00\%$, $P = 0.002$. More detailed information can be seen in [Supplementary Figure 5](#).

4. Discussion

This systematic review and meta-analysis primarily focused on whether there is an association between maternal lead exposure and preeclampsia. The combined results have demonstrated that higher blood lead levels (BLL) are associated with preeclampsia (PE). This is generally consistent with some existing research (25, 50). Lead exposure is associated to adverse maternal and fetal outcomes. The Center for Disease Control and Prevention (CDC) of the USA recommended the safe range of BLL in pregnant women to be 5 $\mu\text{g}/\text{dL}$ or less. Globally, the WHO recommends a safety limit of 10 $\mu\text{g}/\text{dL}$ BLL (51). In the view of geographical sub-group analysis, the associative trend is more prominent in Africa. This may be associated to rapid industrialization, environmental pollution, diet differences, lifestyle (*kohl*, the black eye cosmetic containing lead sulfide), and poor community awareness in the last decade. Asian studies had relatively lower heterogeneity as three out of the four studies originated in the same country. Apart from geographical differences, sample type, methods of measurements, study designs may also affect the overall effect and give rise to the heterogeneity. We therefore applied meta-regression, but no variables were identified to cause the between-study heterogeneity.



Mothers with advanced age were more likely to develop a steeper increase in BLL than younger mothers, particularly in the latter half of their pregnancy. This coincides with the onset of preeclampsia (52). In this view, it is plausible that higher lead accumulated in advanced maternal age women, disposing them to developing preeclampsia. Furthermore, as the lead was known to cross the placenta freely during pregnancy, there are studies focusing on identifying the association between increased maternal BLL and fetal outcomes. A higher risk of spontaneous miscarriage in the early trimester, and stillbirth in the mid-/late-trimester were also observed in several studies (53, 54). Lead has been shown to induce hyperglycemia and glucose intolerance in pregnant women, which has also been observed in animal studies (33, 55). An association between maternal lead exposure and very preterm birth was identified in a large cohort. Moreover, early-life environmental exposure to lead is related to neurodevelopmental disorders, asthma, and obesity. Interestingly, gender differences were found in 949

mother-child pairs research. Male neonates were at higher risk of preterm delivery even if maternal lead exposure was low (56, 57). In an Iranian study, a negative correlation between intrauterine lead exposure and neonatal birth weight was reported recently (58). Furthermore, a study from Mexico demonstrated that girls born from mothers with lead exposure during pregnancy may have delayed puberty, poor pubic hair, and breast growth (59). These show the sustained effect of lead on mothers and offspring, particularly female offspring. A prenatal animal study also supported this opinion as they have found that maternal lead exposure may create a non-genetic adaptive mechanism to protect against reproductive impairment. This process involves imprinting or cell programming and can persist for a long time (52). As lead was believed to interfere with iron absorption, maternal lead exposure was investigated for anemia developed during pregnancy. However, there has been insufficient evidence to consolidate the correlation by far (32).

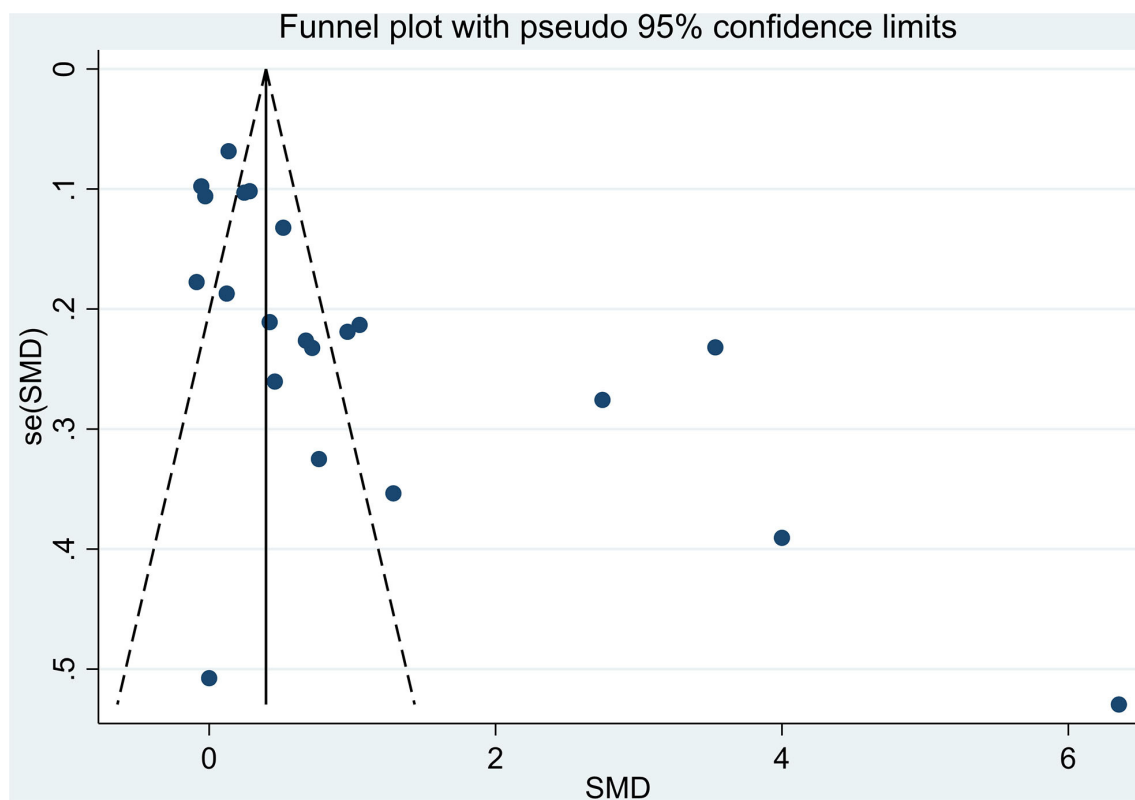


FIGURE 3
The funnel plot to assess publication bias.

Despite several studies demonstrating an association between heavy metals and risks of developing preeclampsia for decades, recent reviews have not included lead or other heavy metals as potential risk factors for preeclampsia (1–3, 17, 20, 44). One reason is that in most western countries, women are at very low risk of suffering from environmental or occupational lead exposure or other heavy metal exposure. In this sense, we divided studies into several groups based on geographical location, assuming there may be differences between each group due to ethnic, economic, lifestyle differences. These differences may also partly explain the significant between-study heterogeneity in this systematic review and meta-analysis. Another reason is that the raised awareness leads to less use of lead-contained food, water, or cosmetics. Fewer women nowadays are exposed to environmental and/or occupational lead without protection. This makes research more difficult to discover significant changes on human beings, and many recent studies turn to focusing on animal study (55).

ACOG stated there is no association between lead exposure and the development of pregnancy-induced hypertension (PIH) (49, 60). However, based on the result we collected, even a low blood lead level ($<5 \mu\text{g/dL}$) can lead to adverse pregnancy outcomes, including preeclampsia (22, 36, 40). This was further

fortified by our sub-group analysis (Supplementary Figure 5). There are different theories to explain the potentially underlying mechanisms. Firstly, the physiological changes in pregnancy facilitate the mobilization of maternal bone lead, contributing to a higher maternal BLL. This triggers a further release of endothelin, a vasoconstrictor involved in the inflammation process, which plays a key role in the pathogenesis of preeclampsia (12, 61). Secondly, an animal study has suggested that long-term lead-contained drinking water can significantly enhance the plasma levels of adrenaline and noradrenaline. This could induce blood hypertension, which is partly responsible for the pathogenesis of preeclampsia (54). Thirdly, high maternal BLL may lead to local changes in miRNA profiles based on research focusing on the cervix (31). As lead can freely cross the placenta, maternal lead exposure during pregnancy could lead to higher *in-utero* lead levels (56). The high levels of umbilical cord blood lead can further trigger changes in fetal miRNA profiles, making it more susceptible to developing maternal preeclampsia and fetal preterm birth or stillbirths. DNA methylation changes were also observed following maternal lead exposure (34, 62). Interestingly, some adverse fetal outcomes are differently associated with fetal sex. DNA methylation is more prominent in female fetuses, while males are at higher risk of pre-term births (34, 57).

Two systematic reviews and meta-analyses focused on the correlation between maternal lead exposure and preeclampsia (25, 50). Kennedy et al. published in 2012, which included only nine original studies. They claimed to search the database from inception to March 2011, but we have found three more articles that met their acclaimed inclusion criteria (14, 24, 45). Poropat et al. study was very well-written, particularly their discussion part. It has been reported that an increment of 1 $\mu\text{g}/\text{dL}$ of blood lead was associated with a 1.6% increase in the likelihood of preeclampsia. However, they share a similar problem of not including all the available studies. They have missed at least five studies collected by Kennedy et al. without explanation, indicating that there might be insufficient search in the research (15, 48, 63–65). Another significant mistake was data accuracy. We have found that the review published in 2018 mishandled the results extracted from Ikechukwu et al. study (25). The SD was 12.8 instead of being mistakenly noted as 24.0, and the number of participants in this study was actually 209 instead of 181 which was written in the systematic review (25, 37). The selection bias and inaccurate data extraction may compromise the reliability of the overall result synthesized in this meta-analysis (25).

Compared to the previous research, we have included the most recent and the largest number of studies reporting maternal lead exposure and preeclampsia. This facilitates detailed analysis from different perspectives. We divided the studies into five sub-groups to see how geographic locations impact the overall result and the heterogeneity between studies. We have included all nine reports since 2018, accounting for the latest trends worldwide (17, 20–22, 36, 40, 43, 44, 47).

However, there are several limits to our study. Firstly, despite the unlimited language requirement during the search, we only extracted one non-English written article (written in Persian) (41). This is a shared problem in the previous meta-analysis (50). Secondly, the size of included reports precludes further analysis. We have tried to investigate the correlation between lead and preeclampsia *via* different perspectives, such as dividing the studies into different geographical groups and applying meta-regression to see whether study design, measurement methods, and blood samples have exerted an effect on the overall result. All methods we tried failed to identify any causal effects, nor to significantly minimize the between-study heterogeneity. Lastly, exposure to heavy metals often occurs in mixtures instead of in single forms (20, 66). However, only nine articles in our studies reported other heavy metals (15, 20, 21, 40, 43–47). With more studies reporting the panel of heavy metals, we would be a step closer to exploring the real-world facts.

5. Conclusion

In summary, we have found that maternal lead exposure is associated with PE during pregnancy, even at very low

levels. More well-designed large cohort studies in the future are needed further to clarify the role of lead on preeclampsia and pregnancy.

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

Conceptualization and modification and writing back to reviewers: ZZ and FZ. Methodology, resources, and data curation: QY and CL. Software and investigation: ZZ and QY. Validation, project administration, and supervision: FZ and XC. Formal analysis: ZZ and XC. Writing—original draft preparation and funding acquisition: ZZ. Writing—review and editing: XC. Visualization: CL. 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.2022.1072052/full#supplementary-material>

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Dietary fiber intake and non-alcoholic fatty liver disease: The mediating role of obesity

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Background and aims: Dietary pattern rich in fiber is negatively associated with the risk of non-alcoholic fatty liver disease (NAFLD). Meanwhile, obesity is a known predisposing factor for NAFLD. Nutrient-focused research can enhance the mechanistic understanding of dietary effects. We thus hypothesized that higher dietary fiber intake was associated with lower risk of NAFLD through the mediating role of obesity.

Methods: In this nationwide cross-sectional study, dietary fiber was surveyed using two 24-h recalls. NAFLD and clinically significant fibrosis (CSF) were determined by vibration-controlled transient elastography. Multivariable logistic and linear regression were applied to investigate the association of dietary fiber with NAFLD, CSF, and liver function parameters. We used counterfactual-based mediation analysis to estimate the direct and indirect effect of dietary fiber on NAFLD.

Results: Of the 3,974 participants, ~36.86% and 7.78% of participants were diagnosed with NAFLD and CSF. Compared with participants among the lowest tertile, the highest tertile of dietary fiber consumption was associated with lower odds of NAFLD (OR = 0.81; 95% CI: 0.66–0.98; $P_{\text{overall}} = 0.019$). Dietary fiber intake appeared to be linked with lower odds of CSF (OR_{Tertile3vs.Tertile1} = 0.81; 95% CI: 0.58–1.14; $P_{\text{overall}} = 0.107$). Mediation analysis showed that obesity fully mediated the association of dietary fiber with NAFLD. Dietary fiber was associated with improved hepatic parameters.

Conclusions: The findings indicated that increasing dietary fiber intake could confer a greater benefit to protect against NAFLD. Translating these findings regarding dietary fiber into dietary advice might be an attractive strategy for NAFLD prevention.

KEYWORDS

dietary fiber, non-alcoholic fatty liver disease, liver function parameters, mediation analysis, cross-sectional study

Introduction

Non-alcoholic fatty liver diseases (NAFLD), characterized by a certain degree of steatosis arising in the absence of excessive alcohol consumption and other known causes of liver disease (1), approximately affects a third of the world population (2). Growing evidence indicates that NAFLD is the hepatic manifestation of metabolic syndrome (MetS) (3). Paralleling the global epidemic of obesity and diabetes, the prevalence of NAFLD is growing dramatically over the past three decades in the US (4), and is projected to increase further. Although there are various existing drugs that have been considered in the management of NAFLD, lifestyle management such as healthy diet remains first-line treatments for NAFLD (5). Diet is the main driver of triglycerides accumulation in hepatocytes (6). Identifying dietary factors that reduce the risk of NAFLD is of importance. However, to date, such research is limited.

A previous study suggested a protective role for higher adherence to plant-based diet (PDI) against NAFLD (6). Furthermore, the Mediterranean diet has been shown to prevent NAFLD (7), and alleviate hepatic steatosis as well as fibrosis in the regression of NAFLD (8). On the other hand, several epidemiological studies demonstrated that high red meat and processed meat consumption might increase the risk of NAFLD (9, 10). PDI and Mediterranean diets are hallmarked by a fiber-rich diet. Consumption of dietary fiber has been associated with lower risk of obesity (11, 12), which was a known predisposing factor for NAFLD. A meta-analysis synthesizing 21 cohort studies with a total of 381,655 participants suggested that obesity independently led to a 3.5-fold increased risk of developing NAFLD compared with normal weight (13). Effect estimate on association between dietary factor and NAFLD appeared to be largely attenuated when obesity was adjusted (7, 14), suggesting that obesity might

be on the causal pathway of the relation (a mediator) (15). Because of complex interplay between diet, nutrient-focused research can enhance the mechanistic understanding of dietary effects (16). Taken together, we hypothesized that intake of dietary fiber lowered the risk of NAFLD, and obesity might play a mediating role in linking dietary fiber to NAFLD. To our knowledge, three studies have reported inverse associations between dietary fiber intake and NAFLD (17–19). One with a nested case-control study identified NAFLD through linkage to the Medicare claims (17), while NAFLD was determined using liver ultrasonography and fatty liver index (FLI) in the other two cross-sectional studies (18, 19). However, there has been no epidemiological study to evaluate the association of dietary fiber intake with NAFLD and clinically significant fibrosis (CSF) determined by vibration-controlled transient elastography (VCTE), one of the most accurate methods to detect hepatic steatosis and fibrosis (20). Meanwhile, given that the association was mediated by obesity, how dietary fiber exerted its effect *via* direct and indirect pathways have not been evaluated.

To add more evidence, we investigated the association of dietary fiber intake with NAFLD and CSF determined by VCTE in a nationally representative sample of US adults. Furthermore, mediation analysis was applied to assess the extent to which the effect of dietary fiber acted on NAFLD through obesity.

Methods

Study population

In this study, participants were selected from the 2017 to 2018 cycle of US National Health and Nutrition Examination Survey (NHANES), which was a cross-sectional survey conducted by the National Center for Health Statistics (NCHS) of the Centers for Diseases Control and Prevention (CDC) in the United States. More details on the survey protocol of NHANES have been described elsewhere (21). The study protocol was approved by NCHS Research Ethics Review Board (IRB: Protocol #2011-17; Protocol #2018-01), and the written informed consent was obtained from all participants.

A total of 9,254 participants were enrolled in the survey. Individuals aged ≥ 18 years old were included in this study. Individuals were excluded if they (i) had a missing dietary data ($n = 873$) or an unreliable energy intake (22) (defined as <600 or $>3,500$ kcal/day for women; <800 or $>4,200$ kcal/day for men, $n = 221$); (ii) had hepatitis B virus infection (the presence of HBsAg, $n = 20$) or hepatitis C virus infection (both hepatitis C antibody and RNA being positive, $n = 82$); (iii) had significant alcohol consumption (>3 drinks/d for men and >2 drinks/d for women, $n = 116$), (iv) underwent VCTE detection with unreliable results ($n = 315$) or did not receive VCTE detection ($n = 255$, Supplementary Figure 1).

Abbreviations: ALT, Aminotransferase; AMPK, Adenosine monophosphate-activated protein kinase; AST, Aspartate aminotransferase; AT, Adipose tissue; BMI, Body mass index; CAP, Controlled attenuation parameter; CDC, Centers for Diseases Control and Prevention; CIs, Confidence intervals; CSF, Clinically significant fibrosis; DBP, Diastolic blood pressure; FLI, Fatty liver index; GGT, Gamma-glutamyl transaminase; HbA1c, Hemoglobin A1c; HBsAg, Hepatitis B surface antigen; HBV, Hepatitis B virus; HCV, Hepatitis C virus; IR, Insulin resistance; LSM, Liver stiffness measurement; MEC, Mobile Examination Center; MET, Metabolic equivalent task; MetS, Metabolic syndrome; NAFLD, Non-alcoholic fatty liver diseases; NASH, Non-alcoholic steatohepatitis; NCHS, National Center for Health Statistic; NHANES, National Health and Nutrition Examination Surveys; ORs, Odds ratios; PDI, Plant-based diet; SBP, Systolic blood pressure; SCFAs, Short chain fatty acids; SD, Standard deviation; USDA, US Department of Agriculture; VCTE, Vibration-controlled transient elastography.

Dietary assessment

Dietary intake was quantified *via* two 24-h dietary recalls. A first 24-h dietary recall was performed in-person in the NHANES Mobile Examination Center (MEC), and the second 24-h dietary recall was conducted by telephone 3–10 days after the first recall. Food energy and nutrients were calculated based on the US Department of Agriculture (USDA), Food and Nutrient Database for Dietary Studies 2017–2018 (FNDDS 2017–2018). Total dietary fiber was calculated by multiplying the weight of each food consumed by the nutrient content of that food and summing it across foods. The intake of dietary fiber was averaged when participants had twice dietary recalls. We adjusted dietary fiber intake for total energy intake using the nutrient density method (intake per 1,000 kcal) to decrease measurement errors and represent the dietary composition.

Definition of mediator

In this study, obesity was hypothesized to be a mediator of the association between dietary fiber and NAFLD. Anthropometric measurement (height and weight) was conducted in MEC. Body mass index (BMI) was calculated as weight (kg) divided by the square of the height (m), and obesity was defined as $BMI \geq 25.0$ (kg/m^2).

Additional covariates

Standardized questionnaires were administrated through household interviews to collect demographic characteristics including age, sex, race/ethnicity, educational level, smoking, physical activity, and income. Physical activity was calculated by the sum of activities every week and was expressed in metabolic equivalent tasks (METs)-hours/week. Family income was defined as the ratio of family income to poverty. Additionally, hypertension was defined if individuals (i) reported a history of hypertension; or (ii) had a systolic blood pressure (SBP) ≥ 140 mmHg; or (iii) had a diastolic blood pressure (DBP) ≥ 90 mmHg. Diabetes was defined if individuals (i) reported a diagnosis of diabetes; or (ii) had a glycohemoglobin A1c (HbA1c) level $\geq 6.5\%$; or (iii) had a fasting glucose level ≥ 126 mg/dl; or (iv) had a random glucose level ≥ 200 mg/dl.

Assessment of NAFLD and CSF

Vibration-controlled transient elastography (VCTE) was conducted by trained and certified technicians in MEC, using the FibroScan[®] model 502 V2 Touch equipped with a medium

or extra-large probe. Hepatic steatosis and fibrosis was assessed by controlled attenuation parameter (CAP) and liver stiffness measurement (LSM).

Non-alcoholic fatty liver diseases (NAFLD) was defined as a CAP score ≥ 285 dB/m in the absence of viral hepatitis and excessive alcohol intake (non-NAFLD vs. NAFLD), and a LSM score ≥ 8.6 kPa was used to define CSF (LSM ≥ 8.6 kPa) vs. non-CSF (LSM < 8.6 kPa) (23, 24).

Laboratory assays and liver function parameters

After drawing and centrifuging the blood samples, the serum was aliquoted and stored at -70°C . Liver function parameters, including serum albumin, globulin, total protein, total bilirubin, alanine aminotransferase (ALT), aspartate aminotransferase (AST), gamma glutamyl transaminase (GGT), were also obtained from participants. All laboratory procedures were shown in detail elsewhere (25).

Statistical analysis

Continuous variables were presented as mean \pm standard deviation (SD) for normal distribution or median (P_{25} , P_{75}) for skewed distribution, and categorical variables were presented as percentages. We summarized characteristics of participants by NAFLD phenotype (non-NAFLD vs. NAFLD) with using *t*-test for continuous variables or chi-square test for categorical variables or Wilcoxon rank-sum test for ordinal variables. Several variables had few missing values, we used the approach of deleting rows to handle missing values (listwise deletion) due to a large sample size.

Dietary fiber intake and total energy were categorized into tertiles, we used multivariable logistic regression to evaluate the odds ratios (ORs) and 95% confidence intervals (CIs) for the association of dietary fiber with NAFLD and CSF. Model 1 did not adjust for the covariates, and Model 2 was performed with adjustment for age (18–39, 40–59, and ≥ 60), sex (male and female), smoking (never smokers and ever smokers), race/ethnicity (non-Hispanic White, non-Hispanic Black, and other races), education (less than high school, high school diploma, and more than high school), ratio of family income to poverty (<1.30 , 1.30 – 3.49 , and ≥ 3.50), physical activity (low level, moderate level, and high level), total energy (Tertile 1, Tertile 2, and Tertile 3), hypertension (yes and no), and diabetes (yes and no). We also applied restricted cubic splines with three knots to depict the dose-response curve between dietary fiber and NAFLD as well as CSF. Furthermore, considering departures from the normal distribution, all liver function parameters were

natural logarithm transformed. Multivariable linear regression was performed to estimate the percentage change and 95% CIs for the associations of dietary fiber intake with liver function parameters.

We assumed that obesity was a mediator among the associations of dietary fiber intake with NAFLD phenotype. Because mediator and outcome were dichotomous events, a counterfactual-based mediation analysis was performed. We derived ORs of natural direct effect and natural indirect effect, and total effect was estimated as the product of the natural direct and indirect effect. Pathway diagram was shown in [Supplementary Figure 2](#).

A two-sided $P < 0.05$ was considered to indicate statistical significance. All statistical analyses were performed using R software (version 4.1.0) and Mplus software (version 8.3).

Results

Characteristics of participants

A total of 3,974 participants aged from 18 to 80 years (mean age: 49.34 years; SD: 18.40 years) were included in this study, with 1,903 (47.89%) men and 2,071 (52.11%) women. The prevalence of NAFLD and CSF was 36.86% (1,465) and 7.78% (309). Compared with those who were free of NAFLD, participants with NAFLD were more likely to be older, male, less physically active as well as Hispanic or other races, and tended to consume more energy, have a history of smoking, hypertension, diabetes, and obesity. In addition, we observed that CAP, LSM, globulin, ALT, AST, and GGT were significantly higher in NAFLD participants than those with non-NAFLD, whereas a slight reduction in albumin was observed among NAFLD participants. More details were shown in [Table 1](#).

The associations of dietary fiber with NAFLD and CSF

In multivariable adjusted analyses, compared with the lowest tertile, the highest tertile of dietary fiber consumption was significantly associated with lower odds of NAFLD (OR = 0.81; 95% CI: 0.66–0.98; $P_{trend} = 0.008$; [Table 2](#)). As shown in the restricted cubic splines analysis ([Figure 1A](#)), the odds of NAFLD seemed to decline with the increase of dietary fiber consumption ($P_{overall} = 0.019$); non-linear trend, however, was not observed ($P_{non-linear} = 0.690$). Dietary fiber intake appeared to be linked with lower odds of CSF, however, this trend was only borderline statistical significance (OR_{Tertile3vs.Tertile1} = 0.81; 95% CI: 0.58–1.14; $P_{overall} = 0.107$ based on restricted cubic splines; [Table 2](#), [Figure 1B](#)).

Mediation effect of obesity on the association of dietary fiber with NAFLD

The results of mediation analysis were presented in [Figure 2](#). Obesity was associated with increased odds of NAFLD (OR = 4.20, 95% CI: 3.61–5.01). Compared with those with the lowest tertile intake of dietary fiber, individuals within medium tertile (OR = 0.83, 95% CI: 0.70–0.98) and the highest tertile (OR = 0.61, 95% CI: 0.51–0.72) had lower odds of obesity. Note that the direct effect of dietary fiber on NAFLD was not statistically significant. However, there were significant and protective indirect effect of dietary fiber (OR = 0.94, 95% CI: 0.89–0.99 for tertile 2 vs. tertile 1; OR = 0.85, 95% CI: 0.81–0.90 for tertile 3 vs. tertile 1) against NAFLD by affecting obesity. Taken together, the total effect of dietary fiber on NAFLD was statistically significant (OR = 0.82, 95% CI: 0.67–0.99) for tertile 3 vs. tertile 1. Because of non-significant association of dietary fiber with CSF, mediation analysis was not performed.

The associations of dietary fiber with liver function parameters

Furthermore, it was worth noting that liver function parameters varied across tertiles of dietary fiber consumption. After multivariable adjusted, the concentration of albumin and total bilirubin increased by 2.00% (95% CI: 1.33–2.67%) and 6.20% (1.43–11.20%) from the lowest tertile to the highest tertile of dietary fiber consumption, while concentration of globulin and GGT dropped by 1.53% (0.40–2.66%) and 9.80% (4.66–14.66%), respectively ([Table 3](#)). For the liver biochemical indicators, obesity mediated the association of dietary fiber with albumin, globulin, total bilirubin, and GGT ([Figure 3](#)). In comparing tertile 3 with tertile 1, 21.05%, 26.67%, 24.53%, and 24.04% of the total effects of dietary fiber on albumin, globulin, total bilirubin, and GGT were mediated by obesity, respectively. In addition, there were no statistically significant total effect of dietary fiber on total protein, ALT, and AST ([Supplementary Figure 3](#)).

Discussion

When no mediator was considered, dietary fiber was inversely associated with NAFLD, and striking dose-response curves suggested that higher intake of dietary fiber could confer even greater benefit to protect against NAFLD. After obesity was controlled for the mediator, we found significant total and indirect association, yet there was no longer a significant direct association of dietary fiber with NAFLD. Our results indicated that obesity fully mediated the association of dietary fiber with NAFLD in this large cross-sectional study. Given the limited

TABLE 1 The characteristics of participants according to NAFLD phenotypes^a.

| Characteristics | Overall ^b | NAFLD phenotypes | | <i>P</i> |
|---|-------------------------------|-------------------------------|-------------------------------|----------|
| | | Non-NAFLD | NAFLD | |
| No. of participants | 3,974 | 2,509 | 1,465 | |
| Age (%) | | | | <0.001 |
| 18–39 | 1,357 (34.15) | 1,015 (40.45) | 342 (23.34) | |
| 40–59 | 1,190 (29.94) | 669 (26.66) | 521 (35.56) | |
| ≥60 | 1,427 (35.91) | 825 (32.88) | 602 (41.09) | |
| Male (%) | 1,903 (47.89) | 1,098 (43.76) | 805 (54.95) | <0.001 |
| Smoking (%) | | | | 0.006 |
| Never | 2,429 (61.12) | 1,590 (63.37) | 839 (57.27) | |
| Former | 936 (23.55) | 519 (20.69) | 417 (28.46) | |
| Current | 609 (15.32) | 400 (15.94) | 209 (14.27) | |
| Race (%) | | | | <0.001 |
| Non-Hispanic white | 1,385 (34.85) | 848 (33.80) | 537 (36.66) | |
| Non-Hispanic black | 897 (22.57) | 635 (25.31) | 262 (17.88) | |
| Hispanic or other | 1,692 (42.58) | 1,026 (40.89) | 666 (45.46) | |
| Education (%) | | | | 0.239 |
| Less than high school | 719 (18.12) | 445 (17.76) | 274 (18.75) | |
| High school diploma | 979 (24.68) | 610 (24.34) | 369 (25.26) | |
| More than high school | 2,269 (57.20) | 1,451 (57.90) | 818 (55.99) | |
| Physical activity (%) | | | | <0.001 |
| Low level | 1,387 (35.18) | 815 (32.77) | 572 (39.29) | |
| Moderate level | 379 (9.61) | 240 (9.65) | 139 (9.55) | |
| High level | 2,177 (55.21) | 1,432 (57.58) | 745 (51.17) | |
| Family income to poverty ratio (%) | | | | 0.699 |
| 0–1.29 | 977 (27.82) | 631 (28.50) | 346 (26.66) | |
| 1.30–3.49 | 1,423 (40.52) | 876 (39.57) | 547 (42.14) | |
| ≥3.50 | 1,112 (31.66) | 707 (31.93) | 405 (31.20) | |
| Hypertension (%) | 1,632 (41.78) | 847 (34.38) | 785 (54.44) | <0.001 |
| Diabetes (%) | 751 (18.90) | 281 (11.20) | 470 (32.08) | <0.001 |
| Obesity (%) | 1,595 (40.14) | 649 (25.87) | 946 (64.57) | <0.001 |
| Total energy (kcal) | 1,873.75 (1,443.12, 2,426.88) | 1,845.50 (1,421.00, 2,402.50) | 1,941.00 (1,483.50, 2,457.00) | 0.014 |
| Dietary fiber (g/1,000 kcal) | 7.70 (5.73, 10.70) | 7.72 (5.70, 10.70) | 7.66 (5.82, 10.69) | 0.862 |
| CAP (dB/m) | 262.00 (217.00, 307.00) | 229.00 (199.00, 257.00) | 323.00 (302.00, 352.00) | <0.001 |
| LSM (kPa) | 4.90 (4.00, 6.10) | 4.60 (3.80, 5.60) | 5.70 (4.50, 7.00) | <0.001 |
| Albumin (g/dl) | 4.10 (3.90, 4.30) | 4.10 (3.90, 4.30) | 4.10 (3.80, 4.30) | 0.001 |
| Globulin (g/dl) | 3.10 (2.80, 3.30) | 3.00 (2.80, 3.30) | 3.10 (2.80, 3.40) | 0.001 |
| Total protein (g/dl) | 7.20 (6.90, 7.40) | 7.20 (6.90, 7.40) | 7.10 (6.90, 7.40) | 0.939 |
| Total bilirubin (g/dl) | 0.40 (0.30, 0.60) | 0.40 (0.30, 0.60) | 0.40 (0.30, 0.50) | 0.180 |
| ALT (IU/L) | 17.00 (13.00, 25.00) | 16.00 (12.00, 21.00) | 22.00 (16.00, 32.00) | <0.001 |
| AST (IU/L) | 19.00 (16.00, 23.00) | 18.00 (16.00, 22.00) | 20.00 (16.00, 26.00) | <0.001 |
| GGT (IU/L) | 21.00 (14.00, 31.00) | 18.00 (13.00, 26.00) | 26.00 (18.00, 41.00) | <0.001 |

ALT, Alanine aminotransferase; AST, Aspartate aminotransferase; CAP, Controlled attenuation parameter; GGT, Gamma-glutamyl transaminase; LSM, Liver stiffness measurement; NAFLD, Non-alcoholic fatty liver disease.

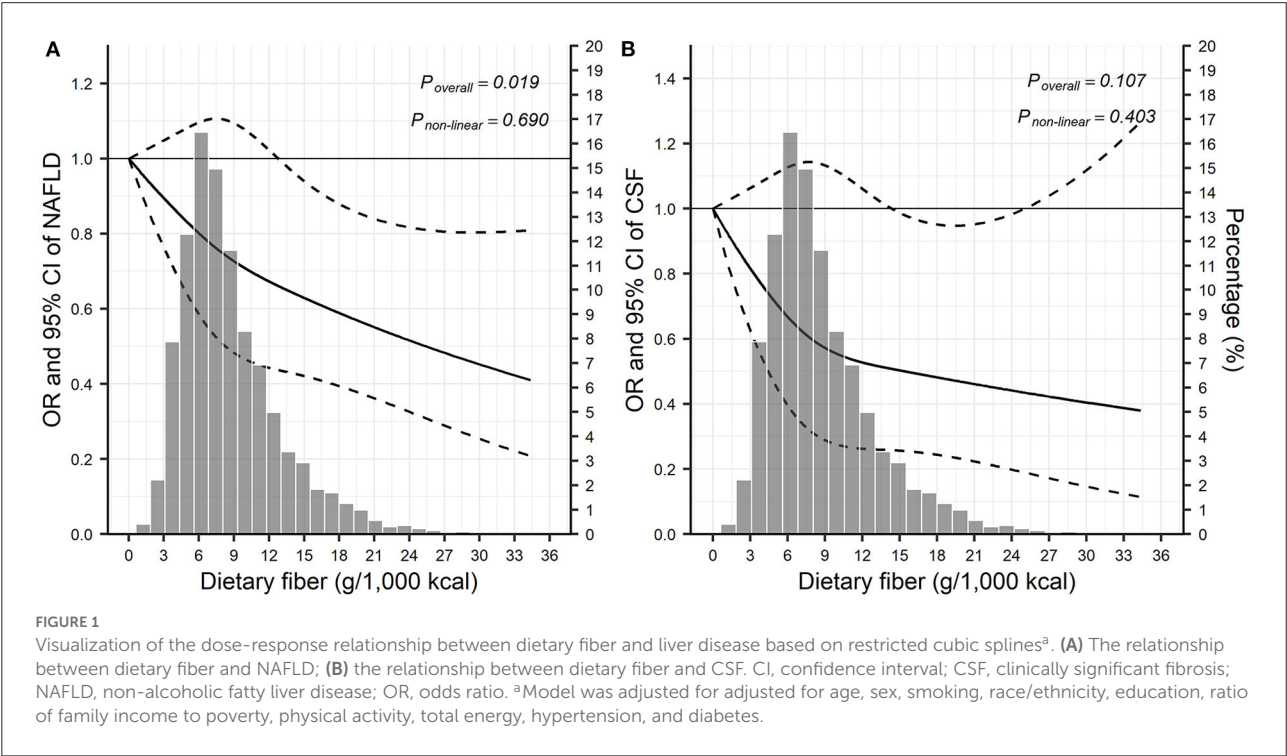
^aValues were presented as median(P₂₅, P₇₅) or percentages.

^bThe summing number for some categories are not 3,974 due to missing values.

TABLE 2 The associations of dietary fiber consumption with NAFLD and CSF.

| Liver diseases | Dietary fiber consumption | | | Per 1-SD | P_{trend}^c |
|-------------------------|---------------------------|------------------|------------------|------------------|---------------|
| | Tertile 1 | Tertile 2 | Tertile 3 | | |
| NAFLD (ORs and 95% CIs) | | | | | |
| Model 1 ^a | 1 | 1.07 (0.91–1.25) | 1.01 (0.86–1.19) | 0.98 (0.92–1.04) | 0.458 |
| Model 2 ^b | 1 | 0.95 (0.79–1.14) | 0.81 (0.66–0.98) | 0.89 (0.82–0.97) | 0.008 |
| CSF (ORs and 95% CIs) | | | | | |
| Model 1 ^a | 1 | 1.09 (0.82–1.44) | 0.95 (0.71–1.26) | 0.91 (0.80–1.03) | 0.131 |
| Model 2 ^b | 1 | 0.91 (0.67–1.25) | 0.81 (0.58–1.14) | 0.87 (0.75–1.01) | 0.068 |

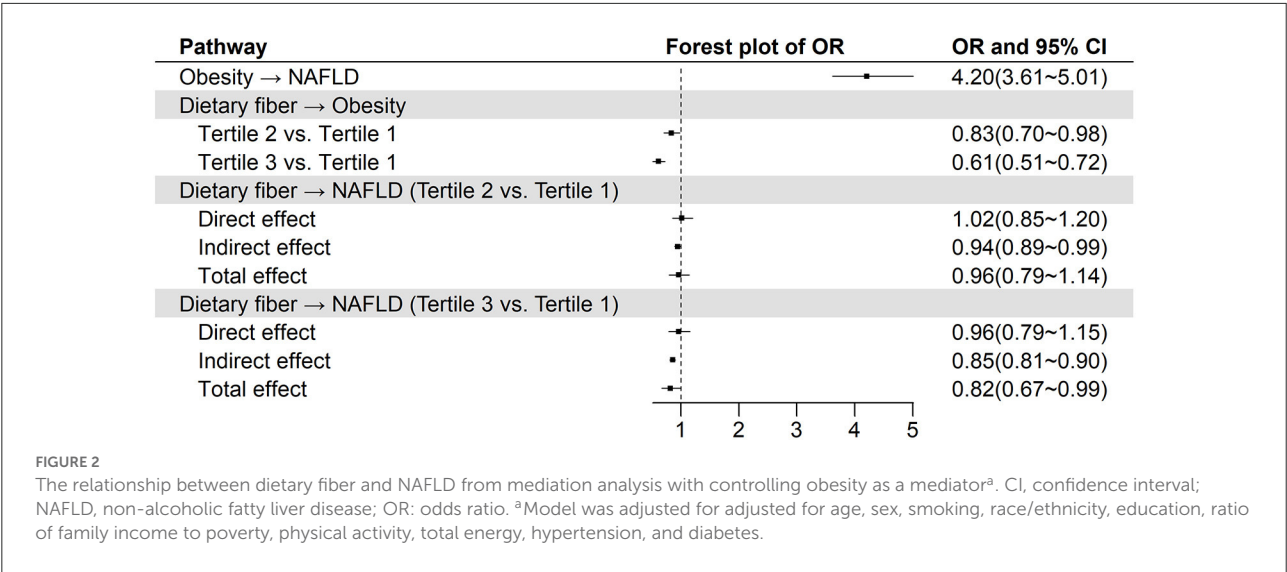
CIs, confidence intervals; CSF, clinically significant fibrosis; ORs, odds ratios.
^aModel 1 did not adjust for the covariates.
^bModel 2 was adjusted for age, sex, smoking, race/ethnicity, education, ratio of family income to poverty, physical activity, total energy, hypertension, and diabetes.
^cThe trend test was performed by using z-score in the models.



number of CSF, we did not observe a statistically significant association between dietary fiber and CSF.

We observed that obesity was strongly linked to NAFLD with an approximately fourfold increased risk. A previous study reported the prevalence of NAFLD up to 80% among obese adults, while 16% among adults with a normal BMI (26), and a meta-analysis reported a significant dose-response relationship between BMI and the risk of NAFLD (13). Even more alarming is that a similar association of obesity with NAFLD was observed among children and adolescents. A recent study of 408 US adolescents with obesity reported that the prevalence of NAFLD was up to 26.0% (27), and the findings from 1,900 adolescents in Italy showed that

adolescents with high waist-to-height ratio had a significantly higher risk of NAFLD and elevated ALT (28). Although the underlying mechanisms of the associations between obesity and NAFLD are not yet fully understood, the adipose tissue (AT) expandability hypothesis can link obesity with the development of NAFLD (29). AT and liver share an evolutionary origin, when each individual adipose tissue reaches the limit of lipid storage, lipids are redirected toward liver and will begin to be deposited ectopically. One consequence of ectopic lipid accumulation is insulin resistance (IR), which is involved in the pathogenesis of NAFLD (30). Ectopic lipid accumulation in the hepatocytes leads to simple steatosis followed by the immune cells infiltrating in liver further contributing to a chronic



intrahepatic inflammatory process and consequent fibrosis, a condition characterized as non-alcoholic steatohepatitis (NASH) (31).

Carbohydrates are the main source of energy. Recent studies have concerned about the health effects of carbohydrates quality rather than quantity (32). Thereinto, dietary fiber has long been thought to have health benefits. The evidence from epidemiology to clinical intervention trials suggested that a generous intake of dietary fiber could lower the risk of obesity and its related non-communicable diseases (33, 34). Observational data suggested an ~30% decrease in incidence of obesity when comparing the highest dietary fiber consumers with the lowest consumers (33). Furthermore, dietary fiber contributes to weight loss for obese population. A meta-analysis of clinical trials showed that higher intake of dietary fiber could help weight loss (34). Our finding of a reduction in risk of obesity among the highest dietary fiber consumers was consistent with previous epidemiological studies. This association is supported by some biological evidence. Fiber-rich food usually requires more chewing times, leading to increased satiety and reduced food intake (35), which may be beneficial in controlling body weight. On the other hand, dietary fiber lowers the risk of obesity through microbiome-related mechanisms. Dietary fiber is fermented by microbiota within gastrointestinal tract and converted to short chain fatty acids (SCFAs), predominantly acetate, propionate, and butyrate. SCFAs can activate G-protein-coupled receptors (*GPR41* and *GPR43*), ultimately suppressing appetite to prevent weight gain. Likewise, butyrate has been shown to be protective against obesity through increasing energy expenditure (34).

A previous study suggested that BMI might be a mediator in the pathway between dietary factor and NAFLD (14), however, the indirect effect mediated through obesity was not

considered in the analysis. According to the present study, the mediation analysis showed that most of the association appeared to have been an indirect effect mediated through obesity. Although we did not observe a statistically significant direct effect of dietary fiber on NAFLD, several evidences supported that dietary fiber could also influence the onset of NAFLD through other biological mechanisms. First, the gut and liver bidirectionally communicate through the biliary tract and portal vein, termed gut-liver axis. A well-known effect of dietary fiber is to regulate gut microbiota, a component of the intestinal barrier. When the gut barrier is compromised, liver is the first extraintestinal organ to be exposed to bacteria and bacterial products, causing inflammation and hepatic injury (36). Second, SCFAs, produced by fermentation of dietary fiber by intestinal microbiota, protect against NAFLD primarily through modulation of inflammation. SCFAs binding to *GPR43* recruit immune cells and regulate inflammatory responses. The progression of NAFLD is often hallmarked by immune cell infiltration. SCFAs reduce liver inflammatory responses by inhibiting the activity of histone acetyltransferases (37). In addition to reducing hepatic inflammatory responses, SCFAs can regulate hepatic lipid metabolism. SCFAs promoted energy expenditure and lipid oxidation through an adenosine monophosphate-activated protein kinase (AMPK) dependent mechanism, reducing the risk of NAFLD (37). Conversely, SCFAs also appear to promote NAFLD. Increased SCFAs flow into the liver through the portal vein, contributing to triglyceride accumulation and gluconeogenesis in the liver (38).

Liver disease develops silently with no signs or symptoms until the late stages. Liver function tests may contribute to the early detection of diseases. A cross-sectional study with 265 healthy adults reported that individuals in the highest quartile of vegetable intake were less likely to have elevated ALT (39),

TABLE 3 The associations of dietary fiber consumption with liver function parameters.

| Liver function parameters ^a | Dietary fiber consumption | | | Per 1-SD | P_{trend}^d |
|--|---------------------------|------------------------|-------------------------|------------------------|---------------|
| | Tertile 1 | Tertile 2 | Tertile 3 | | |
| Albumin | | | | | |
| Model 1 ^b | 0 | 0.62 (−0.02 to 1.25) | 1.36 (0.72 to 2.00) | 0.58 (0.33 to 0.84) | <0.001 |
| Model 2 ^c | 0 | 1.08 (0.46 to 1.71) | 2.00 (1.33 to 2.67) | 0.84 (0.56 to 1.12) | <0.001 |
| Globulin | | | | | |
| Model 1 ^b | 0 | −1.67 (−2.71 to −0.62) | −0.68 (−1.73 to 0.38) | −0.25 (−0.69 to 0.18) | 0.253 |
| Model 2 ^c | 0 | −1.61 (−2.68 to −0.53) | −1.53 (−2.66 to −0.40) | −0.74 (−1.21 to −0.26) | 0.003 |
| Total protein | | | | | |
| Model 1 ^b | 0 | −0.41 (−0.88 to 0.06) | 0.42 (−0.05 to 0.90) | 0.20 (0.01 to 0.39) | 0.044 |
| Model 2 ^c | 0 | −0.13 (−0.61 to 0.36) | 0.42 (−0.10 to 0.94) | 0.14 (−0.07 to 0.36) | 0.198 |
| Total bilirubin | | | | | |
| Model 1 ^b | 0 | 8.65 (4.22 to 13.27) | 7.39 (3.02 to 11.95) | 2.25 (0.52 to 4.00) | 0.011 |
| Model 2 ^c | 0 | 8.63 (3.99 to 13.47) | 6.20 (1.43 to 11.20) | 1.53 (−0.40 to 3.50) | 0.121 |
| ALT | | | | | |
| Model 1 ^b | 0 | 1.19 (−2.97 to 5.52) | 2.38 (−1.82 to 6.76) | 1.29 (−0.43 to 3.04) | 0.143 |
| Model 2 ^c | 0 | 0.82 (−3.38 to 5.20) | −0.08 (−4.46 to 4.50) | 0.88 (−0.99 to 2.79) | 0.360 |
| AST | | | | | |
| Model 1 ^b | 0 | 1.42 (−1.34 to 4.27) | 2.35 (−0.44 to 5.21) | 1.05 (−0.08 to 2.20) | 0.069 |
| Model 2 ^c | 0 | 0.64 (−2.29 to 3.66) | 0.80 (−2.29 to 3.98) | 0.65 (−0.65 to 1.96) | 0.331 |
| GGT | | | | | |
| Model 1 ^b | 0 | −2.55 (−7.45 to 2.61) | −7.84 (−12.47 to −2.97) | −3.83 (−5.84 to −1.78) | <0.001 |
| Model 2 ^c | 0 | −2.45 (−7.44 to 2.82) | −9.80 (−14.66 to −4.66) | −4.22 (−6.43 to −1.96) | <0.001 |

ALT, alanine aminotransferase; AST, aspartate aminotransferase; GGT, gamma-glutamyl transaminase.

^aLiver function parameters were natural logarithm transformed. Percentage change (%) and 95% confidence intervals were calculated as $(e^{\beta} - 1) \times 100\%$ based on multivariable linear regression.

^bModel 1 did not adjust for the covariates.

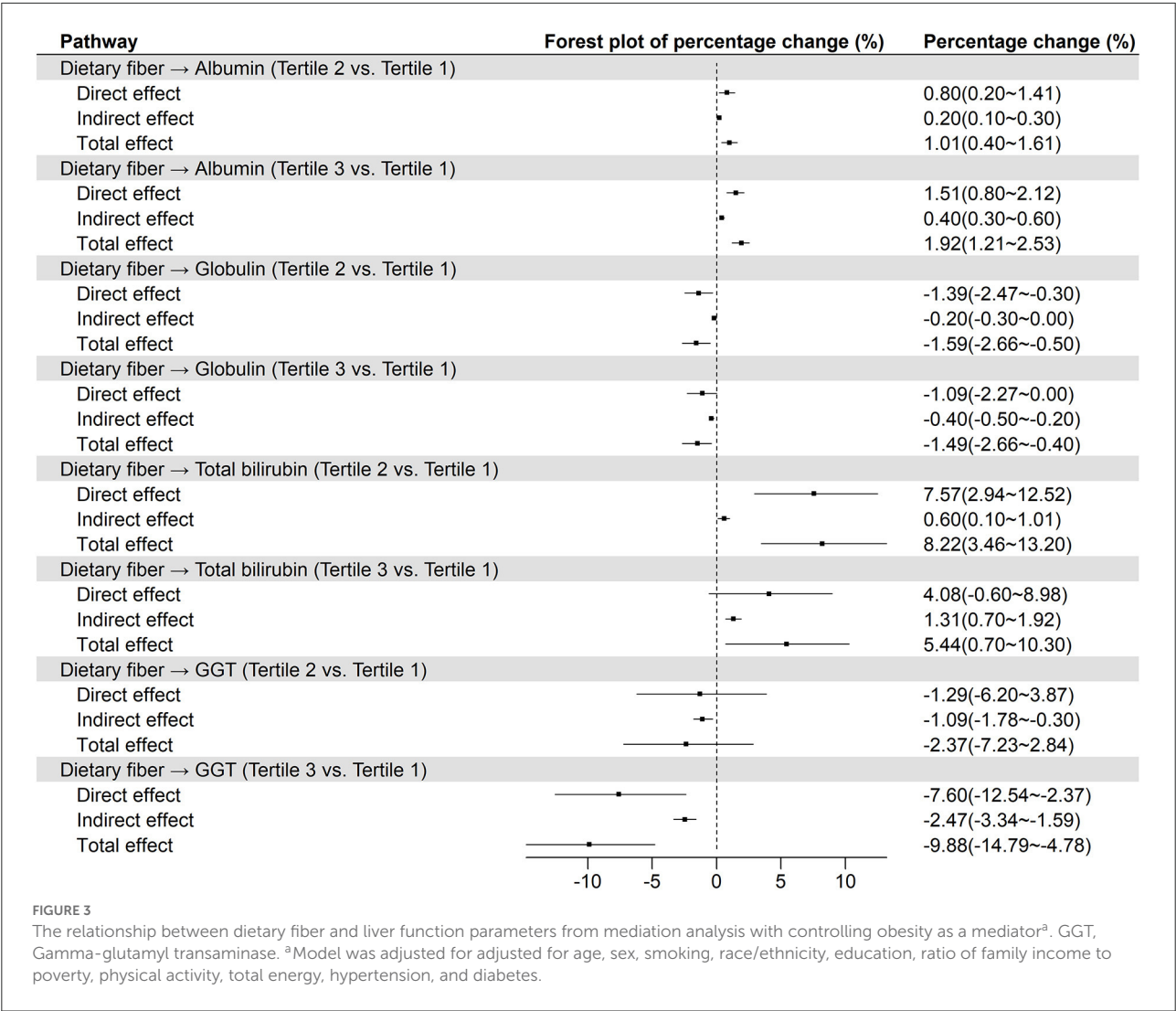
^cModel 2 was adjusted for age, sex, smoking, race/ethnicity, education, ratio of family income to poverty, physical activity, total energy, hypertension, and diabetes.

^dThe trend test was performed by using z-score in the models.

and animal experiment also demonstrated that supplements of insoluble and soluble fibers lowered serum levels of ALT and AST, while increasing serum level of albumin and total protein (40). In current study, we also evaluated the association between dietary fiber and NAFLD at the biochemical level, and observed a similar result that dietary fiber could ameliorate liver function. Elevated serum albumin and total bilirubin were observed among participants with high intake of dietary fiber. Albumin is exclusively synthesized by the liver, and low albumin levels may be a marker of advanced diseases in chronic liver inflammation or cirrhosis (41). Bilirubin is the end product of the breakdown of red blood cells. Accumulating evidence indicates that higher bilirubin levels are inversely associated with NAFLD (42, 43), although the underlying mechanisms are not well elucidated. On the other hand, decline of serum globulin, and GGT was observed among adults with high intake of dietary

fiber. Globulin is a group of proteins synthesized mainly in liver by immune system, increase in globulin may indicate inflammatory diseases (44). GGT is released from damaged liver cells into the blood after hepatocellular injury or death (45, 46), those are considered to serve as reliable non-invasive biomarkers of liver injury.

By combining the findings, dietary fiber intake was associated with lower odds of NAFLD through obesity. Consumption of whole grains and vegetables are important contributors to dietary fiber intake. A case-control study enrolled 940 NAFLD and 940 age- and sex-matched controls from Chinese adults, reported that risk of NAFLD gradually decreased across increasing tertiles of plasma 3-(3,5-dihydroxy phenyl)-1-propanoic acid, a biomarker of whole-grain wheat and rye intake (47). In a randomized controlled clinical trial, 112 patients with NAFLD were randomly assigned to obtain at



least half of cereal each day from whole-grain foods or usual cereals for 12 weeks. A substantial decrease in grades of fatty liver, ALT, AST, and GGT was observed after 12 weeks on the whole-grain foods (48). Furthermore, a cross-sectional study of 18,345 US adults suggested that plant-based diet index (PDI), *a priori* dietary pattern, was negatively associated with ALT, AST, and FLI. Moreover, the highest tertile of PDI was found to reduce a fifth odd of NAFLD (6).

A major strength of the present study was that it has related dietary fiber to NAFLD through controlling obesity. Several limitations should be addressed. First, dietary information was surveyed using two 24-h recalls, it was difficult to capture the long-term dietary intake of dietary fiber. Second, limited data were available regarding specific sources of dietary fiber, we did not estimate the associations of fiber according to diverse food sources with NAFLD risk. Third, we did not observe the obvious threshold effect, although there was a negative

association between dietary fiber intake and NAFLD as well as CSF. Moreover, the *Dietary Guidelines for Americans, 2020–2025* recommend dietary fiber intake of 34 g/1,000 kcal per day for American adult men, and 28 g/1,000 kcal per day for American adult women, respectively (49). This was consistent with the findings of our study that the risk of both NAFLD and CSF was gradually decreased when the fiber intake was within 34 g/1,000 kcal per day. Additionally, the cross-sectional design in the current study did not allow the determination of causality. Through this study alone, it was difficult to provide an exact cut-off value, and large-scale prospective studies or clinical intervention trials in the future were warranted to provide more accurate guidance on the proportion of dietary fiber intake.

Taken together, the present study demonstrated that increased intake of dietary fiber was associated with lower odds of NAFLD. The findings contribute to the growing body of evidence that increasing dietary fiber intake from plant foods

or supplements could confer a greater benefit to protect against NAFLD and improve liver function. Translating these findings regarding dietary fiber into dietary advice might be an attractive strategy for NAFLD prevention.

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 below: <https://wwwn.cdc.gov/nchs/nhanes/continuousnhanes/default.aspx?BeginYear=2017>.

Ethics statement

The studies involving human participants were reviewed and approved by NCHS Research Ethics Review Board. The patients/participants provided their written informed consent to participate in this study.

Author contributions

Full access to all the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis and drafting of the manuscript: YZhu and HY. Study concept and design: WY and YZhu. Statistical analysis: YZhu. Obtained funding, administrative, technical, or material support, and study supervision: WY. Acquisition, analysis, or interpretation of data, and critical revision of the manuscript for important intellectual content: all authors. 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.2022.1038435/full#supplementary-material>

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Exploring the association between circulating trace elements, metabolic risk factors, and the adherence to a Mediterranean diet among children and adolescents with obesity

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Diet is one of the most important modifiable lifestyle factors for preventing and treating obesity. In this respect, the Mediterranean diet (MD) has proven to be a rich source of a myriad of micronutrients with positive repercussions on human health. Herein, we studied an observational cohort of children and adolescents with obesity ($N = 26$) to explore the association between circulating blood trace elements and the degree of MD adherence, as assessed through the KIDMED questionnaire. Participants with higher MD adherence showed better glycemic/insulinemic control and a healthier lipid profile, as well as raised plasma levels of selenium, zinc, cobalt, molybdenum, and arsenic, and increased erythroid content of selenium. Interestingly, we found that these MD-related mineral alterations were closely correlated with the characteristic metabolic complications behind childhood obesity, namely hyperglycemia, hyperinsulinemia, and dyslipidemia ($p < 0.05$, $|r| > 0.35$). These findings highlight the pivotal role that dietary trace elements may play in the pathogenesis of obesity and related disorders.

KEYWORDS

childhood obesity, trace elements, Mediterranean diet, KIDMED, multi-elemental analysis

1. Introduction

Obesity is a condition characterized by an excessive accumulation of body fat, which may have significant negative repercussions on health and raise the risk of developing other chronic diseases. The main driver of obesity is an imbalance between total energy intake and expenditure within the framework of a complex interplay involving multiple players, including genetic, metabolic, environmental, and behavioral factors (1). In this context, diet is well-recognized to be one of the most important modifiable lifestyle factors in the prevention and treatment of obesity and related disorders. On the one hand, the over-consumption of calories is the most likely cause of the obesity epidemic, mainly because of the loss of traditional dietary patterns and the increasingly frequent intake of energy-dense foods (e.g., high-fat foods) and added sugar-containing foods (2). Moreover, the adherence to unhealthy diets has also been

linked to deficiencies of multiple micronutrients, including vitamins, carotenoids, and trace elements, which in turn may contribute and aggravate the pathogenic events behind obesity (3). Trace elements are essential micronutrients that are primarily acquired through the diet and participate in a myriad of primary biological processes, including the antioxidant defense, immune system, hormonal regulation, and many others. In particular, growing evidence points to essential and toxic metals as pivotal regulators of a myriad of biological processes underlying the development and progression of obesity and its comorbidities (4). For instance, it has been repeatedly reported that numerous trace elements (e.g., zinc, chromium, vanadium, molybdenum, cobalt) play central roles in the synthesis, storage, and action of insulin, and consequently influence carbohydrate and lipid metabolism (5). Their correct homeostasis, both in terms of potentially pro-oxidant species (e.g., iron, copper) and elements participating in the antioxidant defense (e.g., selenium, manganese), is also crucial to maintain an adequate redox status (6, 7). Furthermore, it is noteworthy that metals and the immune system are bidirectionally interrelated, being mineral deficiencies associated with impaired immune function, whereas the inflammatory response (e.g., secretion of cytokines) can modulate the metabolism and bioavailability of trace elements and other nutrients (8). On the other hand, the exposure to toxic heavy metals (e.g., cadmium, mercury, lead) has been demonstrated to disrupt the endocrine system and to induce chronic inflammation and oxidative stress (9).

A few authors have previously studied the influence of diet determinants on the levels of specific metals among healthy children (10, 11), but this remains unexplored in obese populations. Obesity is nowadays the most prevalent chronic disease among children and adolescents, which can be often accompanied by different comorbidities related to abnormal insulin-mediated glucose control (e.g., insulin resistance, impaired fasting glucose, impaired glucose tolerance) and dyslipidemia (12). As mentioned above, insulin, glucose, and lipid metabolisms are tightly regulated by essential elements and may be impaired by exposure to toxic heavy metals. Accordingly, investigating the interplay between dietary habits, the homeostasis of trace elements, and the characteristic metabolic complications concurring with obesity is crucial to get new insights into the pathogenesis of this disorder. In this study, we aimed to explore the association between circulating trace elements and the degree of adherence to a Mediterranean diet (MD), as assessed through the Mediterranean Diet Quality Index for children and adolescents (KIDMED), in a Spanish observational cohort of children and adolescents with obesity. To this end, we applied multi-elemental analysis to plasma and erythrocyte samples with the aim of comprehensively characterizing the biodistribution of trace elements in peripheral blood. Furthermore, we also investigated the association between blood metals and other biochemical variables (e.g., glycemia, insulinemia, lipid profile) to better understand the role of diet-related micronutrient alterations in the pathogenic events behind childhood obesity.

2. Materials and methods

2.1. Study design

The study design consisted of an observational cohort of children and adolescents with obesity recruited at “Hospital

Universitario Puerta del Mar” (Cádiz, Spain), who underwent an oral glucose tolerance test (OGTT) under medical prescription. The inclusion criteria were children and adolescents of both sexes, aged between 6 and 14 years, presenting a body mass index (BMI) over two standard deviations above the mean of the reference population, adjusted for sex and age (13). Subjects with other known chronic systemic diseases or suffering of acute infectious processes were not eligible for the study. Using a sample size of 26 children and considering an alpha risk of 0.05, the statistical power of our comparisons was above 80%, as calculated using the GRANMO 7.12 webtool. The study was performed in accordance with the principles contained in the Declaration of Helsinki. The Ethical Committee of “Hospital Universitario Puerta del Mar” (Cádiz, Spain) approved the study protocol (Ref. PI22/01899), and all participants and/or legal guardians provided written informed consent.

2.2. Sampling and determination of anthropometric and biochemical variables

From all participants, venous blood samples were obtained by venipuncture of the antecubital region using an intravenous catheter, BD Vacutainer tubes, and Advance vacuum system. All baseline blood samples were collected in the morning after overnight fasting to minimize the influence of the circadian rhythm and dietary factors. Additional blood samples were collected at the different time points along the OGTT curve (i.e., 30, 60, 90, and 120 min) to evaluate glycemia- and insulinemia-related variables. After withdrawal, blood tubes were gently inverted several times and placed horizontally on ice to prevent red cell lysis and to reduce protease activity. Then, blood samples were centrifuged at 1,500 g for 10 min at 4°C to separate the plasma. Finally, the resulting pellets were washed with cold saline solution (9 g/L NaCl, 4°C) and centrifuged at 1,500 g for 5 min at 4°C (three times) to obtain the erythrocyte fraction. The plasma and erythrocyte samples were aliquoted and stored at −80°C until analysis.

Anthropometric variables (i.e., weight, height, BMI, and waist circumference) were evaluated by pediatric endocrinologists (J.D.-R. and A.M.L.-S.). The height was measured using a fixed wall stadiometer, whereas weight was determined in a SECA 5,000 balance. Blood glucose and insulin concentrations along the OGTT curve (i.e., 0, 30, 60, 90, and 120 min), as well as the fasting lipid profile, were measured using an Alinity automatic analyzer (Abbot, Spain) located at the Clinical Analysis Laboratory of the “Hospital Universitario Puerta del Mar.” Briefly, plasma levels of glucose and insulin were determined using the hexokinase method (CV < 3%) and an electrochemiluminescence microparticle immunoassay (CV < 2.2%), respectively. Enzymatic colorimetric assays were applied to quantify total cholesterol (TC, CV < 1.4%), high-density lipoprotein cholesterol (HDL-C, CV < 2.1%), low-density lipoprotein cholesterol (LDL-C, CV < 1.7%), and triglycerides (TG, CV < 1.1%). The homeostasis model assessment of insulin resistance (HOMA-IR) was calculated by applying the following formula: $HOMA-IR = (Glc_0 \times Ins_0) \times 0.055/22.5$, where Glc_0 and Ins_0 refer to the fasting plasma levels of glucose (mg/dL) and insulin (μU/mL), respectively.

2.3. Dietary assessment

The adherence to a MD of the study participants was assessed using the updated version of the Mediterranean Diet Quality Index for children and adolescents (KIDMED) (14, 15). This dietary questionnaire consists of 16 dichotomous items (i.e., yes/no), of which 12 questions denote a positive connotation with respect to the MD (e.g., consumption of fruits, vegetables, fish, pulses, pasta or rice, cereals, nuts, olive oil, cereals, dairy products) and 4 questions denote a negative connotation (e.g., consumption of fast food, baked goods, sweets, skipping breakfast). Items with a positive connotation were assigned a value of +1, and those with a negative connotation a value of −1. The total KIDMED score is obtained by summing these individual values, which allows classifying subjects into three different groups according to their degree of MD adherence: very low diet quality (≤ 3), improvement needed to adjust intake to Mediterranean patterns (4–7), and optimal MD (≥ 8). For further analyses, these categories were collapsed into low MD adherence (“improvement needed to adjust intake to Mediterranean patterns” and “very low diet quality”) or high MD adherence (“optimal MD”) (16). This dietary assessment was conducted *via* interview by pediatric endocrinologists (J.D.-R.).

2.4. Multi-elemental analysis of plasma and erythrocyte samples

Trace elements (i.e., vanadium, chromium, manganese, iron, cobalt, copper, zinc, arsenic, selenium, molybdenum, cadmium, and lead) were determined by diluting aliquots of 150 μ L of plasma or 50 μ L of the erythrocyte fraction to a final volume of 3 mL using an alkaline solution containing 2% 1-butanol (w/v), 0.05% EDTA (w/v), 0.05% Triton X-100 (w/v), and 1% NH_4OH (w/v) (17). As the internal standard, rhodium was added to sample extracts to reach a final concentration of 1 μ g/L. Samples were filtered through 0.45 μ m pore size hydrophilic PTFE filters before analysis. Then, multi-elemental determinations were performed using an Agilent 7900 inductively coupled plasma mass spectrometer (ICP-MS) equipped with collision/reaction cell system and with nickel sampling and skimmer cones (Agilent Technologies, Tokyo, Japan). High-purity grade helium ($>99.999\%$) was employed as the collision gas. The ICP-MS working conditions were set as follows (18): sampling depth, 7 mm; forward power, 1,550 W; plasma gas, 15 L/min; auxiliary gas, 1 L/min; carrier gas, 1 L/min; make-up gas, 0.10 L/min; collision gas, 5 mL/min. The isotopes monitored were ^{51}V , ^{52}Cr , ^{53}Cr , ^{55}Mn , ^{56}Fe , ^{57}Fe , ^{59}Co , ^{63}Cu , ^{66}Zn , ^{75}As , ^{77}Se , ^{78}Se , ^{82}Se , ^{95}Mo , ^{98}Mo , ^{103}Rh , ^{111}Cd , and ^{208}Pb , using a dwell time of 0.3 s per isotope. Multi-elemental calibration curves were prepared within the concentration range 0.5–2,500 μ g/L, containing 1 μ g/L rhodium as the internal standard.

2.5. Statistical analysis

Data normality and skewness was first tested by inspecting normal probability plots and by performing the Kolmogorov–Smirnov test. Clinical, biochemical, and dietary

variables were subjected to Mann-Whitney U test (continuous variables) and chi-square test (dichotomous variables) to compare the study groups. The pre-processing and statistical analysis of the multi-elemental data were performed using the MetaboAnalyst 5.0 web tool (<https://www.metaboanalyst.ca/>), as follows. First, variables with more than 20% missing values were removed, and the remaining missing values were imputed using the kNN algorithm. Then, the data were log transformed and Pareto scaled. To look for differences between the study groups, data were subjected to Mann-Whitney U test. Finally, correlation analysis was applied to look for associations between trace elements and the KIDMED scores, as well as between trace elements and other biochemical variables (i.e., glucose, insulin, lipids). *P*-Values below 0.05 were considered as statistically significant.

3. Results

In our study population of children and adolescents with obesity, the demographic and anthropometric characteristics were similar between participants with low and high MD adherence as assessed through the KIDMED questionnaire (Table 1). Evidently, the average KIDMED scores significantly differed between the study groups (low MD adherence: 5.2, high MD adherence: 8.3), and similar results were observed for the frequency of consumption of some of the individual items conforming the questionnaire (e.g., Q2: consumption of fruits; Q9: consumption of cereals). Furthermore, it should be noted that subjects reporting higher KIDMED scores showed lower blood levels of glucose and insulin along the OGTT curve, as well as lower fasting levels of total cholesterol.

The degree of adherence to a MD also had a significant influence on the multi-elemental profile of plasma and erythrocyte samples of the study participants, as shown in Table 2. The plasma contents of selenium, zinc, cobalt, molybdenum, and arsenic were higher among subjects adhering to an optimal MD, and the same trend was observed for other trace elements without reaching statistical significance (e.g., chromium, manganese, iron). Similarly, increased erythroid selenium was observed in children and adolescents from the high MD adherence group, but no significant differences were found for the rest of elements under investigation. These findings were further corroborated in a large extent through correlation analyses. The KIDMED index was found to be positively associated with plasma levels of selenium ($r = 0.58$), zinc ($r = 0.57$), molybdenum ($r = 0.40$), and arsenic ($r = 0.39$) (Figure 1A). Furthermore, circulating trace elements were also correlated with the frequency of consumption of some of the individual items conforming the KIDMED questionnaire (Supplementary Figure 1). A consistent negative association was observed between unhealthy dietary habits (e.g., consumption of fast food, baked goods, sweets) and the plasma contents of zinc and/or molybdenum. On the other hand, the intake of food products with a healthy connotation with respect to the MD was positively associated with a myriad of plasmatic micronutrients: the consumption of a second fruit with molybdenum and iron, fish with selenium and arsenic, pulses with molybdenum, and dairy products with copper. Conversely, correlation analysis between dietary intake data and erythroid mineral profiles yielded less robust results, with only a few individual food items being significantly associated with copper

TABLE 1 Demographic, anthropometric, dietary, and biochemical characterization of the study population.

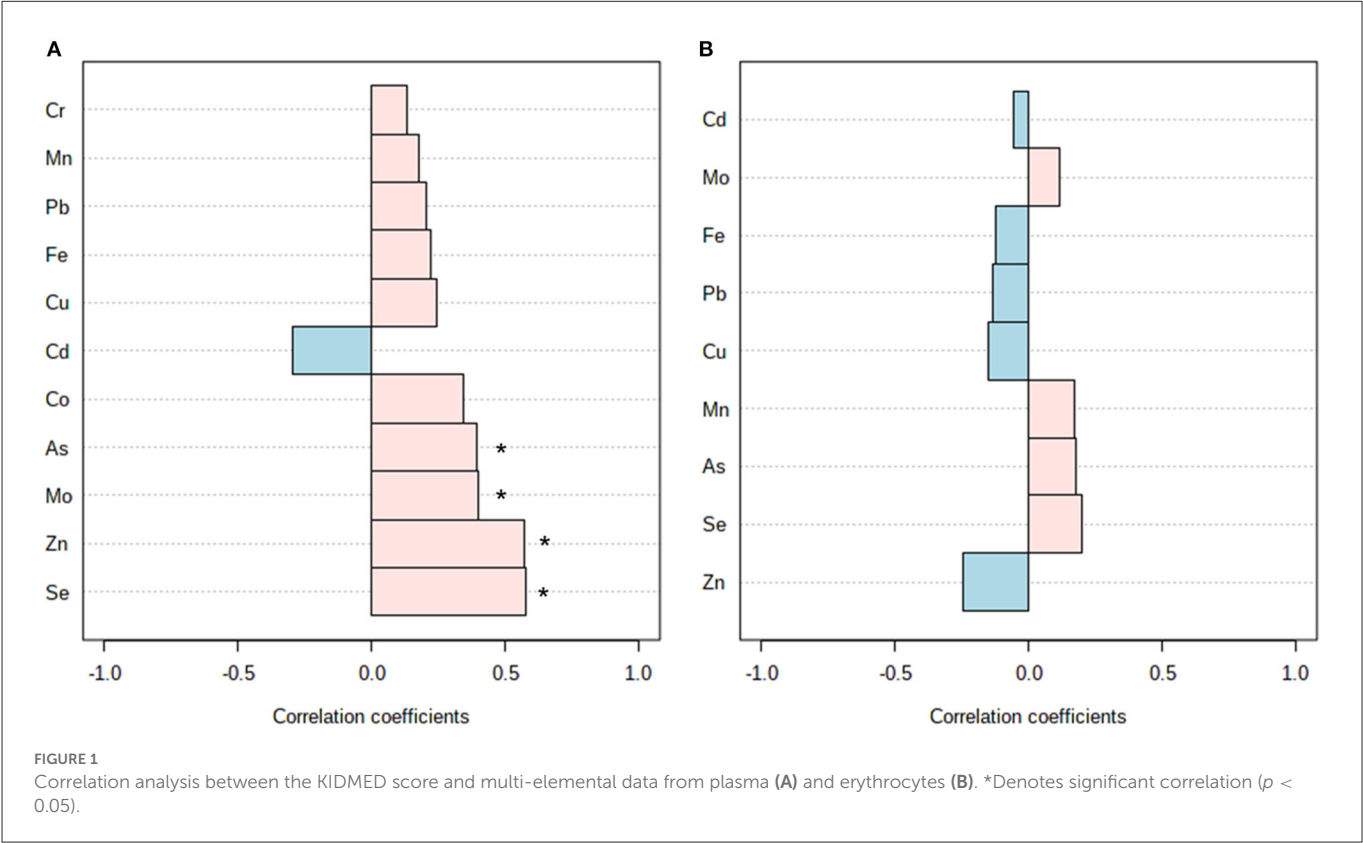
| | Low MD adherence | High MD adherence | <i>p</i> -value |
|--|---------------------|---------------------|----------------------|
| Demographics and anthropometry | | | |
| <i>N</i> | 12 | 14 | |
| Age (years) | 10.4 [9.3–11.8] | 11.7 [10.3–13.0] | NS |
| Sex (% male) | 56.7 | 57.1 | NS |
| Weight (kg) | 68.8 [58.0–80.2] | 70.8 [67.8–80.4] | NS |
| Body mass index (kg/m ²) | 31.1 [28.8–35.2] | 30.5 [27.7–32.0] | NS |
| Waist circumference (cm) | 103.5 [95.0–113.0] | 102.0 [98.0–105.5] | NS |
| Dietary assessment | | | |
| KIDMED score | 5.5 [4.0–6.0] | 8.0 [7.0–9.0] | 1.6·10 ^{−7} |
| Q1. Consumes a fruit every day (%) | 58.3 | 85.7 | NS |
| Q2. Consumes a second fruit every day (%) | 25.0 | 71.4 | 1.8·10 ^{−2} |
| Q3. Consumes fresh/cooked vegetables regularly once a day (%) | 41.7 | 64.3 | NS |
| Q4. Consumes fresh/cooked vegetables more than once a day (%) | 16.7 | 28.6 | NS |
| Q5. Consumes fish regularly (%) | 58.3 | 78.5 | NS |
| Q6. Consumes fast-food more than once a week (%) | 25.0 | 7.1 | NS |
| Q7. Consumes pulses more than once a week (%) | 83.3 | 100.0 | NS |
| Q8. Consumes pasta/rice almost every day (%) | 8.3 | 0.0 | NS |
| Q9. Consumes cereals/grains for breakfast (%) | 41.7 | 78.6 | 4.9·10 ^{−2} |
| Q10. Consumes nuts regularly (%) | 25.0 | 42.9 | NS |
| Q11. Consumes olive oil at home (%) | 100.0 | 100.0 | NS |
| Q12. Skips breakfast (%) | 25.0 | 14.3 | NS |
| Q13. Consumes a dairy product for breakfast (%) | 66.7 | 92.9 | NS |
| Q14. Consumes commercially baked goods or pastries for breakfast (%) | 25.0 | 14.3 | NS |
| Q15. Consumes two yogurts and/or some cheese daily (%) | 66.7 | 78.6 | NS |
| Q16. Consumes sweets and candy several times every day (%) | 16.7 | 14.3 | NS |
| Biochemical variables | | | |
| Glucose, <i>t</i> = 0' (Glc ₀ , mg/dL) | 81.0 [76.2–84.2] | 83.5 [78.0–86.0] | NS |
| Glucose, <i>t</i> = 30' (Glc ₃₀ , mg/dL) | 139.0 [122.2–157.2] | 143.0 [130.0–159.0] | NS |
| Glucose, <i>t</i> = 60' (Glc ₆₀ , mg/dL) | 143.0 [126.5–161.2] | 120.0 [112.0–138.8] | 3.8·10 ^{−2} |
| Glucose, <i>t</i> = 90' (Glc ₉₀ , mg/dL) | 128.0 [118.8–148.5] | 108.5 [100.0–115.8] | 2.6·10 ^{−3} |
| Glucose, <i>t</i> = 120' (Glc ₁₂₀ , mg/dL) | 134.0 [124.0–144.5] | 124.5 [115.0–127.8] | 2.6·10 ^{−2} |
| Insulin, <i>t</i> = 0' (Ins ₀ , μU/mL) | 17.0 [14.0–21.5] | 16.5 [13.0–20.8] | NS |
| Insulin, <i>t</i> = 30' (Ins ₃₀ , μU/mL) | 125.1 [84.4–156.5] | 132.6 [77.0–185.7] | NS |
| Insulin, <i>t</i> = 60' (Ins ₆₀ , μU/mL) | 135.7 [96.0–226.6] | 143.4 [126.5–162.2] | NS |
| Insulin, <i>t</i> = 90' (Ins ₉₀ , μU/mL) | 127.4 [96.6–159.7] | 109.3 [74.0–141.8] | NS |
| Insulin, <i>t</i> = 120' (Ins ₁₂₀ , μU/mL) | 147.1 [123.4–280.2] | 107.9 [106.5–170.4] | 2.2·10 ^{−2} |
| Homeostasis model assessment of insulin resistance (HOMA-IR) | 3.3 [2.8–3.9] | 3.2 [2.6–3.8] | NS |
| Total Cholesterol (TC, mg/dL) | 159.5 [149.8–166.2] | 149.5 [119.0–152.0] | 4.0·10 ^{−3} |
| High-density lipoprotein cholesterol (HDL-C, mg/dL) | 43.5 [40.0–49.8] | 40.0 [38.0–45.8] | NS |
| Low-density lipoprotein cholesterol (LDL-C, mg/dL) | 96.5 [93.2–102.5] | 96.5 [70.0–98.0] | NS |
| Triglycerides (TG, mg/dL) | 86.0 [60.0–103.5] | 92.0 [82.0–117.5] | NS |

Results are expressed as median [IQR] (except for dichotomous variables, expressed as percentages). NS, non-significant.

TABLE 2 Plasma and erythroid concentrations of trace elements in the two study groups.

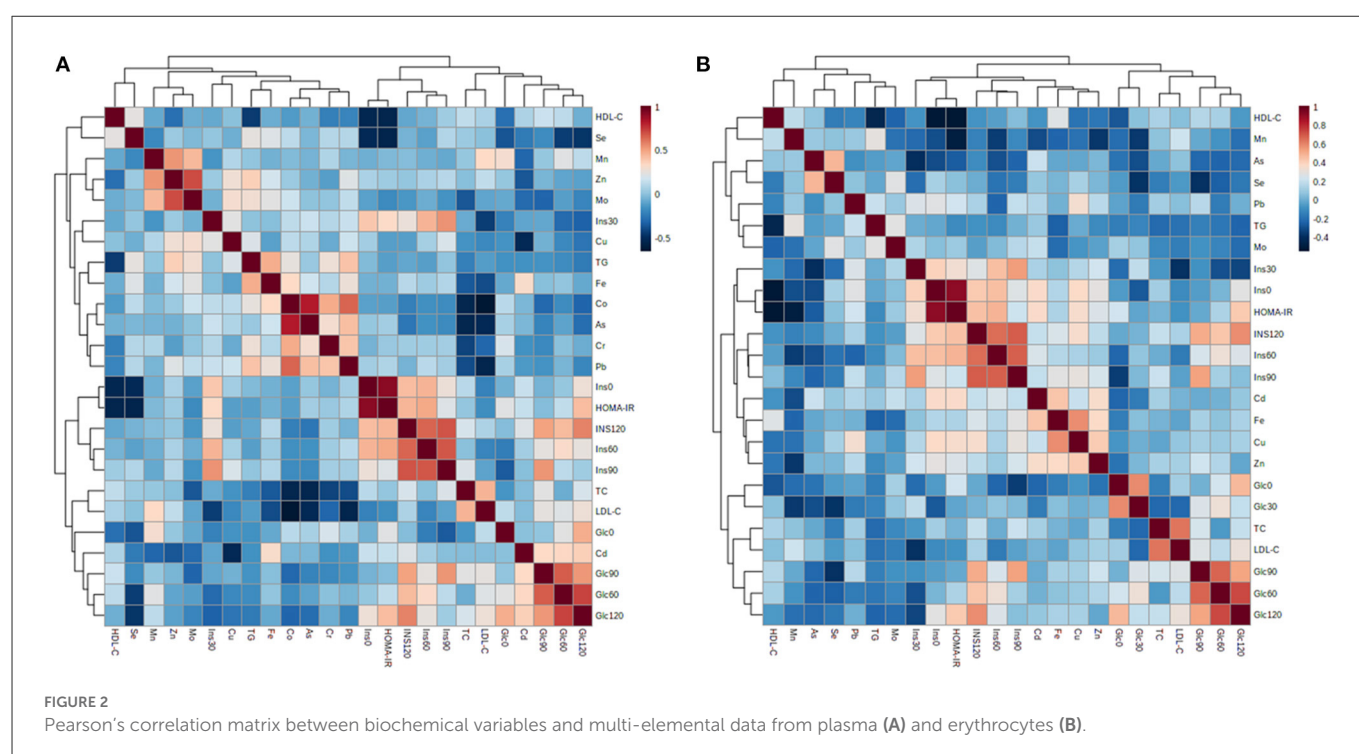
| | Plasma | | | Erythrocytes | | |
|------------|---------------------------|---------------------------|----------------------|---|---|----------------------|
| | Low MD adherence | High MD adherence | <i>p</i> -value | Low MD adherence | High MD adherence | <i>p</i> -value |
| Chromium | 3.4 [2.9–4.9] | 4.8 [3.7–7.5] | NS | ND | ND | - |
| Manganese | 4.0 [3.7–4.5] | 4.7 [3.6–5.0] | NS | 20.2 [10.9–27.7] | 18.7 [14.2–28.0] | NS |
| Iron | 676.8 [622.8–723.7] | 779.0 [659.8–835.4] | NS | 5.8·10 ⁵ [5.6·10 ⁵ –6.1·10 ⁵] | 5.6·10 ⁵ [5.4·10 ⁵ –5.8·10 ⁵] | NS |
| Cobalt | 0.57 [0.53–0.62] | 0.68 [0.61–0.73] | 2.2·10 ^{−2} | ND | ND | - |
| Copper | 1,207.6 [1,122.8–1,311.0] | 1,228.9 [1,171.2–1,301.8] | NS | 640.5 [590.6–666.6] | 631.9 [584.7–687.7] | NS |
| Zinc | 689.2 [566.7–1,176.0] | 1,215.5 [1,156.3–1,273.5] | 9.5·10 ^{−3} | 10,047.3 [9,479.1–10,787.2] | 9,547.5 [9,184.2–9,753.2] | NS |
| Arsenic | 0.57 [0.33–0.90] | 0.85 [0.71–1.09] | 2.8·10 ^{−2} | 42.0 [31.2–50.1] | 47.0 [40.0–50.9] | NS |
| Selenium | 124.4 [120.7–133.5] | 137.6 [131.5–143.9] | 3.3·10 ^{−3} | 164.4 [136.1–186.1] | 179.5 [159.6–242.2] | 3.7·10 ^{−2} |
| Molybdenum | 1.9 [1.4–2.4] | 2.7 [2.3–3.1] | 2.8·10 ^{−2} | 19.3 [17.2–22.7] | 21.1 [19.9–24.8] | NS |
| Cadmium | 0.0026 [0.00074–0.0036] | 0.0018 [0.00099–0.0027] | NS | 1.9 [1.3–2.6] | 1.8 [1.1–2.6] | NS |
| Lead | 0.021 [0.020–0.022] | 0.021 [0.020–0.023] | NS | 62.4 [58.3–71.3] | 58.4 [56.5–60.2] | NS |

Results are expressed as median [IQR] (μg/L). ND, non-detected; NS, non-significant.



(e.g., consumption of a second vegetable, cereals) and zinc (e.g., consumption of pasta/rice) levels (Supplementary Figure 2).

Finally, Pearson's correlations were computed between multi-elemental and biochemical variables with the aim of investigating the relationship between diet-related micronutrient alterations and the typical metabolic complications behind childhood obesity (Figure 2). Glucose and insulin concentrations along the OGTT, as well as the HOMA-IR scores, showed a consistent negative association with selenium ($r = -0.36$ with Glc₀, $r = -0.43$ with Glc₆₀, $r = -0.45$ with Glc₁₂₀, $r = -0.50$ with Ins₀, $r = -0.54$ with HOMA-IR) and a positive association with cadmium ($r = 0.35$ with Glc₆₀, $r = 0.35$ with Glc₉₀, $r = 0.38$ with Glc₁₂₀) in plasma. The same trend of association was observed for erythroid selenium ($r = -0.37$ with Glc₃₀, $r = -0.38$ with Glc₉₀) and cadmium ($r = 0.37$ with Ins₀, $r = 0.37$ with HOMA-IR). Similarly, erythroid levels of manganese were negatively correlated to the HOMA-IR score ($r = -0.48$). Regarding lipid parameters, various plasmatic trace elements, including iron, cobalt, chromium, arsenic, and lead, were negatively associated with



LDL-C ($r = -0.38$ with Fe, $r = -0.63$ with Co, $r = -0.51$ with As, $r = -0.54$ with Pb) and/or TC ($r = -0.34$ with Fe, $r = -0.53$ with Co, $r = -0.40$ with Cr, $r = -0.53$ with As, $r = -0.38$ with Pb) levels. Conversely, a positive association was found between TGs and plasma iron ($r = 0.48$), zinc ($r = 0.38$), and lead ($r = 0.42$).

4. Discussion

Dietary assessment through self-reporting methods (e.g., food frequency questionnaires, dietary recalls) has repeatedly evidenced a positive association between the adherence to a MD, micronutrient intake, and nutritional adequacy in young (19, 20), adult (21), and older populations (22). However, the measurement of dietary biomarkers in biological matrices has emerged in recent years as a more reliable strategy to get a closer and more objective understanding of the crosstalk between diet and health within the complex meshwork of bioavailability, metabolism, biodistribution, and excretion processes. In this respect, some observational studies have previously reported that subjects adhering to a MD usually have increased blood levels of multiple micronutrients, including vitamins (23), lipid-soluble compounds (e.g., carotenoids, tocopherols) (24), n-3 polyunsaturated fatty acids (25), and other essential food bioactives. To get a more comprehensive exploration about the influence of nutrition on human health, other authors have proposed the application of wide-coverage metabolomics approaches for identifying food intake biomarkers and biomarkers of adherence to specific dietary patterns (26, 27). Nevertheless, the number of studies relying on the determination of trace elements are still scarce (28), and normally focused on investigating single food items rather than complex dietary patterns (10, 11, 29).

In the present study, we have demonstrated for the first time that circulating blood trace elements can reflect the degree of MD adherence, as assessed through the KIDMED index, among

children and adolescents with obesity. Herein, we found that subjects reporting higher KIDMED scores showed increased selenium, zinc, cobalt, molybdenum, and arsenic in plasma, as well as increased erythroid levels of selenium (Table 2). These findings could be explained by the rich content of minerals present in the major food products that conform the traditional MD, including vegetables, fruits, cereals, and fish (30). Indeed, nutritional interventions with healthy dietary habits (e.g., MD) have proven to significantly raise the serum concentrations of various essential minerals (31). Further correlation analyses corroborated our results (Figure 1) and shed more light on the specific food items that could contribute the most to the characteristic multi-elemental profiles observed among participants with optimal MD adherence (Supplementary Figures 1, 2). Circulating contents of essential trace elements (e.g., selenium, iron, molybdenum, copper) were positively correlated with the frequency of consumption of healthy food products, such as fruits, vegetables, fish, pulses, and dairy products, in line with previous data (29, 32). Conversely, plasma levels of zinc and molybdenum were negatively associated with unhealthy dietary habits, such as the intake of fast foods, baked goods, and sweets. Therefore, we hypothesize that metal differences between the study groups could be regarded as a direct reflection of the MD adherence, since participants consuming healthy diets are expected to have higher circulating levels of minerals than subjects who replace healthy food items with micronutrient-poor ones. Altogether, these results highlight the crucial role that diet may play on maintaining an adequate micronutrient status. Nevertheless, it is worth mentioning here that levels of metal elements may be influenced by many exogenous (e.g., diet, pollution, smoking, medications) and endogenous factors (e.g., sex, age, hormonal factors) (33), which considerably limits their reliability as sensitive and specific dietary biomarkers.

To conclude, it should also be noted that participants with the higher MD adherence showed better glycemic and insulinemic control (i.e., lower blood levels of glucose and insulin along the

OGTT curve) and a healthier lipid profile (i.e., lower fasting TC levels) compared to those reporting lower KIDMED scores. For this reason, we decided to explore possible associations between the MD-related mineral profile and the pathogenic hallmarks behind childhood obesity, namely abnormal insulin-mediated carbohydrate metabolism and dyslipidemia (Figure 2). On the one hand, we found a strong negative association between selenium and manganese levels (this latter only in erythrocytes) and different variables related to hyperglycemia and hyperinsulinemia. In this respect, other authors have previously reported reduced activity of selenium- and manganese-dependent antioxidant enzymes, such as glutathione peroxidase (34, 35) and manganese superoxide dismutase (36), in children with obesity. This exacerbated oxidative stress may in turn perturb insulin secretion in pancreatic β cells (5), which could explain the direction of association between the above-mentioned trace elements and glycemia/insulinemia-related variables. Conversely, the association was positive with plasma and erythroid contents of cadmium, plausibly because of its capacity to disrupt the endocrine system, provoke insulin resistance, and consequently increase the circulating concentrations of insulin and glucose (9). In line with previous studies describing the involvement of trace elements and heavy metals in the development of dyslipidemia factors, we found a negative correlation between various blood cholesterol fractions (e.g., TC, LDL-C) and plasmatic minerals (e.g., iron, cobalt, chromium, arsenic, lead). This could be attributed to the essential roles that trace elements seem to play in ameliorating atherogenic dyslipidemia through a myriad of mechanisms (e.g., lipid β -oxidation, expression of peroxisome proliferator-activated receptors), and particularly by interfering with cholesterol metabolism (37–39). Surprisingly, a positive association was found between plasma TG, iron, zinc, and lead, which could be due to their dietary co-occurrence (e.g., animal-based products).

5. Conclusions

Herein, we have demonstrated that the degree of MD adherence, as assessed through the KIDMED score, has a significant impact on the biochemical profile and trace element status among children and adolescents with obesity. Subjects adhering to an optimal MD showed better glycemic/insulinemic control and a healthier lipid profile, as well as increased metal contents in plasma (selenium, zinc, cobalt, molybdenum, arsenic) and erythrocytes (selenium). Further correlation analyses evidenced close interrelationships between these diet-related mineral alterations and the typical metabolic complications behind childhood obesity (i.e., abnormal insulin-mediated carbohydrate metabolism, dyslipidemia), thereby highlighting the crucial role that diet might play on health through maintaining an adequate micronutrient status.

The main limitation of the present study was the relatively small sample size of the population under investigation and the lack of an independent cohort for validation purposes. Although this pilot exploration lays the foundation for better understanding the interplay between dietary habits, homeostasis of trace elements, and obesity-related pathogenic events, future studies are needed in larger cohorts to further validate our findings. In this respect, the analysis of other complementary biological matrices could be of great interest to investigate short-term (e.g., urine) and long-term (e.g., hair, nails) exposure.

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 Ethical Committee of “Hospital Universitario Puerta del Mar” (Cádiz, Spain). Written informed consent to participate in this study was provided by the participants’ legal guardian/next of kin.

Author contributions

RG-D: conceptualization, project administration, and supervision. ÁG-D and RG-D: data curation and roles/writing-original draft. ÁG-D, MM-M, and RG-D: formal analysis. RG-D and AL-S: funding acquisition and resources. ÁG-D, MM-M, JD-R, AL-S, and RG-D: investigation and writing-review and editing. MM-M and RG-D: methodology. 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.2022.1016819/full#supplementary-material>

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Comparison of copper concentration between non-alcoholic fatty liver disease patients and normal individuals: A meta-analysis

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Non-alcoholic fatty liver disease (NAFLD) is the most prevalent chronic liver disease worldwide. Copper metabolism plays an important role in the pathogenesis of NAFLD. However, the relationship between serum/hepatic copper concentration and NAFLD is still debated. A literature search was performed using electronic databases to find publications up to September 2022, where the relationship between serum/hepatic copper or ceruloplasmin concentration and NAFLD was evaluated. Finally, 6 articles with 9 unique outcomes involving 2,607 NAFLD patients and 1,441 non-NAFLD normal individuals were included. The pooled results showed that hepatic copper concentration was significantly decreased in NAFLD patients (SMD = -0.98 , 95% CI = $[-1.21; -0.74]$, $p < 0.0001$), and the sensitivity analysis also confirmed this. Nevertheless, serum copper (SMD = -0.02 , 95% CI = $[-0.32; 0.28]$, $p = 0.88$) and ceruloplasmin (SMD = -0.03 , 95% CI = $[-0.69; 0.63]$, $p = 0.93$) were not associated with NAFLD. This meta-analysis revealed that low hepatic copper concentration was found in NAFLD patients and serum copper and ceruloplasmin were not associated with NAFLD. Larger cohort studies and related trials are needed to further validate the result of this meta-analysis in the future.

KEYWORDS

non-alcoholic fatty liver disease, copper, serum, hepatic, meta-analysis

Introduction

Non-alcoholic fatty liver disease (NAFLD) is a multistage disease affecting 30% of the global population, which will supposedly emerge as the most common cause of the end-stage liver disease (1, 2). Substantial studies have demonstrated that NAFLD contributes to several disease processes containing hepatic/extrahepatic diseases and an overall increase in mortality (3). Currently, it is well recognized that the double-hit theory plays a vital role in the pathogenesis of NAFLD (4). However, no medicine is consented yet for this condition by the US Food and Drug Administration or the European Medicines Agency (5).

Serum copper is mainly transported by binding to ceruloplasmin which regulates the distribution and release of copper and later played its biological roles (6). Copper is an indispensable trace element that serves as a structural and enzymatic cofactor for various antioxidant proteins, including cytochrome c oxidase (COX), superoxide dismutase (SOD),

and ceruloplasmin (7). Excessive or deficient copper can lead to mitochondrial dysfunction or dyslipidemia (8). A typical example is Wilson's disease, an overloaded hepatic copper accumulation and insufficient ceruloplasmin with liver steatosis, inflammation, and cuprotosis (9). However, recent studies demonstrate the association between copper deficiency and the accumulation of fat in the liver, and NAFLD patients with hepatic copper deficiency show more severe liver steatosis, inflammation, and clinical symptoms (10, 11). Besides, rats fed with a restricted copper diet will spontaneously develop liver steatosis (12). These results indicate that copper plays an essential role in the pathogenesis of NAFLD.

Whereas, the relationship between serum/hepatic copper levels and NAFLD is still unclear. What's more, it is reported that lower hepatic copper is associated with NAFLD, but excessive copper also impairs hepatocytes (8, 13). Lan, et al. find that blood copper concentration is lower in NAFLD patients (14). Chen, et al. and Wang, et al. indicate that serum copper level is higher in NAFLD patients compared with normal individuals (15, 16).

Given that the role of copper in NAFLD is still controversial, this study performed a meta-analysis of the studies on serum/hepatic copper or ceruloplasmin levels to clarify their relationship.

Materials and methods

Data sources and search strategy

We searched Pubmed, EMBASE, Cochrane Library, Web of Science, and China National Knowledge Internet (CNKI)

databases from inception to September 20, 2022. Key search terms included: ("NAFLD" or "fatty liver") AND ("copper" or "cuprum" or "ceruloplasmin"). We also manually screened the citation of selected articles to identify the eligible articles. The present systematic review was conducted in accordance with the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) checklist, and the protocol had been registered at PROSPERO (www.crd.york.ac.uk/PROSPERO, ID: CRD42022361187).

Study selection

Abstracts and titles were reviewed to filter out irrelevant studies. Potentially relevant studies had their full text extracted and two reviewers (Guanhong Li and Wen Wang) independently assessed articles for inclusion/exclusion criteria. Dissonance was resolved by discussion. Inclusion criteria included: (1) studies comparing serum copper or hepatic copper levels between NAFLD patients and non-NAFLD normal individuals (control group); (2) diagnosis of hepatic steatosis by imaging or biopsy; (3) full-text articles available. Exclusion criteria included: (1) studies using overlapping samples; (2) those did not contain non-NAFLD normal control; (3) researches about copper intake; (3) incomplete or unextractable raw data; (4) reviews, letters, case reports, editorials, conference abstracts, and animal experiments. A summary of studies identified, screening, eligibility, and exclusion is shown in the PRISMA flow diagram (Figure 1).

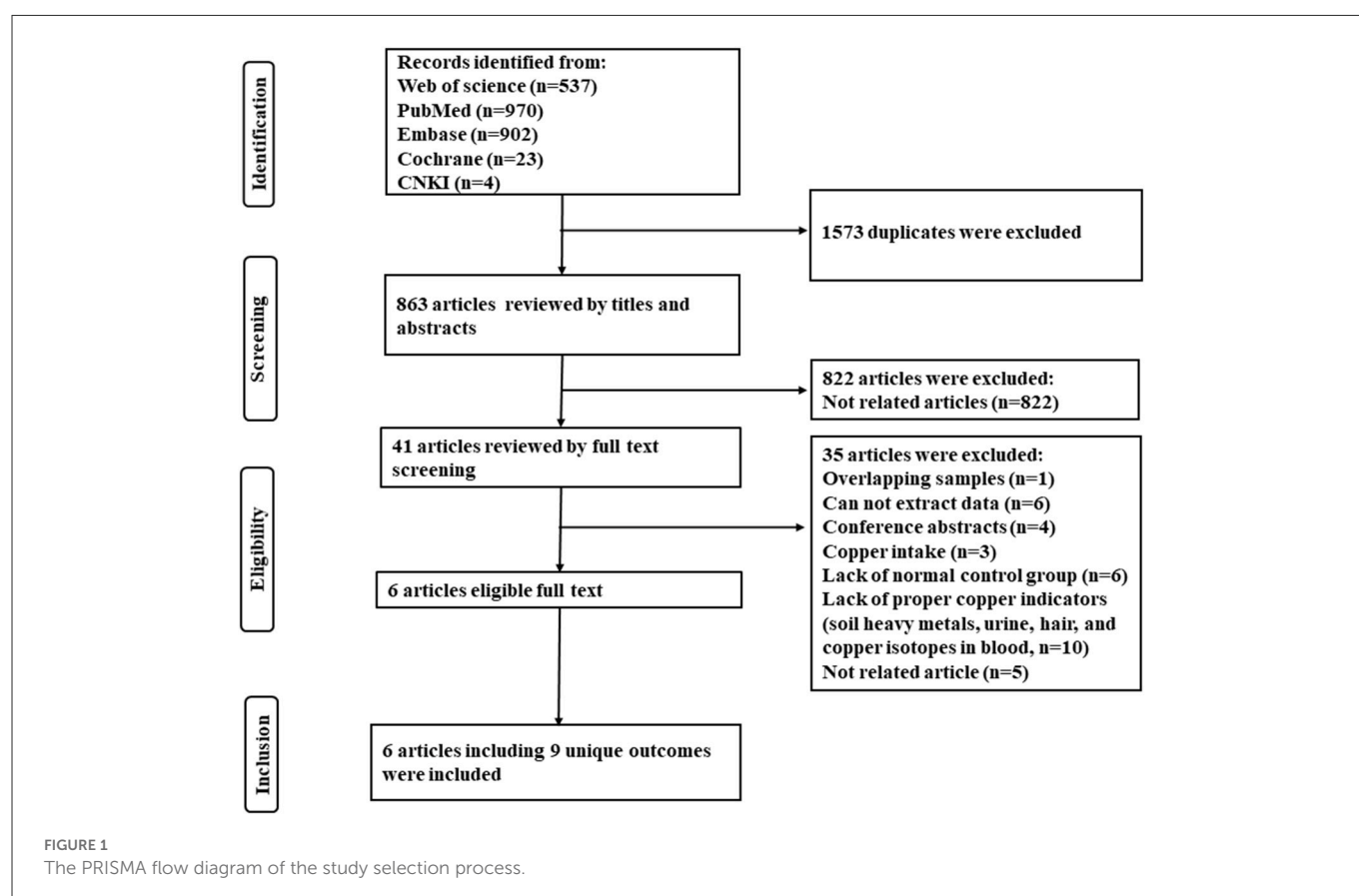


TABLE 1 The characteristics of included study.

| Author | Country | year | Copper detection | Diagnostic method | Type | Study design | NAFLD | | | CNTR | | | NOS |
|---------------------------|---------|------|--------------------|-------------------|------|-----------------|---------------|-------------|---------------|-------------|-----|-------------|-----|
| | | | | | | | Age | Male/Female | Age | Male/Female | Age | Male/Female | |
| Arefhosseini, et al. (10) | Iran | 2022 | Commercial kits | US | SCu | Cross-sectional | 37.07 ± 11.78 | 18/67 | 26.48 ± 13.19 | 19/37 | | | 6 |
| Lan, et al. (14) | China | 2021 | ICP-MS | US | SCu | Case-control | 56.3 ± 12.12 | 970/846 | 56.4 ± 12.3 | 598/513 | | | 9 |
| Wang, et al. (16) | China | 2011 | FAAS | US | SCu | Case-control | 39.13 ± 10.69 | 105/45 | 38.94 ± 10.66 | 105/45 | | | 7 |
| Aigner, et al. (18) | Austria | 2008 | Laboratory methods | Biopsy | SCu | Case-control | 52.4 ± 11.1 | 84/56 | 43.6 ± 9.3 | 8/16 | | | 6 |
| Arefhosseini, et al. (10) | Iran | 2022 | Commercial kits | US | SCer | Cross-sectional | 37.07 ± 11.78 | 18/67 | 26.48 ± 13.19 | 19/37 | | | 6 |
| Mendoza, et al. (19) | America | 2017 | NA | Biopsy | SCer | Case-control | 13.9 ± 3 | 77/25 | 13.4 ± 5 | 16/32 | | | 7 |
| Mendoza, et al. (19) | America | 2017 | NA | Biopsy | HCu | Case-control | 13.9 ± 3 | 77/25 | 13.4 ± 5 | 16/32 | | | 7 |
| Stattermayer, et al. (13) | Austria | 2017 | FAAS | Biopsy | HCu | Case-control | 49.1 ± 3 | 120/54 | 34.1 | 14/12 | | | 6 |
| Aigner, et al. (18) | Austria | 2008 | MS | Biopsy | HCu | Case-control | 52.4 ± 11.1 | 84/56 | 43.6 ± 9.3 | 8/16 | | | 6 |

The data were shown as mean ± standard deviation (SD). NAFLD, non-alcoholic fatty liver disease; CNTR, control group; ICP-MS, inductively coupled plasma mass spectrometry; FAAS, flame atomic absorption spectroscopy; MS, mass spectrometry; NA, not available; US, ultrasound; NOS, Newcastle-Ottawa Quality Assessment Scale; SCu, serum copper; SCer, serum ceruloplasmin; HCu, hepatic copper.

Data extraction and quality assessment

Two reviewers (YF and CT) independently performed data extraction and solved dissonance by discussion. Data extracted for eligible studies included the first author, publication year, study location, study design, the diagnostics method of NAFLD, serum/hepatic copper levels or ceruloplasmin and standard deviation (SD), sample size, the method for the detection of copper, age, and sex.

Newcastle-Ottawa Quality Assessment Scale (NOS) was used to assess the methodological quality of included studies. Risk of bias was categorized as follows: 7–9 was regarded as high-quality, 4–6 as moderate-quality, and under 4 as low-quality (17).

Statistical analysis

All statistical analyses were performed using “meta” R package in R software (Version 4.2.1, www.r-project.org). Heterogeneity was evaluated using the χ^2 test, tau² test, and Higgins I² test. If p value ≥ 0.05 and $I^2 \leq 50\%$, suggesting little homogeneity among the studies, the fixed effect model was used for analysis. If p value < 0.05 and $I^2 > 50\%$, indicating substantial heterogeneity among different studies, the random effects model was used for analysis. If there was heterogeneity among the studies, subgroup analysis was conducted to detect the source of heterogeneity. Standardized mean differences (SMD) and a 95% confidence interval (CI) of serum/copper levels were calculated for continuous variables. A sensitivity analysis was performed by omitting one study and pooling the SMD for the others in each turn. Publication biases were determined by Egger’s test. A p value < 0.05 was considered statistically significant.

Results

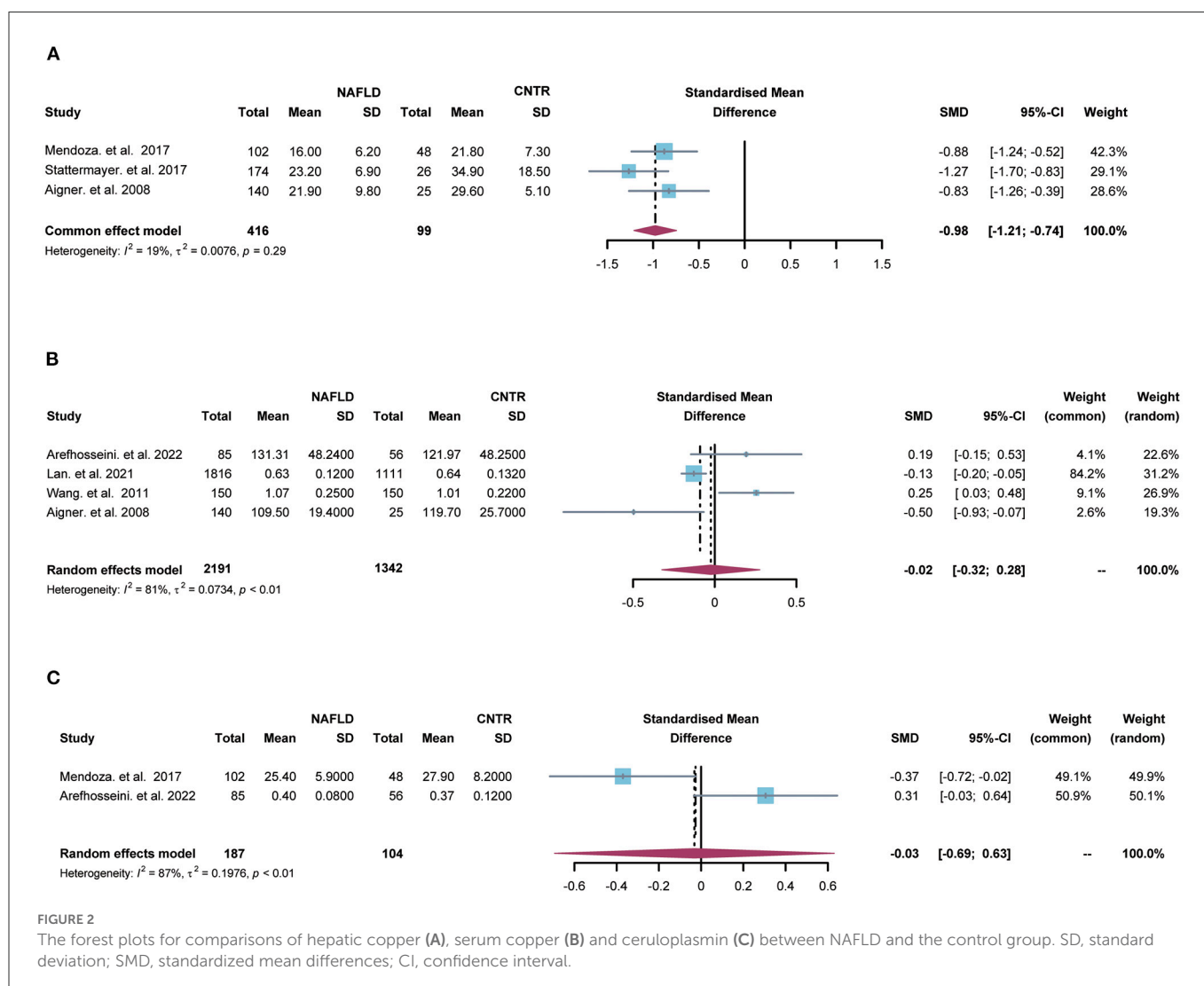
Study selection and characteristics

As shown in Figure 1, 2,436 articles were included in the initial research, and 6 articles with 9 unique outcomes were finally retrieved after filtration. The 9 researches were published from 2008 to 2022, involving 2,607 NAFLD patients and 1,441 non-NAFLD normal individuals (control group). One study was cross-sectional and 5 studies were case-control. Two studies were carried out in China, 2 in Austria, 1 in America, and 1 in Iran. The NOS scores shown that 3 studies were rated as high quality and 3 were considered as moderate quality (Table 1).

Overall meta-analysis

Meta-analysis was performed to compare the hepatic/serum copper or ceruloplasmin levels between NAFLD patients and the control group. There were 3 researches about hepatic copper and the heterogeneity among the studies was little ($I^2 = 19\%$, tau² = 0.0076, $p = 0.29$). The hepatic copper level was significantly decreased in NALFD patients (SMD = −0.98, 95% CI = [−1.21; −0.74], $p < 0.0001$, Figure 2A) using the fixed effects model.

Moreover, 4 studies contained serum copper levels and substantial heterogeneity was found among the studies ($I^2 = 81\%$, tau² = 0.0734, $p < 0.01$). There was no significant relationship between serum copper



and NAFLD (SMD = -0.02 , 95% CI = $[-0.32; 0.28]$, $p = 0.88$, Figure 2B) using the random effects model. Besides, 2 studies included serum ceruloplasmin and large heterogeneity was found among the studies ($I^2 = 87\%$, $\tau^2 = 0.1976$, $p < 0.01$). There was no significant relationship between serum ceruloplasmin and NAFLD (SMD = -0.03 , 95% CI = $[-0.69; 0.63]$, $p = 0.93$, Figure 2C) by the random effects model.

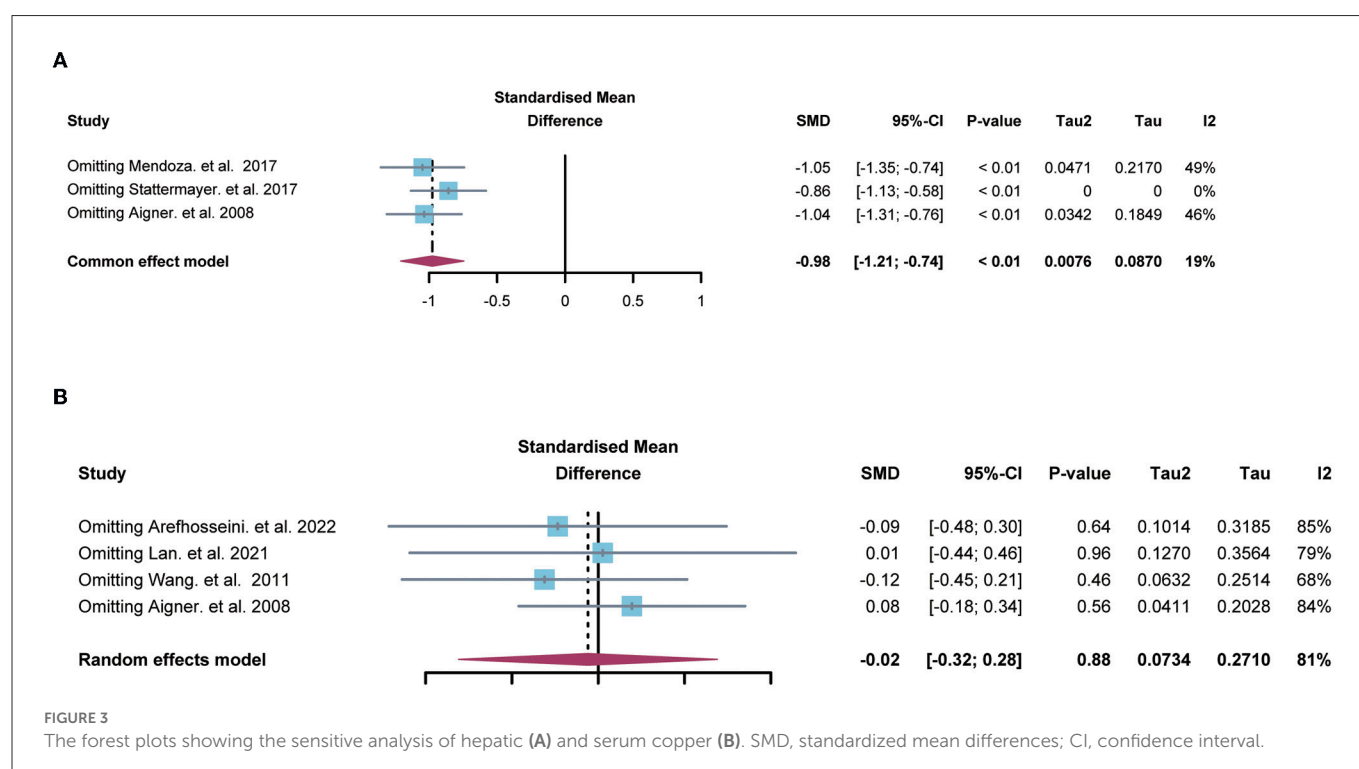
Sensitivity analysis and publication bias

Sensitivity analysis suggested that no single study significantly influenced the difference on hepatic copper (Figure 3A) and serum copper (Figure 3B) in the comparison between NAFLD patients and normal individuals. Also, Egger's test indicated that there was no publication bias in the pooled estimates of hepatic copper ($p = 0.73$), and serum copper ($p = 0.65$). Given only 2 studies about serum ceruloplasmin, the sensitivity analysis and publication bias were not performed in the comparison of serum ceruloplasmin.

Discussion

This meta-analysis revealed that hepatic copper concentration was significantly decreased in NAFLD patients and the sensitivity analysis also confirmed this. Nevertheless, serum copper and ceruloplasmin were not associated with NAFLD.

The total copper content in the adult body is 50–120 mg, and balancing the concentration of copper in the liver is essential for biological processes (20). It is reported that downregulation of hepatic ceruloplasmin results in the restoration of hepatic copper level, which attenuates liver steatosis by targeting copper-SOC1-AMPK signaling pathway in NAFLD mice model (20). What's more, copper inhibits the activation of PDE3B, a key enzyme of lipolysis, and the latter promotes c-AMP-dependent lipolysis (21). In addition, excessive copper induces lipogenesis and lipolysis through the activation of Nrf2-PPAR γ pathway and autophagy (22). Moreover, copper induces cell death by targeting the lipoylated components of the tricarboxylic acid (TCA) cycle, which is called "cuproptosis" (9). Obviously, the homeostasis of copper plays an important role in lipid metabolism. Given the different results between liver copper and serum copper in our



study, the biological function of liver copper and serum copper may be varying, and a positive correlation is found between serum copper and hepatic copper concentration in NAFLD patients, but there is no research contributing to the difference biological function between them (18).

Our research had the following advantages: (1) this study was the first to conduct a meta-analysis of the relationship between copper and NAFLD. However, our research also had some disadvantages: (1) the included studies were mainly case-control and cross-sectional study; (2) the meta-analysis of serum copper concentration still had substantial heterogeneity, and the removal of any literature did not improve this; (3) as few literatures on copper and NAFLD at present, few literatures were included; (4) the measurements used varied among the included studies, which increased the heterogeneity; (5) there was a strong possibility of sampling bias in our study. The liver copper cases were predominantly male (only 32% of subjects were female in the subset of studies with liver tissue data) which was not representative. Further, it was unclear what the severity of the disease was in the included studies (as an invasive test, people who were biopsied might have increased pre-test probability of more severe disease). According to Mendoza et al., advanced fibrosis may increase hepatic copper concentrations, which was part of the reason why that study excluded patients with high liver tissue copper (19); (6) we were unable to address the competing etiologies of copper deficiency and excess; (7) in the liver tissue studies, there are few controls for comparison, in part because of the invasive biopsy procedure required.

Therefore, this study suggests that the hepatic copper concentration is significantly decreased in NAFLD patients, and no difference is found in serum copper and ceruloplasmin between NAFLD patients and the normal individuals. Larger cohort studies and related trials are needed to further validate the result of this meta-analysis in the future.

Author contributions

YC and CW contributed equally to this paper. YC, CW, and GL were involved with the study concept and design, acquisition of data, analysis, interpretation of data, drafting of the manuscript, and critical revision of the manuscript for important intellectual content. WW was involved in the acquisition of data. ST supervised the research and edited 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.

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Spatial distribution and trends of anemia among pregnant women in Ethiopia: EDHS 2005–2016

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Background: Anemia is a public health problem affecting both developed and developing nations worldwide with a significant consequence on health and economic growth. The problem is more severe in pregnant women. Hence, the main purpose of this study was to determine the factors of anemia levels among pregnant women in zones in Ethiopia.

Methods: We utilized data from 2005, 2011, and 2016 Ethiopian demographic and health survey (EDHSs), a population-based cross-sectional study. The study includes 8,421 pregnant women. An ordinal logistic regression model with spatial analysis was used to explore factors of anemia levels among pregnant women.

Result: About 224 (2.7%), 1,442 (17.2%), and 1,327 (15.8%) pregnant women were mild, moderate, and severely anemic, respectively. The spatial autocorrelation of anemia among the administrative zones of Ethiopia for the three consecutive was not significant. The middle wealth index of 15.9% (OR = 0.841, CI: 0.72–0.983) and richest wealth index of 51% (OR = 0.49, CI: 0.409–0.586) were less likely anemic compared to the poorest wealth index, age group of mother 30–39 was 42.9% (OR = 0.571, CI: 0.359–0.908) times less likely to be moderate and above anemic compared to <20 years, several household members 4–6 were 51% (OR = 1.51, CI: 1.175–1.94 more likely moderate and above anemic compared to 1–3.

Conclusion: Over one-third of the pregnant women (34.5%) were anemic in Ethiopia. Wealth index, age group, religion, region, number of household members, source of drinking water, and EDHS were significant factors in anemia levels. The prevalence of anemia among pregnant women varied among Ethiopian administrative zones. North West Tigray, Waghimra, Oromia special woreda, West shewa, and East shewa were a high prevalence of anemia.

KEYWORDS

anemia levels, ordinal logistic regression, partial proportional odds model, Ethiopia, spatial, zones

Introduction

Anemia is considered a condition in which the hemoglobin (Hb) concentration falls below an established cut-off value, as evidenced by a reduced quality or quantity of red blood cells which minimizes oxygen-carrying capacity to tissue. Even the hemoglobin concentration decreases with dilution as the volume of circulating blood increases (1). According to the World Health Organization (WHO), anemia in pregnancy is defined as a Hb concentration of fewer than 11 grams per deciliter (2). Anemia during pregnancy is a major cause of morbidity and mortality in pregnant women in developed and developing

countries. Although it can occur among any human population, pregnant women and young children are common victims of this hematological abnormality (3).

Anemia reduces levels of hemoglobin and favors changes in placental angiogenesis, limiting the availability of oxygen to the fetus and consequently causing potential restriction of intrauterine growth and low birth weight (4). Anemia is evaluated by measuring hemoglobin levels, rather than by clinical signs, which are less observable than for vitamin A deficiency and disorders of iodine scarcity (5). The hemoglobin deprivation due to anemia during pregnancy has serious maternal-fetal complications, which could even lead to maternal mortality (6). The main causes of anemia during pregnancy are nutritional deficiencies of iron, vitamin B12, and parasitic diseases in addition to this excessive menstrual bleeding, acute or chronic blood loss, chronic diseases, parasites infestation, hemolytic anemia, and frequent pregnancies, and also the evidence shows that anemia contributes to 20% of deaths among pregnant women (1, 7).

It is one of the most common nutritional deficiency diseases observed, globally 1.62 billion people of the world's population are anemic and about 38% accounts for pregnant women of which 46.3% of them are in Africa (8). Sub-Saharan Africa took the greatest share, where 17.2 million pregnant women were reported as anemic (9), and 41.82% were accounted for by east Africa (6). Anemia is a major and one of the greatest prevalent nutritional deficiency problems disturbing pregnant women. During pregnancy, anemia prevalence differs significantly due to the reasons of differences in socioeconomic conditions, lifestyles, and health-seeking behaviors concerning different cultures (10).

The Global data shows that 56% of pregnant women in low- and middle-income countries (LMIC) have anemia due to the absence of balanced nutrients (11). Anemia is one of the major and highly spread public health problems in developing countries including Ethiopia. It leads to different complications and difficulties for the fetus and mother during the pregnancy period. The prevalence of anemia among women decreased from 27% in 2005 to 17% in 2011 but climbed to 24% in 2016, according to the Ethiopian demography and health survey report (12). In Ethiopia, different studies were done on factors associated with anemia among pregnant women using classical models such as binary logistic regression. However, binary logistic regression cannot deliver sufficient information for studying the pattern of different anemia levels (12, 13). Hence, we used the ordinal logistic regression model to show the pattern of anemia levels among pregnant women. Several studies in Ethiopia studied the risk factor of anemia based on the regional level (14, 15), but this study focused on the second level of administrative area (zones) in Ethiopia.

To the best of our knowledge, some research using EDHS data on the causes of anemia in Ethiopia has been done. However, they failed to display the spatial distribution among zones and its trend over time (11–13). Therefore, this study would investigate the determinants, distribution, and trends of anemia among pregnant women in Ethiopian administrative zones based on 2005, 2011, and 2016 EDHS data.

Operational definition

Hemoglobin

An iron-containing respiratory pigment of vertebrate red blood cells that consists of a globin composed of four subunits each of which is linked to a heme molecule, that functions in oxygen transport to the tissues after conversion to oxygenated form in the gills or lungs, and that assists in carbon dioxide transport back to the gills or lungs after the surrender of its oxygen.

Anemia

A condition in which you lack enough healthy red blood cells to carry adequate oxygen to your body's tissues.

Methods

Study area

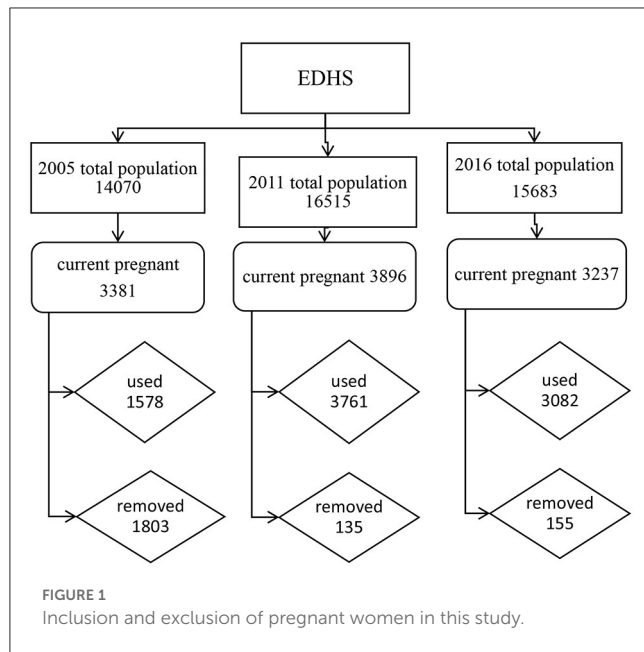
The study was conducted in Ethiopia, located at 3° and 14.8° latitude, 33 and 48° longitude in the Eastern part of Africa laying between the Equator and the Tropic of Cancer (16). Ethiopia is the largest and most populated country in the Horn of Africa and the capital city is Addis Ababa ("New Flower"), located almost at the center of the country (17). Ethiopia is administratively structured into nine regional states and two city administrations. Moreover, the country has 72 administrative zones Central Statistical Agency (CSA) report, 2007.

Source of data

This study used 2005, 2011, and 2016 EDHS data conducted in Ethiopia for the last 12 years. It was worked by the Central Statistical Agency (CSA) at the inquiry of the Federal Ministry of Health (FMOH). The EDHS is drafting every 5 years to provide health and health-related indicators at the national and regional levels in Ethiopia. The data was collected from all the nine regions and the two city administrations of Ethiopia in a representative manner. The DHS data is freely available after permission has been obtained from: <https://dhsprogram.com> and can be accessed following the protocols.

Inclusion criteria

The three surveys were conducted and anemia was included as a key indicator since the 2005 survey. In 2005, 2011, and 2016 surveys years respectively, 540 (139 urban and 401 rural areas), 624 (187 urban and 437 rural), and 645 (202 urban and 443 rural) enumeration areas (EAs) are selected using a stratified, two-stage cluster design. A total of 13,721 households (14,070 eligible reproductive age women), 16,702 households (16,515 eligible reproductive age women), and 16,650 households and 15,683 eligible reproductive-age women are selected, respectively. A total of 8,421 pregnant women of which 1,578 from 2005, 3,761 from 2011, and 3,082 from 2016 were included in this study, Figure 1.



Exclusion criteria

All non-pregnant women whose ages are 15–49 years were excluded.

Study variables

Response variable

The response variable in this study was the anemia status of pregnant women which is measured by hemoglobin level (18). The status of anemia was determined based on hemoglobin concentration in blood adjusted to the altitude. Adjusted concentration 10.0–10.9 g/dl was considered as mild anemia, 7.0–9.9 g/dl as moderate anemia, and <7.0 g/dl as severe anemia ≥11 g/dl as not anemic (6).

Explanatory variables

These variables were obtained based on insight from previous studies (1–6) (see Figure 2).

Methods of statistical analysis

Descriptive measures were used to summarize the characteristics of the study participants using frequencies and percentages for variables.

Ordinal logistic regression

It is applicable when a dependent variable has values with having natural order or rank (19). It is used to model the

relationship between an ordinal dependent variable and a set of independent variables (20).

Proportional odds model (POM)

It is also known as the cumulative logit model. It is used for modeling the response variable that has more than two levels with K set of explanatory variables by defining the cumulative probabilities, cumulative odds, and cumulative logits for the $J-1$ categories of the response, this model simultaneously uses all cumulative logits (13).

Assumed the response variable Y is a vector of an ordinal scale with J categories and $\mathbf{X} = (x_1, x_2, \dots, x_p)$ is the vector of covariates, then the probability of the variable response of the j th category of explanatory variable X , in particular, can be expressed by P ,

$$p[y = j/x_1, \dots, x_p] = \pi_{j(x)} \quad (1)$$

Where Y and X are vectors.

When the response categories are ordered, the logits can utilize the ordering that results in greater power and simple interpretations. Hence, the cumulative probability of Y is the probability that falls at or below particular outcome category j is given by:

$$p(Y \leq j) = \pi_1(x) + \pi_2(x) + \dots + \pi_j(x) \quad (2)$$

$j = 1, 2, \dots, J-1$. Where J is categories for the response variable Y . Then the odds of the first $J-1$ cumulative probability are,

$$\text{odds}(p(y \geq j)) = \left(\frac{p(y \geq j)}{1 - p(y \geq j)} \right) = \frac{\pi_j}{1 - \pi_j} \quad (3)$$

Where $j = 1, 2, \dots, J-1$.

The cumulative logit model (21).

$$\begin{aligned} \text{logit}(y_i \geq j/x) &= \log \left(\frac{\text{pr}(y_i \geq j/x)}{\text{pr}(y_i < j/x)} \right) \\ &= \log \left(\frac{\pi_{j+1} + \pi_{j+2} + \dots + \pi_J}{\pi_1 + \pi_2 + \dots + \pi_j} \right) \\ &= \beta_{0j} + X' \beta, j = 1, \dots, J-1 \end{aligned} \quad (4)$$

Where β_{0j} is a threshold value.

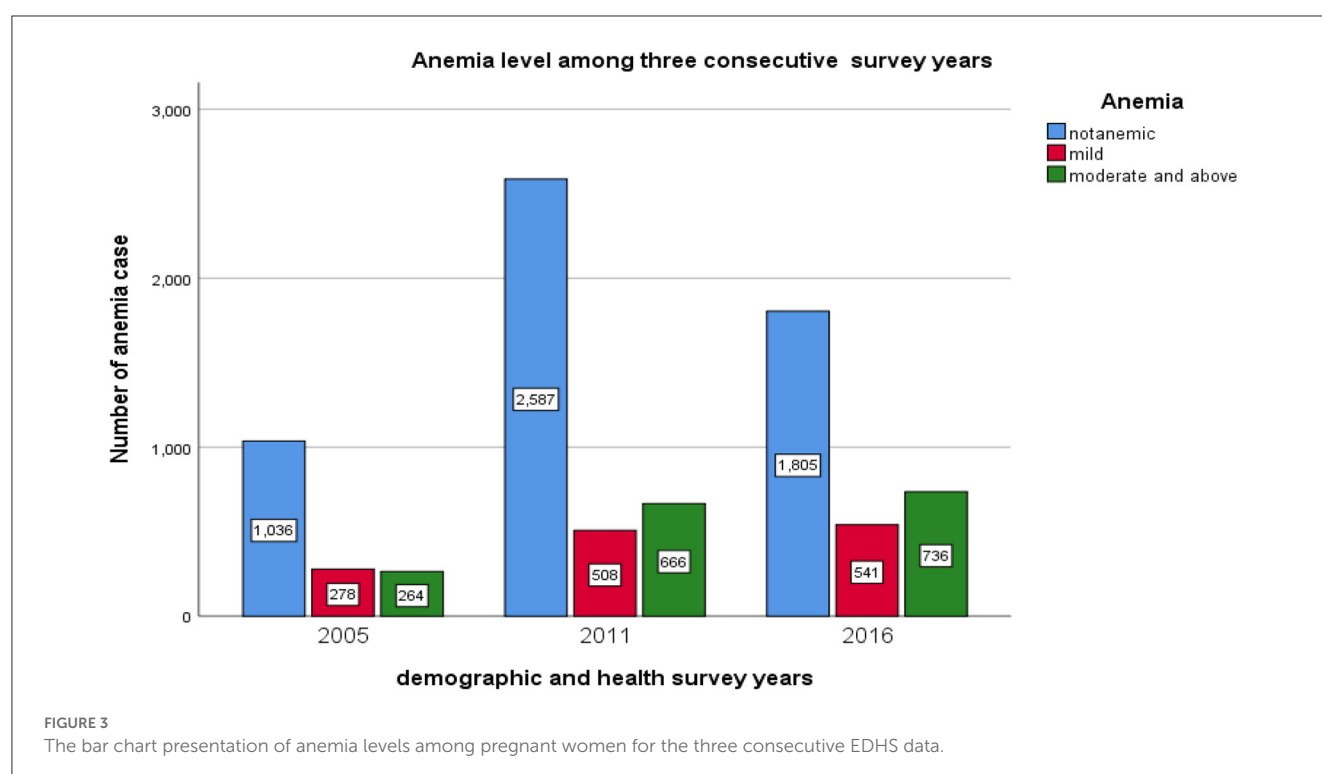
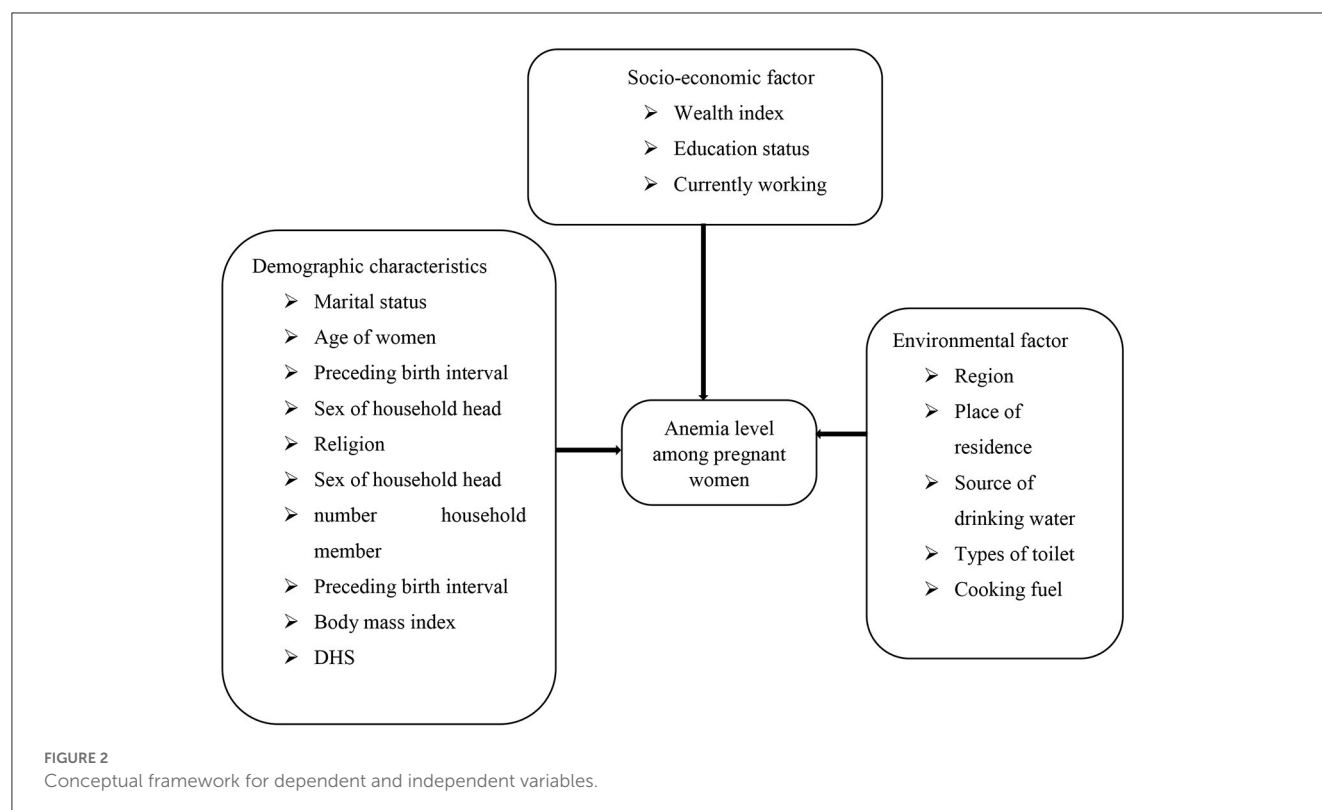
x = set of factors or predictors.

Each cumulative logit uses all J response categories.

Manually the probability $p(Y \geq j)$, can be estimated as:

$$p(Y \geq j) = \frac{\exp(\beta_{0j} + x' \beta)}{1 + \exp(\beta_{0j} + x' \beta)} \quad (5)$$

The cumulative probabilities do not use the final one, $P(Y \leq j)$, since it must equal 1. The parameter β is a vector of regression coefficient describing the effect of corresponding covariates X on the log odds of response in category j or below. When this model good fit, it requires a single parameter rather than $J-1$ parameters to describe the effect of X . Because the model assumes that the effect of X is identical, proportional odds, for all $J-1$ cumulative logits (22).



For the ordinal regression model to hold, the assumption of parallel lines of all levels of the categorical data is satisfied since the model does not assume normality and constant variance (23).

To fit an ordinal logistic regression using the proportional odds model the assumption is that the relationship between independent variables and the dependent

variable does not change for the dependent variable's categories (13).

Partial proportional odds model

It is rare for all the explanatory variables included in the model to display the proportional odds property. A partial proportional odds model can be used when the parallel lines assumption holds or not. The partial proportional odds model bears the same characteristics as the proportional odds model but now the coefficients are associated with each category of the response variable (24). This model allows some predictors to be modeled with the POM assumption, but for those variables in which this assumption is not satisfied is with PPOM.

$$\text{logit}(\gamma_j) = \log\left(\frac{\gamma_j}{1 - \gamma_j}\right) = \eta_i = \theta_j + X_i'\beta + \tau\psi_j \quad (6)$$

Where X is a vector containing the full set of independent variables and τ is a vector of a subset of independent variables not violating and violating parallel line assumption; β and ψ are the regression coefficients of those predictors, respectively.

TABLE 1 The prevalence of anemia among pregnant women in 2005, 2011, and 2016 EDHS data.

| Year | Frequency (%) |
|------|----------------|
| 2005 | 542 (34.35%) |
| 2011 | 1,277 (33.9%) |
| 2016 | 1,174 (38.09%) |

Generalized ordered model

The generalized ordered logit model is an ordinal logistic regression that considers the order of category of the response variable with a k set of explanatory variables. This model results in $J-1$ logits without constraining the effect of each explanatory variable is equal across the logit (25).

The model can be expressed as:

$$\log(\text{pr}(Y > j/X)) = \log\left(\frac{\text{pr}(Y > j/X)}{\text{pr}(Y \leq j/X)}\right) = \alpha_j + \beta_{1j}x_1 + \beta_{2j}x_2 + \dots + \beta_{kj}x_k \quad (7)$$

In this model defines $J - 1$ sets of model parameters, one for each of the $J - 1$ generalized logit.

This means the model has a separate intercept (α_j) and a separate set of regression parameters (β_j).

This model estimates the odds of being beyond a certain category relative to being at/below that category. A positive β -value indicates that an individual is less likely to be low in the category as compared to beyond the category of the outcome variable. The generalized ordered logit model estimates the regression parameter for each independent variable on $j-1$ logit of the probability being at or below j th category in every logit to have different estimated values.

Spatial analysis

Spatial analysis is an analysis that includes the influence of space in the analysis (26). It is a statistical method that

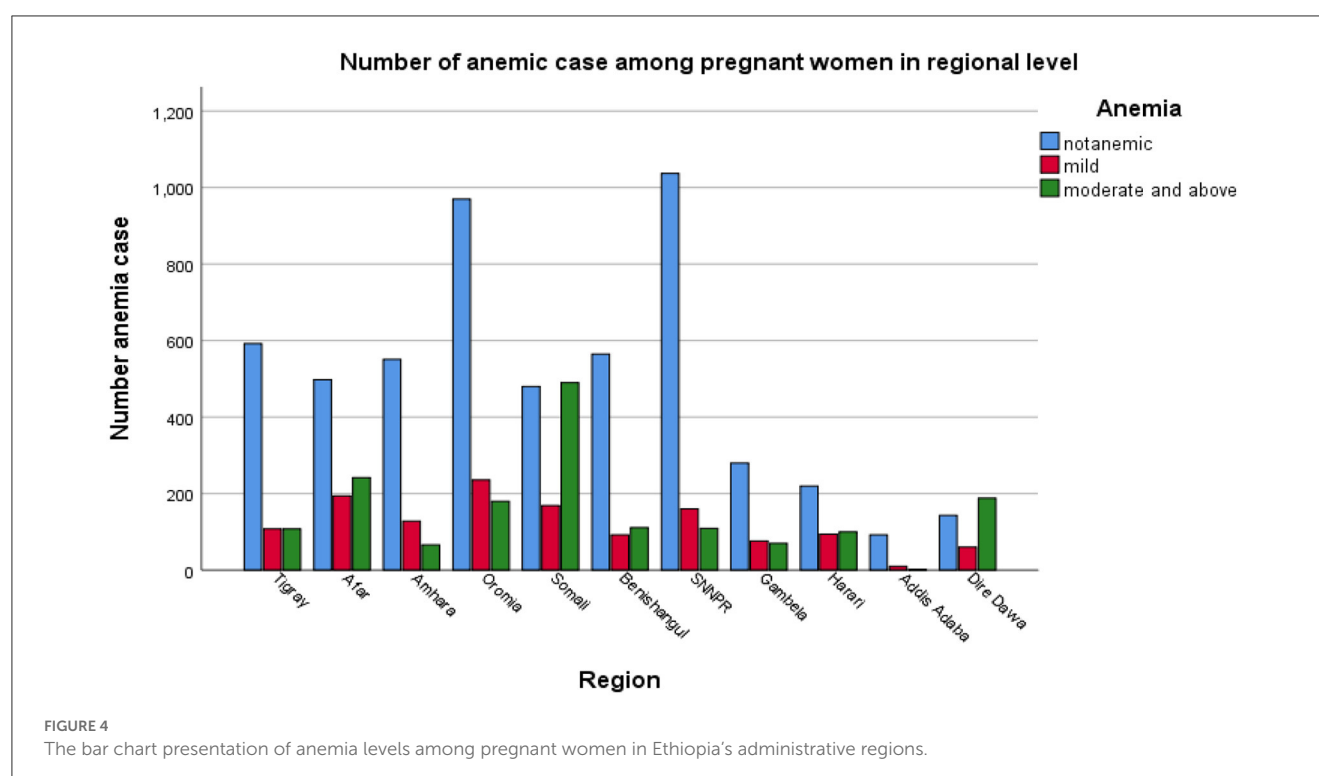


TABLE 2 Frequency distribution of independent variables among the 3 survey years.

| Variables | Categories | 2005 Frequency (%) | 2011 Frequency (%) | 2016 Frequency (%) |
|--------------------------|------------------|-----------------------|-----------------------|-----------------------|
| Residence | Urban | 44 (2.8) | 273 (7.6) | 359 (11.6) |
| | Rural | 1,534 (97.2) | 3,343 (92.4) | 2,723 (88.4) |
| Education | No education | 1,320 (83.7) | 2,644 (73.2) | 2,313 (75) |
| | Primary | 231 (14.6) | 928 (25.7) | 616 (20) |
| | Secondary | 49 (1.2) | 25 (0.7) | 106 (3.5) |
| | Higher | 8 (0.5) | 16 (0.5) | 47 (1.5) |
| Religion | Orthodox | 554 (35.1) | 1,102 (30.5) | 680 (22.1) |
| | Catholic | 10 (0.6) | 84 (2.3) | 13 (0.4) |
| | Protestant | 320 (20.3) | 919 (25.4) | 479 (15.5) |
| | Muslim | 638 (40.4) | 1,446 (40) | 1,836 (59.6) |
| | Traditional | 37 (2.3) | 33 (0.9) | 55 (1.8) |
| | Other | 19 (1.2) | 33 (0.9) | 19 (0.6) |
| Wealth | Poorest | 498 (31.6) | 938 (25.9) | 1,347 (43.7) |
| | Poorer | 334 (21.2) | 819 (22.6) | 534 (17.3) |
| | Middle | 314 (19.9) | 697 (19.3) | 423 (13.7) |
| | Richer | 258 (16.3) | 735 (20.3) | 423 (13.7) |
| | Richest | 174 (11) | 428 (11.8) | 355 (11.5) |
| Region | Tigray | 214 (13.6) | 249 (6.9) | 196 (6.4) |
| | Afar | 70 (4.4) | 49 (1.4) | 358 (11.6) |
| | Amhara | 158 (10) | 576 (15.9) | 280 (9.1) |
| | Oromiya | 387 (24.5) | 1,590 (44) | 459 (14.9) |
| | Somali | 111 (7) | 155 (4.3) | 640 (20.8) |
| | Benishangul-gumz | 128 (8.1) | 55 (1.5) | 224 (7.3) |
| | SNNP | 318 (20.2) | 886 (24.5) | 413 (13.4) |
| | Gambella | 71 (4.5) | 8 (0.2) | 177 (5.7) |
| | Harari | 87 (5.5) | 7 (0.2) | 169 (5.5) |
| | Addis Ababa | 17 (1.1) | 28 (0.8) | 42 (1.4) |
| | Dire-Dawa | 17 (1.1) | 13 (0.4) | 124 (4) |
| | | | | |
| Working | Yes | 321 (20.3) | 976 (26%) | 730 (23.7) |
| | No | 1,257 (79.7) | 2,785 (74%) | 2,352 (76.3) |
| Age category mother | <20 | 18 (1.1) | 52 (1.4) | 31 (1) |
| | 20–29 | 515 (32.6) | 1,349 (35.8) | 1,202 (39) |
| | 30–39 | 845 (53.5) | 1,861 (49.5) | 1,584 (51.4) |
| | 40–49 | 200 (2.7) | 500 (13.3) | 265 (8.6) |
| Nhmember | 1–3 | 114 (7.2) | 304 (8.1) | 287 (9.3) |
| | 4–6 | 734 (46.5) | 1,888 (50.2) | 1,405 (45.6) |
| | 7–9 | 594 (37.6) | 1,264 (33.6) | 1,142 (37.1) |
| | ≥10 | 136 (8.6) | 305 (6.1) | 246 (8) |
| Preceding birth interval | ≤24 | 432 (27.4) | 1,078 (28.7) | 1,005 (32.6) |
| | ≥25 | 1,146 (72.6) | 2,683 (71.3) | 2,077 (67.4) |

(Continued)

TABLE 2 (Continued)

| Variables | Categories | 2005 Frequency (%) | 2011 Frequency (%) | 2016 Frequency (%) |
|--------------------------|--------------|-----------------------|-----------------------|-----------------------|
| Source of drinking water | Improved | 782 (49.6) | 1,729 (46) | 1,248 (40.5) |
| | Unimproved | 796 (50.4) | 2,032 (54) | 2,834 (59.5) |
| Body mass index | Under weight | 106 (6.7) | 368 (10.2) | 423 (13.7) |
| | Normal | 1,409 (89.3) | 2,947 (81.5) | 2,251 (73.1) |
| | Overweight | 63 (4) | 301 (8.3) | 408 (13.2) |
| Toilet | Improved | 110 (7) | 537 (14.3) | 412 (13.4) |
| | Unimproved | 1,468 (93) | 3,224 (85.7) | 2,670 (86.6) |
| Cooking fuel | Wood/straw | 36 (2.3) | 3,318 (88.2) | 2,715 (88.1) |
| | Other | 1,542 (97.2) | 443 (11.8) | 367 (11.9) |

is useful to identify geographical areas with the highest prevalence of anemia among pregnant women and its variability over the Ethiopian administrative zones. Ignoring such information during analysis may offer faulty results and conclusions (27).

Spatial autocorrelation

The idea of spatial autocorrelation was proposed by Tobler in the first geography law, “Everything is related to everything else, however nearest things are related than distant things (28).” A Moran’s I was used to measure spatial autocorrelation (29). The value of Global Moran’s I is range from -1 to 1 . When the index was distributed around -1 , the overall spatial distribution displayed is not similar and the reverse is true when the index is 1 (26, 30).

A statistically significant Moran’s I ($p < 0.05$) leads to rejecting the null hypothesis (anemia among pregnant women randomly distributed) and indicates the existence of spatial autocorrelation.

The global Moran’s I expressed as follows:

$$I = n \frac{\sum_{i=1}^n \sum_{j=1}^n w_{ij} (x_i - \bar{x}) (x_j - \bar{x})}{\sum_{i=1}^n w_{ij} (x_i - \bar{x})^2} \quad (8)$$

Where n is the number of observations in the whole cluster, x_i and x_j are the observations at locations of i and j , \bar{x} is the mean of x , and W_{ij} , a component of spatial weights matrix W , is the spatial weight between locations of i and j . The Local Moran’s I expressed as follows:

$$I = n \frac{\sum_{i=1}^n \sum_{j=1}^n w_{ij} (x_i - \bar{x}) (x_j - \bar{x})}{\sum_{i=1}^n (x_i - \bar{x})^2} \quad (9)$$

Contiguity matrix

A contiguity matrix is a matrix that explains the relationship between zones, giving the value 1 if the area i neighbor with area j , while a value of 0 is given if area i is not adjacent to the area j (31). In our study, it is referred to as matrix W containing w_{ij} for

row i and column j , based on the contiguity of the area units. It is a square symmetric $n \times n$ matrix with (i, j) and the diagonal elements of the spatial weight matrix are zeros (32). The most common ways to construct such a matrix are as follows.

$$w_{ij} = \begin{cases} 1, & \text{if area } i \text{ and } j \text{ are neighboring} \\ 0, & \text{Otherwise} \end{cases}$$

$$W = \begin{bmatrix} 0 & w_{12} & w_{13} & \cdots & w_{1N} \\ w_{21} & 0 & w_{23} & \cdots & w_{2N} \\ w_{31} & w_{32} & 0 & \cdots & w_{3N} \\ \vdots & \vdots & \vdots & \ddots & \vdots \\ w_{N1} & w_{N2} & w_{N3} & \cdots & 0 \end{bmatrix}$$

Spatial interpolation

Kriging is the most commonly used geostatistical approach for spatial interpolation (33). It is relied on a spatial model between observations to predict attribute values at unsampled locations. One of the specificities of kriging methods is that they do not only consider the distance between observations but they also intend to catch the spatial structure in the data by comparing observations separated by specific spatial distances two at a time. In this study, the Ordinary Kriging geostatistical interpolation method was used to predict the prevalence of anemia in unobserved areas of Ethiopian administrative zones.

Spatial ordinal logistic regression

Spatial Ordinal Logistic Regression is an analysis which is adding of spatial effects into the ordinal logistic regression model. Uses the spatial ordinal logistic regression model of the form (34):

$$\text{logit} (P(Y \leq j|X)) = \log \left[\frac{P(Y \leq j|X)}{1 - P(Y \leq j|X)} \right] = \alpha_j + X\beta + \rho W_y \quad (10)$$

Where $\rho W_y = \sum_{j=1}^k w_{ij} \hat{y}_i / \sum_{j=1}^k w_{ij}$.

TABLE 3 The association of socio-demographic, socio-economic, and environmental variables with anemia level among pregnant women.

| Predictor | Category | Not anemic Frequency (%) | Mild Frequency (%) | Moderate and above Frequency (%) | χ^2 and p -value |
|----------------------------|--------------|-----------------------------|-----------------------|-------------------------------------|-------------------------|
| Place of residence | Urban | 547 (66.5) | 109 (13.2) | 167 (20.3) | 4.347 (0.114) |
| | Rural | 4,881 (64.2) | 1,218 (16) | 1,499 (19.7) | |
| Education | No education | 4,088 (62.5) | 1,070 (16.4) | 1,380 (21.1) | 56.928 (<0.001) |
| | Primary | 1,163 (70.9) | 219 (13.3) | 259 (15.8) | |
| | Secondary | 122 (71.3) | 32 (18.7) | 17 (9.9) | |
| | Higher | 55 (77.5) | 6 (8.5) | 10 (14) | |
| Religion | Orthodox | 1,580 (74.2) | 307 (14.4) | 245 (11.5) | 407.205 (<0.001) |
| | Catholic | 70 (89.7) | 4 (5.1) | 4 (5.1) | |
| | Muslim | 2,503 (55.7) | 778 (17.3) | 1,214 (27) | |
| | Protestant | 1,121 (73.8) | 218 (14.4) | 180 (11.8) | |
| | Traditional | 84 (70) | 20 (16.7) | 16 (13.3) | |
| | Other | 70 (90.9) | 0 | 7 (9.1) | |
| Wealth index | Poorest | 1,888 (58) | 537 (16.5) | 828 (25.5) | 150.22 (<0.001) |
| | Poorer | 984 (64.1) | 260 (16.9) | 292 (19) | |
| | Middle | 899 (70.3) | 187 (14.6) | 192 (15) | |
| | Richer | 891 (68.4) | 216 (16.6) | 196 (15) | |
| | Richest | 766 (72.9) | 127 (12.1) | 158 (15) | |
| Region | Tigray | 592 (73.3) | 108 (13.4) | 108 (13.4) | 008.533 (<0.001) |
| | Afar | 498 (53.3) | (194 (20.8) | 242 (25.9) | |
| | Amhara | 551 (74) | 128 (17.2) | 66 (8.9) | |
| | Oromiya | 970 (70) | 236 (17) | 180 (13) | |
| | Somali | 480 (42.1) | 169 (14.8) | 490 (43) | |
| | Ben-gumz | 565 (73.6) | 92 (12) | 111 (14.5) | |
| | SNNP | 1,037 (79.4) | 160 (12.3) | 109 (8.3) | |
| | Gambella | 280 (65.7) | 76 (17.8) | 70 (16.4) | |
| | Harari | 220 (53.1) | 94 (22.7) | 100 (24.2) | |
| | Addis Ababa | 92 (88.5) | 10 (9.6) | 2 (1.9) | |
| | Dire Dawa | 143 (36.6) | 60 (15.3) | 188 (48.1) | |
| Pregnant currently working | Yes | 1,370 (67.6) | 311 (15.3) | 346 (17.1) | 14.29 (<0.001) |
| | No | 4,058 (63.5) | 1,016 (15.9) | 1,320 (20.6) | |
| Age group of mother | <20 | 62 (61.4) | 12 (11.9) | 27 (26.7) | 24.022 (<0.001) |
| | 20–29 | 1,994 (65.0) | 455 (14.8) | 616 (20.1) | |
| | 30–39 | 2,699 (62.9) | 718 (16.7) | 873 (20.3) | |
| | 40–49 | 673 (69.7) | 142 (14.7) | 150 (15.5) | |
| Number of household member | 1–3 | 456 (64.7) | 124 (17.6) | 125 (17.7) | 29.863 (<0.001) |
| | 4–6 | 2,560 (63.6) | 674 (16.7) | 793 (19.7) | |
| | 7–9 | 1,964 (65.5) | 459 (15.3) | 577 (19.2) | |
| | ≥10 | 448 (65) | 70 (10.2) | 171 (24.8) | |
| Preceding birth interval | ≤24 | 1,546 (61.5) | 383 (15.2) | 586 (23.3) | 28 (<0.001) |
| | ≥25 | 3,882 (65.7) | 944 (16) | 1,080 (18.3) | |

(Continued)

TABLE 3 (Continued)

| Predictor | Category | Not anemic Frequency (%) | Mild Frequency (%) | Moderate and above Frequency (%) | χ^2 and p -value |
|--------------------------|-------------|-----------------------------|-----------------------|-------------------------------------|-------------------------|
| Body mass index | Underweight | 653 (66) | 150 (15.2) | 186 (18.8) | 12.589 (0.013) |
| | Normal | 4,280 (64.8) | 1,045 (15.8) | 1,280 (19.4) | |
| | Overweight | 495 (59.9) | 132 (15.9) | 200 (24.2) | |
| Toilet | Unimproved | 4,784 (65.1) | 1,153 (15.7) | 1,407 (19.2) | 13.452 (<0.001) |
| | Improved | 635 (60) | 174 (16.4) | 250 (23.6) | |
| Source of drinking water | Improved | 2,440 (64.9) | 629 (16.7) | 690 (18.4) | 11.31 (0.004) |
| | unimproved | 2,988 (64.1) | 698 (15) | 976 (20.9) | |
| Cooking fuel | Other | 1,509 (64.2) | 404 (17.2) | 439 (18.7) | 6.279 (0.043) |
| | Wood/straw | 3,919 (64.2) | 923 (15.2) | 1,227 (20.2) | |

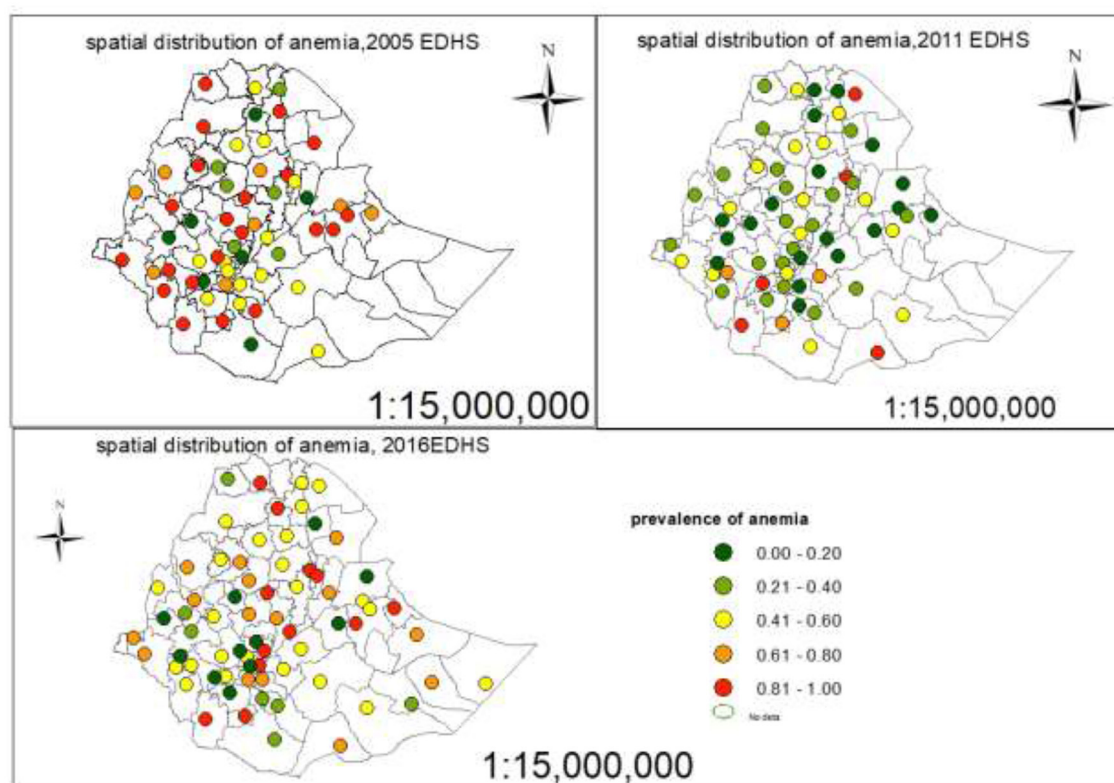


FIGURE 5
Spatial distribution of anemia on pregnant women in Ethiopian administrative zones.

Where ρW_y is a form of auto-covariance and is a weighted average of the number of events among the k_i neighbors. The weighting of the average location of the i th is through $W_{ij} = 1/h_{ij}$ where h_{ij} is the Euclidean distance between the village i and j . \hat{y}_i is the alleged existence of an event and β is the regression coefficient.

The data were analyzed on SAS version 9.4 with Proc logistic command; ArcGIS version 10.4, and SPSS version 26 were used in the data management.

Result

Explanatory analysis

Among a total sample of 8,421 pregnant women considered 224 (2.7%) were severe, 1,442 (17.2%) moderate, anemic 1,327 (15.8%) mild anemic, and while among all pregnant women 5,428 (64.5%) were non-anemic, see Figure 3.

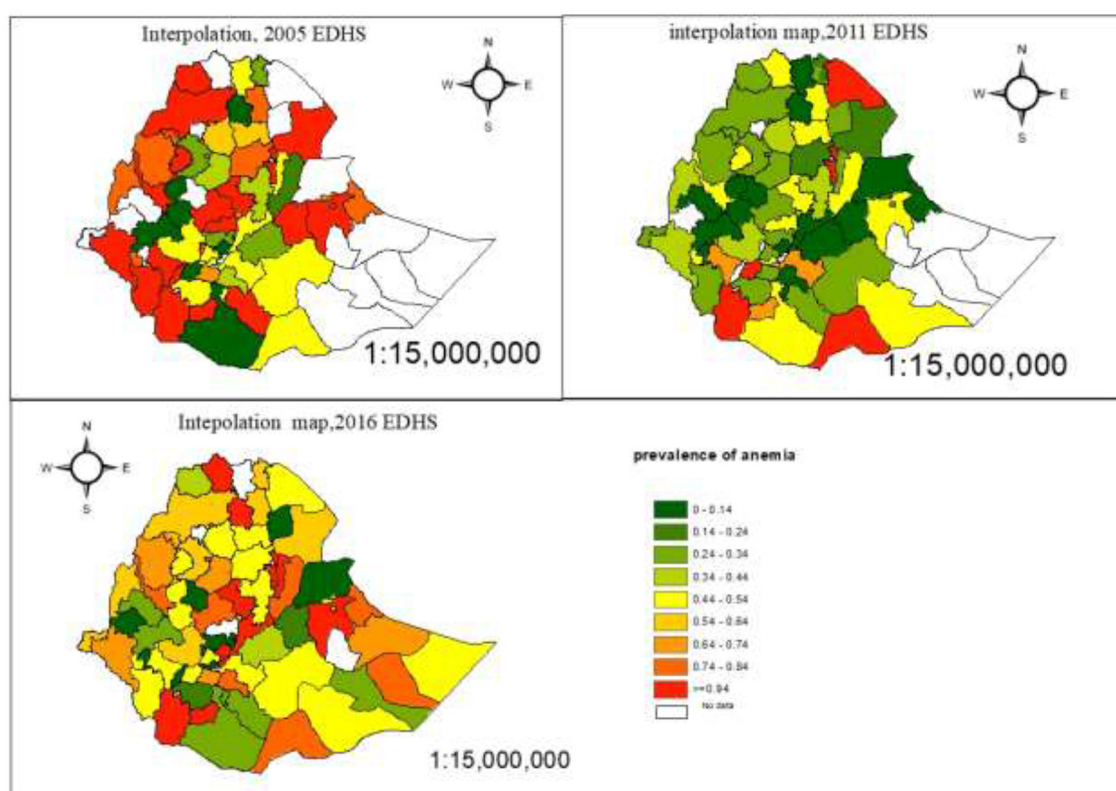


FIGURE 6

The estimated prevalence of anemia among pregnant women in Ethiopian administrative zones.

The prevalence of pregnant anemic women in the survey year 2005, 2011, and 2016 were 542 (34.35%), 1,277 (33.9%), and 1,174 (38.09%), respectively (see Table 1). Besides, Figure 4 revealed the prevalence of anemic pregnant women across the region. The highest number of anemic women was noticed in Somali, Afar, and Dire Dawa.

About 4,290 (50.94%) were found to be aged 30–39 years. The majority of 6,277 (74.54%) mothers did not have formal education. Among the total, 6,394 (76%) of the pregnant women were not working, and 3,920 (46.55%) were Muslim. Out of the total, 67.24% used unimproved water sources. The majority of the pregnant women (90.25%) lived in rural areas, and 2,783 (33.04%) had the poorest wealth index. Concerning the number of household members, having four up to six-member households covers 4,027 (47.82%) of the total and more than half of the pregnant women used wood/straw cooking fuel (Table 2).

The chi-square statistics presented in Table 3 indicate anemia level among pregnant women was significantly associated with categorical predictor variables such variables are highly associated with anemia levels among pregnant women. The region, wealth index, number of household members, age category, religion, pregnant currently working, education level of the mother, cooking fuel, survey year, source of drinking water, types of toilet, body mass index, and preceding birth interval (p -values < 0.05).

Spatial analysis

Spatial distribution of anemia

Each point on the map was characterized by the prevalence of anemia in each zone. The leaf green color indicates the zones with a low distribution of anemia, whereas the mars red color indicates zones with a high distribution of anemia (Figure 5).

Spatial interpolation of anemia

Ordinary Kriging interpolation technique, the mars red ramp color on the map indicates the predicted highest prevalence of anemia and the leaf green ramp color on the map indicates the lowest predicted prevalence of anemia (see Figure 6). Based on the result of predicted values for the prevalence of anemia in 2005, the southern part of Ethiopia would be affected by anemia. The prevalence of anemia has decreased since the 2011 EDHS, but the predicted prevalence of anemia in 2016 shows that the distribution of anemia has increased in Ethiopian administrative zones.

Hot spot analysis

A hot spot analysis was performed to identify the high-risk and low-risk areas for the prevalence of anemia among pregnant women in Ethiopian administrative zones. The red color

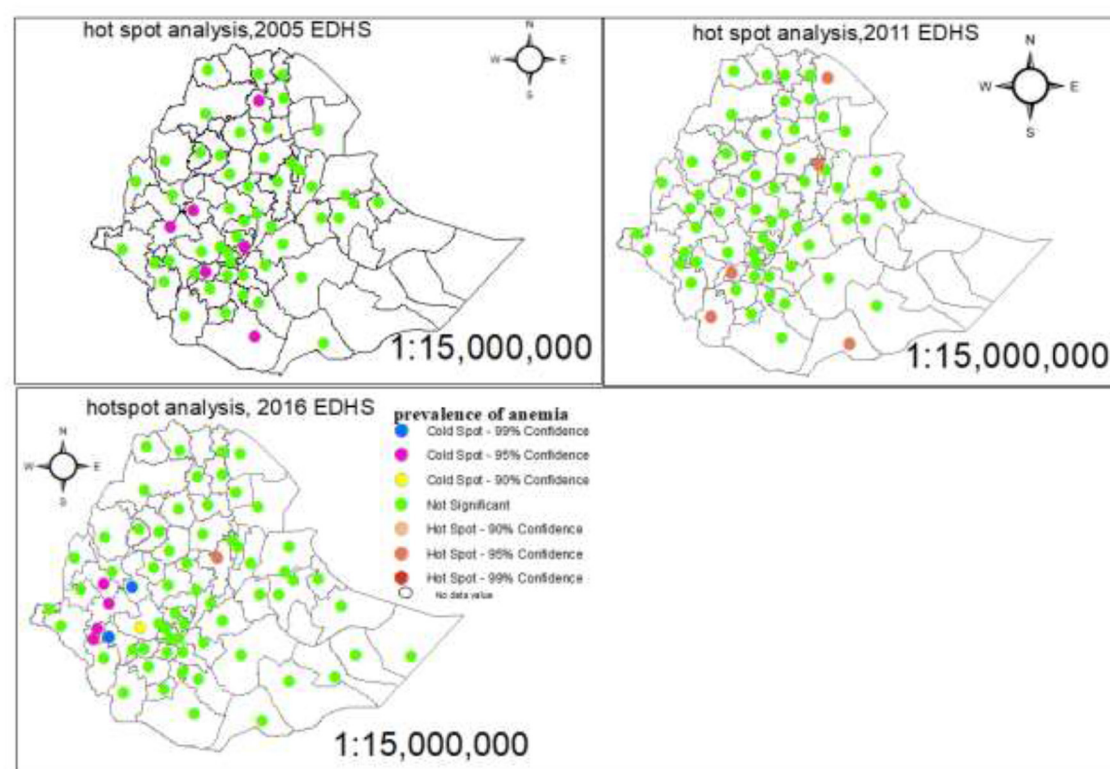


FIGURE 7

Hot spot and cold spot identification of anemia among pregnant women in Ethiopia administrative zones.

TABLE 4 Variogram output of autocorrelation statistics across 2005, 2011, and 2016.

| Survey year | Moran's index | Statistic (z-value) | p-value |
|-------------|------------------------|---------------------|---------|
| 2005 | -7.44×10^{-2} | -1.090 | 0.2755 |
| 2011 | 8.1×10^{-3} | 0.478 | 0.3699 |
| 2016 | -1.1×10^{-2} | 0.0471 | 0.9431 |

TABLE 5 Model comparison.

| Type of model | Observation | AIC values | LRT | p-value |
|---------------|-------------|------------|-----------|---------|
| POM | 8,421 | 14,033.633 | 1,140.796 | 0.001 |
| PPOM | 8,421 | 13,877.24 | 1,345.001 | 0.001 |
| ACLM | 8,421 | 14,016.853 | 1,157.576 | 0.001 |
| GOLM | 8,421 | 13,898.918 | 1,316.429 | 0.001 |

indicates significant hot spot (high-risk) areas for anemia and the blue color indicates the cold spot (low-risk) areas of anemia (Figure 7).

Spatial autocorrelation, Moran's tests

We can test the spatial autocorrelation by using global moans and variogram tests. The Global Moran's test revealed that there is no spatial autocorrelation of anemia among the administrative zones of Ethiopia for the three consecutive EDHSs data sources (Table 4).

Table 4 depicted that the neighboring zones are independent (p -value > 0.05) for the three consecutive survey years.

Ordinal logistic regression

There are different types of an ordinal logistic regression models. These are the proportional odds model (POM), partial proportional odds model (PPOM), adjacent category logit model (ACLM), and generalized ordered logit model (GOLM). Thus, before estimating parameters using the appropriate statistical approach, the model comparison was carried out using AIC and LRT (see Table 5).

All models were significant in the final fit relative to their intercept and covariate models, as indicated by a significant LR test. A model with the smallest value of AIC or with the largest values of LRT was considered a good model and preferable. PPOM has the lowest AIC and highest LR values (13,877.24 and 1,345.001, respectively) compared to the others (Table 5). For ordinal dependent variables with J categories, there are J-1 binary

models to conduct a series of comparisons. In this study, the response variable has three categories and there are two possible binary comparisons: moderate anemia and above vs. mild anemia and non-anemic and moderate and mild anemia vs. non-anemic.

Partial proportional models

The logistic command was used to fit the partial proportional model. In this model, restrictions of parallel lines were imposed on some variables to meet the assumption while others, like wealth and EDHS, were not. The PPOM used a series of Wald tests to check the assumption of proportionality for the categories of all explanatory variables (see [Tables 6, 7](#)).

The result of this study revealed that the age of pregnant women aged 20–29, 30–39, and 40–49 was 46.9% (OR = 0.531, CI: 0.334–0.842), 42.9% (OR = 0.571, CI: 0.359–0.908), and 45.8% (OR = 0.542, CI: 0.33–0.89) less likely to be moderate anemic rather than normal and mild anemic pregnant women as compared to pregnant women whose age was <20 years, respectively.

For pregnant women who lived in Afar, Somalia, Harari, and Dire Dawa region the odds of being moderate and above anemic increased by a factor of 3.556 (OR = 3.556, CI: 1.735–7.286), 2.745 (OR = 2.745, CI: 1.465–5.158), 2.433 (OR = 2.433, CI: 1.467–4.033), and 5.469 (OR = 5.469, CI: 3.407–9.043) times rather than not anemic and mild anemic pregnant women compared to who lived in Amhara region keep all other variables constant. Compared to pregnant women from the household who had consumed water from an improved source, the odds of being moderate and above anemia increased by a factor of 1.192 times percent (OR = 1.192, CI: 1.038–1.369) rather than mild and normal anemia among pregnant women from households who had consumed unimproved source of water holding all other variables constant. Pregnant women from Afar, Tigray, Gambella, and Harari regions were 2.116 (OR = 2.116, CI: 1.143–3.918), 3.53 (OR = 3.53, CI: 2.393–5.211), 11.766 (OR = 11.766, CI: 1.164–2.679), and 2.052 (OR = 2.052, CI: 1.404–3.00), respectively times more likely mild and above anemia rather than non-anemic pregnant women compared to pregnant women from Amhara holding all other variable constant. Compared pregnant women from 1 to 3 number household members, the pregnant women from 4 to 6 and 7 to 9 number household members were 51% (OR = 1.51, CI: 1.175–1.94) and 43.2% (OR = 1.432, CI: 1.183–1.734) more likely to be mild and above anemia, holding all other variables constant. The pregnant women whose religions were catholic and other 61.5% (OR = 0.385, CI: 0.183–0.813) and 88.7% (OR = 0.113, CI: 0.029–0.436) were less likely to be mild and above anemic rather than not anemic compared to pregnant women whose religion was Muslim keep all other variables were constant.

Predictors that satisfied parallel line assumption

The result of PPOM revealed that holding all other variables constant, compared to the pregnant women from households with

the poorest wealth index, the risk of anemia decreased by 15.9% (OR = 0.841, CI: 0.72–0.983) and 51% (OR = 0.49, CI: 0.409–0.586) for the pregnant women whose household wealth index was middle and richest respectively rather than normal. As compared to the pregnant women from the 2016 survey year, the pregnant women from the 2011 survey year were 29.9% (OR = 0.701, CI: 0.631–0.777) less likely to be moderate, severe, and mild anemic rather than not anemic, keeping all other variables constant.

Discussion

The main purpose of this study was to investigate the associated factors and the spatial distribution of anemia among Ethiopian administrative zones using 2005, 2011, and 2016 EDHS. The factors/variables in this study were education level, age group of mother, place of residence, region, religion, source of drinking water, wealth index, cooking fuel, types of toilet, preceding birth interval, body mass index, pregnant currently working, survey years, and number of household members. The prevalence of anemia levels for pregnant women varied among regions. The highest proportion of moderate anemia and above was at Dire Dawa, followed by Afar, and mild anemia at Harari, followed by Afar. Pregnant women from Amhara, followed by Addis Ababa, had the highest proportions of non-anemia. The lowest proportions of moderate anemia and above were observed in Addis Ababa, followed by SNNP and Amhara.

In this study, the adjacent category logit model, generalized ordered logit model, partial proportional odds model, and proportional odds model were fitted to the data, and comparisons of models were made. Thus, the best fit according to AIC and LRT is PPOM, and it was used to identify significant determinants of anemia levels. Parameter estimates of the PPOM are presented and interpreted for the significant predictors (at a 5% significance level). Significant factors associated with anemia level in pregnant women include the mother's age group, region, religion, and source of drinking water; wealth index; the number of household members, and EDHS. The regional differences of distribution of maternal anemia may be because of the health facilities access, weather condition, and types of consumption across region are different.

Our study showed that the wealth index has a significant association with anemia levels among pregnant women. The study found that pregnant women from the richest and middle-class households had a lower risk of anemia than pregnant women from the poorest households. This result is in line with studies done in Ethiopia ([35, 36](#)). Based on spatial analysis, the highest prevalence of anemia was in North West Tigray, Waghimra, Oromia special woreda, West shewa, East shewa, North shewaR4, East harargie, Selti, Alaba, Sidama, Segen people, and South Omo, Afar zone5 and zone3 and Somali Siti, whereas the lowest prevalent zones were Afarzone4, Huru guduru, West harargie, Gurague, Yem, konta, KT, and Gamo Gofa but Shewa (R3) administrative zone was a high-risk area, and Sheka, Majang, Illubabor, and West Wollega were low-risk areas of anemia based on the nearest EDHS data.

The finding of this study revealed that the age of the mother had a significant effect on the anemia level of pregnant women. Pregnant women whose age was <20 years more likely to be

TABLE 6 Maximum likelihood estimates of partial proportional odds model.

| Predictors | Category | Coefficient | p-value | OR | 95% CI (OR) |
|--|-----------------|-------------|----------|-------|--------------|
| Moderate and above vs. mild and not anemic | | | | | |
| Wealth | Poorest (ref.) | 0.0000 | | 1.000 | |
| | Poorer | −0.0233 | 0.7425 | 0.977 | 0.850–1.122 |
| | Middle | −0.17320 | 0.0292* | 0.841 | 0.720–0.983 |
| | Richer | −0.1255 | 0.1103 | 0.882 | 0.756–1.029 |
| | Richest | −0.7140 | <0.0001* | 0.490 | 0.409–0.586 |
| Religion | Muslim (ref.) | 0.0000 | | 1.000 | |
| | Catholic | −0.2591 | 0.6021 | 0.772 | 0.291–2.044 |
| | Orthodox | 0.0456 | 0.7258 | 1.047 | 0.811–1.351 |
| | Other | 1.3793 | 0.0967 | 3.972 | 0.7802–0.221 |
| | Protestant | 0.0111 | 0.9357 | 1.011 | 0.772–1.324 |
| | Traditional | −0.1031 | 0.7243 | 0.902 | 0.509–1.600 |
| EDHS | 2005 | −0.0251 | 0.709 | 0.975 | 0.855–1.113 |
| | 2011 | −0.0356 | <0.001* | 0.701 | 0.631–0.777 |
| | 2016 (ref.) | 0.0000 | | 1.000 | |
| Region | SNNP | −0.1314 | 0.6246 | 0.877 | 0.518–1.484 |
| | Addis Ababa | −0.8263 | 0.1795 | 0.438 | 0.131–1.463 |
| | Afar | 1.2686 | 0.0005* | 3.556 | 1.735–7.286 |
| | Tigray | −0.2688 | 0.4386 | 0.764 | 0.387–1.509 |
| | Ben-Gumz | 0.3499 | 0.1322 | 1.419 | 0.900–2.238 |
| | Gambella | 0.5922 | 0.0419 | 1.808 | 1.022–3.198 |
| | Harari | 0.8888 | 0.0006* | 2.432 | 1.467–4.033 |
| | Oromiya | −0.1309 | 0.5766 | 0.877 | 0.554–1.389 |
| | Somalia | 1.0097 | 0.0017* | 2.745 | 1.460–5.158 |
| | Dire-Dawa | 1.6990 | <0.0001* | 5.469 | 3.307–9.043 |
| | Amhara (ref.) | 0.0000 | | 1.000 | |
| Household member | 1–5 (ref.) | 0.0000 | | 1.000 | |
| | 4–6 | −0.0990 | 0.5076 | 0.906 | 0.676–1.214 |
| | 7–9 | 0.0704 | 0.5234 | 1.073 | 0.864–1.332 |
| | ≥10 | −0.0513 | 0.6418 | 0.950 | 0.765–1.179 |
| SDW | Unimproved | 0.1758 | 0.0128* | 1.192 | 1.038–1.369 |
| | Improved (ref.) | 0.0000 | | 1.000 | |
| Age group | <20 (ref.) | 0.0000 | | 1.000 | |
| | 20–29 | −0.6335 | 0.0072* | 0.531 | 0.334–0.842 |
| | 30–39 | −0.5598 | 0.0179* | 0.571 | 0.359–0.908 |
| | 40–49 | −0.6133 | 0.0155* | 0.542 | 0.33–0.89 |
| | Constant | −1.8966 | <0.0001* | 0.150 | 0.082–0.276 |

*Significant variable.

SDW, Source of Drinking Water; EDHS, Ethiopian Demographic and Health survey year; ref., Reference.

moderate and above anemia as compared to pregnant women whose age was >20 years which is similar to a previous study conducted by Woldegebriel et al. (14). As a result of this research, a number of household members had significantly affected anemia

levels among pregnant women. This indicates that as the number of household members increased, the risk of anemia among pregnant women also increased. This finding fitted with the study done in Tanzania (36).

TABLE 7 Maximum likelihood estimates of partial proportional odds model.

| Predictors | Category | Coefficient | p-value | OR | 95% CI OR |
|-------------------------------|-----------------|-------------|----------|--------|-------------|
| Mild and above vs. not-anemic | | | | | |
| Wealth | Poorest (ref.) | 0.0000 | | 1.000 | |
| | Poorer | −0.0233 | 0.7425 | 0.9770 | 0.850–1.122 |
| | Middle | −0.1732 | 0.0292* | 0.841 | 0.720–0.983 |
| | Richer | −0.1255 | 0.1103 | 0.882 | 0.756–1.029 |
| | Richest | −0.7140 | <0.0001* | 0.490 | 0.409–0.586 |
| Religion | Muslim (ref.) | 0.0000 | | 1.000 | |
| | Catholic | −0.9536 | 0.0123* | 0.385 | 0.183–0.813 |
| | Orthodox | −0.0146 | 0.8762 | 0.986 | 0.820–1.184 |
| | Other | −2.1768 | 0.0015* | 0.113 | 0.029–0.436 |
| | Protestant | −0.0455 | 0.6501 | 0.956 | 0.785–1.163 |
| | Traditional | −0.0967 | 0.6625 | 0.908 | 0.588–1.401 |
| EDHS | 2005 | −0.0251 | 0.709 | 0.975 | 0.855–1.113 |
| | 2011 | −0.0356 | <0.001* | 0.701 | 0.631–0.777 |
| | 2016 (ref.) | 0.0000 | | 1.000 | |
| Region | Amhara (ref.) | 0.0000 | | 1.000 | |
| | SNNP | −0.1092 | 0.5735 | 0.897 | 0.613–1.311 |
| | Addis Ababa | −0.6401 | 0.0702 | 0.527 | 0.264–1.054 |
| | Afar | 0.7497 | 0.0170* | 2.116 | 1.143–3.918 |
| | Tigray | 1.2617 | <0.0001* | 3.531 | 2.393–5.211 |
| | Ben-Gumz | 0.1087 | 0.5249 | 1.115 | 0.797–1.559 |
| | Gambella | 0.5685 | 0.0075* | 1.766 | 1.164–2.679 |
| | Harari | 0.7188 | 0.0002* | 2.052 | 1.404–3.000 |
| | Oromiya | −0.2396 | 0.1588 | 0.787 | 0.564–1.098 |
| | Somalia | 0.2953 | 0.2744 | 1.344 | 0.791–2.282 |
| | Dire-Dawa | 0.3590 | 0.1851 | 1.432 | 0.842–2.435 |
| Number of household member | 1–5 (ref.) | 0.0000 | | 1.000 | |
| | 4–6 | 0.4122 | 0.0013* | 1.510 | 1.175–1.940 |
| | 7–9 | 0.3592 | 0.0002* | 1.432 | 1.183–1.734 |
| | ≥10 | 0.0753 | 0.4015 | 1.078 | 0.891–1.305 |
| SDW | Unimproved | −0.0382 | 0.5515 | 0.962 | 0.849–1.092 |
| | Improved (ref.) | 0.0000 | | 1.000 | |
| Age group | <20 (ref.) | 0.0000 | | 1.000 | |
| | 20–29 | −0.1137 | 0.6101 | 0.892 | 0.576–1.382 |
| | 30–39 | 0.0968 | 0.6663 | 1.102 | 0.709–1.711 |
| | 40–49 | 0.0733 | 0.7560 | 1.076 | 0.678–1.708 |
| | Constant | −1.4175 | <0.0001* | 0.242 | 0.142–0.414 |

*Significant variable.

SDW, Source of Drinking Water; EDHS, Ethiopian Demographic and Health survey year.

The findings of this study revealed that the source of drinking water had a significant effect on the level of anemia among pregnant women. This showed that pregnant women who drank unimproved water were more anemic compared to those who drank improved water, which is similar to a previous study conducted by Berhe et al. (37).

The current study identified that religion was a significant effect on anemia among pregnant women. The risk of anemia among pregnant women whose religion was catholic was high compared to those whose religion was Muslim this result was consistent with the result obtained by Woldegebriel et al. (14).

This study incorporated data from three successive surveys and considered a simultaneous spatial variation of anemia on pregnant women. Thus, the findings generated from this research would improve the findings of cross-sectional studies so far and will help policymakers implement appropriate policy measures. This study has a number of drawbacks. The survey from which the data for this study were gathered was conducted in three waves of 5 years each: in 2005, 2011, and 2016. As a result, prevalence of anemia on pregnant women are noticeable within 5 years. Another weakness of the study might be attributed to the memory bias in the cross-sectional DHS data. Using the most recent survey data, we advise additional research.

Conclusion

About over one-third of the expectant mothers (34.5%) were anemic to varying degrees. The prevalence of anemia in 2005 in the southern region of Ethiopia would be significantly impacted by anemia, according to the outcome of forecasted values. According to data from the fourth EDHS, the areas with the highest prevalence of anemia were West Shewa, Waghimra, Oromia special woreda, and North West Tigray. Anemia levels in pregnant women were significantly influenced by factors including location, wealth index, drinking water source, household size, mother's age, religion, and EDHS. Women who were expecting were less likely to develop moderate or higher levels of anemia if their income index was higher and they drank superior water. Pregnant women with 1–3

family members who were less likely to have mild or above-average anemia.

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 at: <https://dhsprogram.com/data/available-datasets.cfm>.

Author contributions

MA proposed the first draft, conducted data analysis and interpretation, and wrote the manuscript. HF, DZ, and LT edited and revised 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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Association of multi-metals with the risk of hypertension and the interaction with obesity: A cross-sectional study in China

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Background: Environmental exposure to multiple metals have been inconsistently associated with hypertension. Obesity is an important independent risk factor for hypertension, and few studies have assessed the interaction between obesity and metals in this context. We aimed to clarify their association and interaction.

Methods: This cross-sectional study included 3,063 adults from 11 districts or counties, Guangdong. We measured the whole blood levels of 13 metals and used multipollutant-based statistical methods to analyze the association of metals with hypertension. The interaction between metals and obesity on hypertension was assessed on additive and multiplicative scales.

Results: Four metals (manganese, arsenic, cadmium, and lead) were significantly associated with hypertension risk, five metals (manganese, zinc, arsenic, cadmium, and lead) were related to elevated SBP levels, five metals (manganese, zinc, selenium, cadmium, and lead) were associated with elevated DBP levels in single-metal model. Manganese remained significantly related to hypertension risk [odds ratio, 1.35 (1.02–1.78)] after adjusting for these four metals. Significant positive dose-response relationships between manganese, arsenic, cadmium, lead and hypertension risk were observed (P for overall < 0.001, P for non-linearity > 0.05). Compared with those in the lowest quartile, participants in the highest manganese quartile had a 2.83 mmHg (95% CI: 0.71–4.96) (P_{FDR} = 0.040) higher level of SBP. Individuals in the highest quartiles of zinc and lead had a 1.45 mmHg (0.10–2.81) (P_{FDR} = 0.033) and 2.06 mmHg (0.59–3.53) (P_{FDR} = 0.020) higher level of DBP, respectively. The negative interactions between cadmium, lead and obesity influences hypertension risk. BKMR analysis showed a significant joint effect of manganese, arsenic, cadmium and lead on hypertension when the concentrations of four metals were at or above their 55th percentile compared to their median values.

Conclusions: The combined effect of four metals (manganese, arsenic, cadmium and lead) were associated with the prevalence of hypertension. Potential interaction effects of cadmium, lead and obesity on hypertension risk may exist. Further cohort studies in larger population are needed to clarify these findings.

KEYWORDS

multiple metals, hypertension, blood pressure, obesity, interaction, BKMR analysis

1. Introduction

Hypertension is a major risk factors for cardiovascular disease and imposes a heavy burdens of societies internationally (1). It has been estimated that about 1.39 billion people worldwide have hypertension in 2010, and by 2030 the number of people with hypertension will reach to 1.56 billion (2, 3). Over the past decades, the prevalence of hypertension has been increasing in Chinese adults (4). It was reported that the prevalence of Chinese adult hypertension was 17.6% in 2002 (5), and it increased to 23.2% in 2015 (6). Hypertension has become a worldwide public health concern. However, the pathogenesis of hypertension has yet to be elucidated.

The traditional risk factors, including heredity, age, sex, BMI and unhealthy lifestyles, could only partially explain the causes of hypertension (7). Recently, accumulating epidemiological studies have evaluated the potential association between various metal levels from different biological samples and the prevalence of hypertension, but the results are inconsistent (8–10). A prospective study conducted in 3047 American Indian reported that the low-to-moderate levels of urinary cadmium (Cd) was positively correlated with hypertension (11). Nevertheless, a cross-sectional study from the Canadian Health Measures Survey (2007–2013) observed an inverse correlation of urine Cd with the risk of hypertension in current smokers, particularly female current smokers (12). The 2011–2016 US National Health and Nutrition Examination Survey (NHANES) confirmed a positive correlation between blood selenium (Se) levels and hypertension but not serum zinc (Zn) and copper (Cu) in American adults (13). A cross-sectional study conducted in the Cd-polluted and the unpolluted area of southwestern China unveiled that high blood iron (Fe) and lead (Pb) levels in polluted area and high blood manganese (Mg) and

Fe in unpolluted area were related to increasing SBP and DBP levels (14).

In daily life, metals exist in almost all environmental media and people are often exposed to multiple metals simultaneously (15). Excessive visceral fat distribution, especially in obese individuals, can cause inflammatory reaction and endothelial damage, leading to hypertension (16). However, the effects of multiple-metal exposures on the risk of hypertension are largely unknown. A prospective cohort study in the Yangtze River region suggested that based on Bayesian kernel machine regression (BKMR), multiple metals [Cd, Cu, Mn, molybdenum (Mo), and Zn] had a significant joint effect on hypertension (10). Inter-metal interactions may alter the toxicity of single metals. Therefore, the co-exposure effect of multiple metals cannot be ignored in the study of metals.

As a widely known risk factor for higher blood pressure and hypertension, high BMI is reported to be associated with heavy metals (12, 17, 18). The 1999–2014 US NHANES reported that the negative interaction between Cd exposure and obesity influenced systolic hypertension risk (18), however, high urinary Cd levels were associated with significantly high blood pressure among overweight and obese Canadian women (12). Therefore, the effect of the interaction of heavy metals exposure and BMI on hypertension and blood pressure is worthy of further investigation.

In this context, we conducted a cross-sectional study from Guangdong Province located in southern China and measured the whole blood 13 metals levels as internal exposure. And the multipollutant-based statistical methods, including multivariate logistic regression, restricted cubic spline (RCS), the Bayesian kernel machine regression (BKMR) and interaction analysis, were applied to explore the joint effects of multiple metals on hypertension risk and their interaction with obesity.

2. Methods

2.1. Study population

This was a cross-sectional survey and the data obtained in the Guangdong Provincial Residents' Chronic Disease and Nutrition Surveillance Survey (2015). All participants were recruited from general communities and they were not exposed to metal pollutions or occupational factors. Briefly, 11 districts or counties were randomly selected, and about 300 participants were further selected in each district or county using a simple random sampling method. Finally, 3,029 participants aged 18 years or older were included between October 2015 and February 2016 after excluding 34 individuals who did not provide blood samples. The protocol of the present study was approved by the institutional review board of

Abbreviations: As, Arsenic; BKMR, Bayesian kernel machine regression; BMI, Body mass index; Cd, Cadmium; CGMP, Cyclic guanosine monophosphate; Co, Cobalt; condPIP, Conditional posterior inclusion probability; Cr, Chromium; Cu, Copper; DBP, Diastolic blood pressure; FDR, False discovery rate; HbA1c, Glycosylated hemoglobin A1c; HDL, High-density lipoprotein; ICP-MS, Inductively coupled plasma mass spectrometry; IQR, Interquartile range; LDL, Low-density lipoprotein; In, Logarithm; LOD, Limit of detection; Mn, Manganese; Mo, Molybdenum; NHANES, National Health and Nutrition Examination Survey; Ni, Nickel; NO, nitric oxide; ORs, Odds Ratios; Pb, Lead; PPS, Probability proportional to size; Q1, The lowest quartile; RAS, Renin-angiotensin system; RCS, Restricted cubic spline; RERI, Relative excess risk index; ROS, Reactive oxygen species; SD, Standard deviation; Se, Selenium; SBP, Systolic blood pressure; TC, Total cholesterol; TG, total triglycerides; Tl, Thallium; UA, Uric acid; V, Vanadium; WC, Waist circumference; WHt R, Waist-to-height; Zn, Zinc.

Chinese Center for Disease Control and Prevention (No. 201519-B), and all of subjects provided their written informed consent.

2.2. Data collection and outcome definition

A face-to-face questionnaire by a well-trained public health practitioner was applied to collect data. The details of the data collection methods have been described in previous study (19). Information on general demographic information, lifestyle, diseases, medication, and family history of hypertension were gathered. The anthropometric data, including systolic blood pressure (SBP), diastolic blood pressure (DBP), weight, height, and waist circumference (WC), were also measured using standard methods (19). Waist-to-height (WtR) was calculated as waist (cm) divided by height (cm). The levels of fasting blood-glucose (FBG), total triglycerides (TG) and total cholesterol (TC) were also tested following a standard protocol (19).

Drinking status was classified based on alcohol consumption into current drinking (any alcohol consumption in the past 30 days) and not drinking. Smoking status was classified as “non-smokers,” “former smokers” (no tobacco use in the past 30 days) or “current smokers” (any tobacco use in the past 30 days).

Hypertension was defined as a self-reported physician diagnosis (confirmed with medical records from district-level or higher hospitals), or individuals with SBP ≥ 140 mmHg and/or DBP ≥ 90 mmHg for three times, or current use of antihypertensive medication (20). Body mass index (BMI) was classified into four categories following the standards of Chinese adults: low body weight, <18.5 kg/m²; normal, ≥ 18.5 kg/m², and <24 kg/m²; overweight, ≥ 24 kg/m² and <28 kg/m²; obesity, ≥ 28.0 kg/m². Individuals with central obesity were those with WC ≥ 85 cm for males or ≥ 80 cm for females (21).

2.3. Whole blood metal measurements

The whole blood concentrations of 13 metals (As; Cd; Cobalt, Co; Chromium, Cr; Cu; Mn; Mo; Nickel, Ni; Pb; Se; Thallium, Tl; Zn; Vanadium, V) were measured by inductively coupled plasma mass spectrometry (ICP-MS, Agilent 7500ce, Agilent Technologies, USA). The fasting blood samples were collected and stored at -80°C for subsequent detection following a standard detailed protocol (19). Before analysis, frozen whole blood samples were thawed at room temperature ($22 \pm 5^{\circ}\text{C}$). Two hundred and fifty microliter of the sample was diluted with 4.75 mL diluent [including 2.5 mL HNO₃ (Guaranteed reagent, Fisher) and 0.5 mL TritonTMX-100 (Analytical reagent, sigma-alorich)], and the sample was further diluted to 1,000 mL with ultrapure water for final analysis. Trace Element Blood L-1~2 (Seronom, Sero, Oslo, Norway) were applied as quality control samples, and quality control was run in each batch (25 samples). Besides, three blank samples (1% HNO₃) were used to control the potential contaminations and set into each batch. Meanwhile, the spiked recovery method was used by measuring each sample twice and the recovery rates of all metals were from 90.0 to 110.0%. Once the measured values were suggested to be contaminated or differ from

certified values, the instrument was recalibrated and the previous batch of samples was reanalyzed. The data were multiplied by the dilution factor to gain the final concentration. Measurements below the limit of detection (LOD) were replaced with $\text{LOD}/\sqrt{2}$. The LODs for the 12 metals ranged from 0.2 (Co, Cd, Tl) to 20.0 (Zn, Pb) $\mu\text{g/L}$. Since the proportion of participants whose whole blood Tl and Mo with concentrations lower than LOD was 88.7 and 48.04%, Tl and Mo was not further analyzed. For other metals, more than 70.91% of the samples had values above the LOD (Supplementary Table S1).

2.4. Statistical analysis

The demographic characteristics of all the subjects were described as percentages, means \pm standard deviation (SD), or median with interquartile range (IQR). *T*-tests, chi-square test and the Wilcoxon rank sum were used based on data distribution. The levels of metals were transformed to natural logarithm (ln) to approximate a normal distribution. Pearson correlation test was used to evaluate the correlation between whole blood metals.

The blood metal level was divided into quartiles, and the lowest quartile (Q1) was assigned to be the referent group. The relationship between blood metal level and the risk of hypertension was evaluated by using multivariate logistic regression model with 11 districts or coastal/inland as fixed effect and the mixed effects logistic regression model with 11 districts or coastal/inland as a random intercept, respectively, and the optimal model was further selected for analysis. The results of model are shown in Supplementary Tables S2–S5. Multivariable linear model was applied to assessed the associations of metal levels in whole blood and BP/PP levels. Taking into account the potential deviation between taking antihypertensive drugs and blood pressure level, the BP level of participants using antihypertensive drugs increased by an additional constant of 10 mmHg (22). The covariates adjusted in the single- and multiple-metals models were age, sex, region and education level, drinking and smoking status, family history of hypertension, antihypertensive use, BMI, TG, TC, LDL, HDL, HbA1c, UA (10, 23–26). The trend test was performed by taking the median of each metal quartile as a continuous variable in the model, and the false discovery rate (FDR) correction was applied to adjust trend *P*-values.

Restricted cubic spline (RCS) analyses were applied to further explore the dose-response relationship of whole blood metal level with hypertension risk, with the percentiles of five the ln-transformed metal concentrations to be the knots. Adjusted factors were same to multiple-metal models.

Bayesian Kernel Machine Regression (BKMR) based on Gaussian process regression was used to investigate the non-linear dose-response and interactions of metals on hypertension. Metals (Mn, As, Cd, and Pb) with significant or suggestive *P* trend (*P* trend < 0.05 or $0.05 < P$ trend < 0.10) in single-metal models were incorporated into BKMR model. Adjusted factors were same to multiple-metal models. First, four metals were classified into two groups based on the correlation coefficients. The conditional posterior inclusion probability (con PIP) was calculated to select the key metals for hypertension, and the threshold for PIP was 0.5

(27). Then, exposure-response function was performed to explore the association of individual metal with the risk of hypertension while holding the other four metals at median. Moreover, the joint effect of four metals on the risk of hypertension was estimated by comparing a particular percentile of multiple metals against their median value. Finally, an interaction of a particular metal and the remaining metals was estimated when the remaining metals were fixed at a particular percentile (25th, 50th, or 75th percentile). Sensitivity analyses for BKMR were performed by changing its smoothing parameters ($b = 50$ and $b = 200$).

Subgroup analysis and modeled interaction terms was used to examine the modification of associations in logistic regression model according to BMI (low body weight, normal, overweight, and obesity), WHt R (<0.6 or ≥ 0.6), central obesity (yes or no), drinking status (drinking within 30 days, drinking before 30 days, never drinking) and smoke status (current smokers, former smokers, non-smokers). Subgroup analysis was limited to metals that predicted the outcome with $P < 0.10$ in the single-metal models.

All statistical analyses were performed with IBM SPSS 26.0 (Armonk, NY, USA) and R software (v.4.1.0). Two-tailed $P < 0.05$ was reckoned as statistically significant, while P -values in the range of 0.05–0.10 was suggestively significant.

3. Results

3.1. Characteristics of participants

After all individuals who did not provide blood samples ($n=34$) were excluded, 3,029 of the 3,063 eligible individuals were included in the final data analysis. As shown in Table 1, the percentages of non-hypertension and hypertension subjects were 64 and 36%, respectively. Compared to subjects without hypertension, those with hypertension were more likely to be female, older, former smoker and current alcohol consumers, live in island areas, had lower education level and higher SBP, DBP, WHt R, BMI, TC, TG, and FBG (all $P < 0.05$). The whole blood levels of Cu, Zn, Mo, Cd, Pb were significantly higher (all $P < 0.05$) in the hypertension group than in non-hypertension, while no significant differences were observed for other metals (all $P > 0.05$). Pearson correlation coefficients (r) of metals ranged from -0.412 to 0.834 (all $P < 0.05$, Supplementary Table S6).

3.2. Whole blood metals and hypertension risk

In the single-metal model (Table 2), after adjusting for age, sex, region and education level, drinking and smoking status, family history of hypertension and antihypertensive use (Model 1), the multivariate-adjusted ORs (95% CIs) of hypertension were 1.40 (1.07, 1.82) for Mn and 1.44 (1.08–1.94) for As comparing the highest vs. the lowest quartiles of metals. After additionally adjusted for BMI, TC, TG and FBG (Model 2), the ORs (95% CIs) of hypertension were 1.42 (1.08–1.86) for Mn, 1.41 (1.04–1.91) for As, 1.42 (1.05–1.91) for Cd, and 1.38 (1.02–1.86) for Pb. Mn, As, and Cd evaluated in Model 2 had positive and significant increased trends

of ORs for hypertension (all P trend < 0.05), Pb had suggestively significant increased trends (P trend < 0.10), but none of them was retained after FDR-adjustments (all $P_{FDR} > 0.05$).

In the multiple-metal models that simultaneously included four metals, trends for As and Cd remained suggestively significant, but neither of them were retained after FDR-adjustments (both $P_{FDR} > 0.10$). And only the associations of whole blood Mn with hypertension risk remained significant, and the multivariate adjusted ORs (95% CIs) of the highest quartiles of metals was 1.35 (1.02–1.78) for Mn.

3.3. Whole blood metals and blood pressure

The associations of metals in whole blood with the levels of SBP and DBP were investigated in the multivariable linear regression model (Table 3). In the single-metal models, the positive trends of Mn, Cd, and Pb with SBP were found (all P trend < 0.05), and these trends were retained after FDR-adjustments (all $P_{FDR} < 0.10$). Compared with the reference group, participants in the highest Cr, Mn, As, Cd, and Pb groups had a 0.98 mmHg (95% CI, 0.49–1.47), 3.41 mmHg (95% CI, 1.32–5.50), 2.39 mmHg (95% CI, 0.09–4.69), 2.73 mmHg (95% CI, 0.40–5.05) and 2.77 mmHg (95% CI, 0.48–5.06) higher level of SBP, respectively. Increasing trends of Mn, Cd, Pb, and Zn quartiles with elevated DBP levels (both P trend < 0.05) were found, and the trends were remained after FDR-adjustments (all $P_{FDR} < 0.05$). Individuals in the highest group of Mn, Zn, Cd, and Pb had a 1.92 mmHg (95% CI, 0.61–3.23), 2.09 mmHg (95% CI, 0.77–3.41), 2.38 mmHg (95% CI, 0.92–3.83), and 2.63 mmHg (95% CI, 1.21–4.05) higher in DBP level, respectively. In the multi-metals models, significant increasing trends of Mn or Zn and Pb quartiles with SBP or DBP levels were observed, respectively, and the trends remained after FDR-adjustments (all $P_{FDR} < 0.05$). Participants in the highest quartiles of Mn or Zn and Pb had a 2.83 mmHg (95% CI, 0.71–4.96), 1.45 mmHg (95% CI, 0.10–2.81) and 2.06 mmHg (95% CI, 0.59–3.53) higher in SBP or DBP levels, respectively.

3.4. Dose-response relationships of metal level and hypertension risk

For whole blood Mn levels, RCS analyses showed a wave shaped associations with the risk of hypertension (P for overall < 0.001 , P for non-linearity = 0.098). The positive linear dose-response relationships between whole blood As, Cd, Pb and the hypertension risk were presented (all P for overall < 0.001 , P for non-linearity of 0.704, 0.811, and 0.671, respectively) (Figure 1).

3.5. BKMR analyses

Figure 2A found a linear relationship between exposure to single metal (Mn, As, Cd, and Pb) and hypertension risk when other three metals exposure were fixed at the median. The conPIP of As, Cd, Mn and Pb was 0.67, 0.44, 0.40, and 0.44, respectively. A significant joint effect of four metals on the risk of hypertension

TABLE 1 Base characteristics of the study population.

| Characteristics | Hypertension (<i>n</i> = 1,090) | Non-hypertension (<i>n</i> = 1,939) | <i>P</i> -value |
|--|----------------------------------|--------------------------------------|-----------------|
| Age (years) | 62.0 (53.2, 69.3) | 48.6 (36.3, 58.7) | <0.001* |
| Sex, <i>n</i> (%) | | | 0.021* |
| Male | 524 (48.1) | 848 (43.7) | |
| Female | 566 (51.9) | 1,091 (56.3) | |
| Region, <i>n</i> (%) | | | <0.001* |
| Coastal | 428 (39.3) | 932 (48.1) | |
| Inland | 662 (60.7) | 1,007 (51.9) | |
| Education level, <i>n</i> (%) | | | <0.001* |
| ≤9 years | 898 (82.4) | 1,332 (68.7) | |
| 9–12 years | 136 (12.5) | 371 (19.1) | |
| > 12 years | 56 (5.1) | 236 (12.2) | |
| Family history of hypertension, <i>n</i> (%) | | | 0.01* |
| Yes | 293 (26.9) | 440 (22.7) | |
| No | 797 (73.1) | 1,499 (77.3) | |
| Anti-hypertensive use, <i>n</i> (%) | 301 (27.6) | 0 (0) | NA |
| Central obesity, <i>n</i> (%) | | | <0.001* |
| Yes | 604 (55.4) | 641 (33.1) | |
| No | 486 (44.6) | 1,298 (66.9) | |
| Smoking status ^a , <i>n</i> (%) | | | <0.001* |
| Current smokers | 289 (26.5) | 505 (26.0) | |
| Former smokers | 101 (9.3) | 93 (4.8) | |
| Non-smokers | 700 (64.2) | 1,341 (69.2) | |
| Drinking status, <i>n</i> (%) | | | <0.001* |
| Drinking within 30 days | 250 (22.9) | 498 (25.7) | |
| Drinking before 30 days | 91 (8.3) | 271 (14.0) | |
| Never drinking | 749 (68.7) | 1,170 (60.3) | |
| SBP (mmHg) | 149.7 (141.7, 162.3) | 120.3 (111.0, 128.0) | <0.001* |
| DBP (mmHg) | 85.7 (77.3, 92.3) | 73.3 (67.7, 78.3) | <0.001* |
| BMI (kg/m ²) | 24.1 (21.7, 26.5) | 22.5 (20.5, 24.7) | <0.001* |
| WHt R | 0.53 (0.49, 0.57) | 0.49 (0.45, 0.53) | <0.001* |
| TC (mmol/L) | 5.23 (4.63, 5.94) | 4.93 (4.26, 5.54) | <0.001* |
| TG (mmol/L) | 1.21 (0.84, 1.86) | 0.97 (0.69, 1.48) | <0.001* |
| FBG (mmol/L) | 5.38 (4.89, 6.00) | 5.03 (4.67, 5.48) | <0.001* |
| Blood metals (μg/L) | | | |
| V | 0.95 (0.50, 1.54) | 0.99 (0.54, 1.52) | 0.253 |
| Cr | 4.90 (3.68, 6.49) | 4.97 (3.79, 6.46) | 0.408 |
| Mn | 13.83 (11.00, 17.96) | 14.05 (10.69, 18.01) | 0.886 |
| Co | 0.28 (0.18, 0.38) | 0.28 (0.18, 0.40) | 0.111 |
| Ni | 2.25 (1.21, 3.90) | 2.23 (1.31, 4.00) | 0.328 |
| Cu | 962.84 (860.73, 1070.81) | 932.37 (839.59, 1044.96) | <0.001* |
| Zn | 5703.99 (4819.70, 6568.20) | 5534.83 (4762.66, 6410.31) | 0.006* |

(Continued)

TABLE 1 (Continued)

| Characteristics | Hypertension (<i>n</i> = 1,090) | Non-hypertension (<i>n</i> = 1,939) | <i>P</i> -value |
|-----------------|----------------------------------|--------------------------------------|-----------------|
| As | 4.48 (2.71, 7.10) | 4.43 (2.62, 7.05) | 0.518 |
| Se | 165.36 (137.57, 197.17) | 165.72 (139.19, 194.20) | 0.797 |
| Cd | 2.54 (1.44, 4.58) | 2.12 (1.25, 4.21) | <0.001* |
| Pb | 45.51 (30.41, 64.97) | 37.08 (24.58, 55.90) | <0.001* |

BMI, body mass index; DBP, diastolic blood pressure; FBG, fasting plasma glucose; NA, non-available; SBP, systolic blood pressure; TC, total cholesterol; TG, triglycerides; WHt R, waist height ratio.

*Smoking status was classified as “non-smokers,” “former smokers” (Tobacco was used before but not now) or “current smokers” (Tobacco was used daily or occasionally).

**P* < 0.05.

was found when all metals were at or above their 55th percentile compared with their median values (Figure 2B). However, no significant association between single metal (Mn, As, Cd, and Pb) and hypertension risk (50th vs. 25th) was found when other three metals were fixed at different percentiles (25th, 50th, or 75th) (Figure 2C). No interaction effect was found among the four metals in the bivariate exposure-response analysis (Figure 2D). In addition, sensitivity analyses found that our findings were not sensitive to the choice of smoothing parameter.

3.6. Subgroup and interaction analyses

In normal BMI subgroups, significantly increased trends of OR for hypertension with Mn, Cd exposure were observed (both *P* trend < 0.05). Similar trends of OR for hypertension with As, Pb exposure were presented in overweight subgroup (Supplementary Table S7). Nevertheless, no trends of OR for hypertension with four metals (Mn, Cd, As, and Pb) exposure in subgroups of WHt R, central obesity, smoking and drinking status were observed (data not shown). To further explore the effect of the potential interaction between blood metals and BMI on hypertension, whole blood metals and BMI were categorized into two categories [blood metals: \geq geometric mean (GM) vs. <GM; and BMI: \geq 28 kg/m² indicating obesity vs. <28 kg/m² indicating non-obesity]. On the additive scale, remarkable negative interaction between BMI and Cd, Pb on hypertension were discovered [RERI = −2.35 (95% CI, −4.58, −0.12); RERI = −1.17 (95% CI, −2.28, −0.06), respectively] (Table 4). Similar findings were obtained on the multiplicative scale [OR = 0.50 (95% CI, 0.26–0.95); OR = 0.53 (95% CI, 0.28–1.00), respectively].

4. Discussion

In this study, we evaluated the association of 13 blood metals with risk of hypertension and blood pressure in the south Chinese general population by various statistical models. The findings showed that positive trends for increased odds of hypertension with increasing Mn, As, Cd, and Pb quartiles, and these trends were further confirmed in RCS analysis. Increasing Mn, Cd, and Pb quartiles were associated with elevated SBP levels, and increasing Mn, Zn, Cd, and Pb quartiles were associated with elevated DBP levels. BKMR analyses indicated a positive joint effect of mixture of four metals (Mn, As, Cd, and Pb) on hypertension risk.

4.1. Manganese (Mn)

Manganese (Mn) is an essential dietary element and a critical component in dozens of proteins and enzymes that involves several biological processes, including carbohydrate and lipid metabolism, growth and reproduction, and formation of tissues (28). Overexposure to Mn can induce severe neurological damage manganese toxicity (29). Despite it is considered as a toxic heavy metal, Mn could play a potential role in controlling blood pressure due to its anti-oxidative function (30). However, the accumulation of Mn may cause inflammation and endothelial dysfunction, leading to elevated blood pressure or the formation of high blood pressure (31). Besides, Mn exposure can inhibit myocardial contraction, dilates blood vessels and induces hypertension (32). The relationship between the body burden of Mn and blood pressure or hypertension remains somewhat controversial. A cross-sectional study of 957 participants from the Gulf Long-Term Follow-up Study showed that Mn was positively associated with the risk of hypertension (9), while a negatively association of urinary Mn with blood pressure was found in another study (8). Another cross-sectional study included 2,597 Chinese adults with hypertension showed that blood Mn levels were associated with age, region and season, but not with SBP (β = −0.01, *P* > 0.05) and DBP (β = 0.00, *P* > 0.05) (33). In the present study, no significant differences in whole blood Mn levels between hypertensive and non-hypertensive subjects were found, however, a positive trend and association of blood Mn with the risk of hypertensive, levels of SBP and DBP were observed in single-metal model (both *P* trend < 0.05), and this trend is maintained in levels of SBP and DBP after FDR-adjustments (both *P* FDR < 0.10). Similar results were observed in a recent study (34). In the multiple-metal model, the association of blood Mn with hypertensive risk and SBP level were remained. In subgroup analysis, the risk of hypertension in High Mn + BMI \geq 28 kg/m² group was 1.83-folds comparing with the Low Mn + BMI < 28 kg/m² group, but no obvious interaction on hypertension was observed. Taken together, the association between Mn and blood pressure or hypertension remain inconclusive. Further investigations are needed to validate our findings, and establish a standard biomarker of exposure to Mn.

4.2. Cadmium (Cd)

Cadmium (Cd) is a hazardous heavy metal with a long biological half-life of 10–30 years that may exert toxic effects on

TABLE 2 Adjusted odds ratios (95%CI) for hypertension according to quartile of whole blood metals exposure.

| Whole blood metals | Quartiles of whole blood metals (μg/L) | | | | <i>P</i> trend (<i>P</i> _{FDR} ^d) |
|-----------------------|--|--------------------|--------------------|--------------------|---|
| | Q1 | Q2 | Q3 | Q4 | |
| Single-metal model | | | | | |
| V | ≤0.52 | 0.53–0.98 | 0.99–1.55 | ≥1.56 | |
| <i>n</i> (case/total) | 287/755 | 277/747 | 236/741 | 271/736 | |
| Crude ^a | Ref | 0.96 (0.78, 1.18) | 0.76 (0.62, 0.94)* | 0.95 (0.77, 1.17) | 0.254 (0.339) |
| Model 1 ^b | Ref | 1.08 (0.84, 1.40) | 0.93 (0.71, 1.22) | 1.00 (0.77, 1.30) | 0.716 (0.820) |
| Model 2 ^c | Ref | 1.10 (0.85, 1.43) | 0.92 (0.70, 1.22) | 1.00 (0.76, 1.31) | 0.714 (0.857) |
| Cr | ≤3.75 | 3.76–4.94 | 4.95–6.47 | ≥6.48 | |
| <i>n</i> (case/total) | 286/760 | 272/758 | 258/756 | 274/755 | |
| Crude ^a | Ref | 0.93 (0.75, 1.14) | 0.86 (0.70, 1.06) | 0.94 (0.77, 1.16) | 0.104 (0.250) |
| Model 1 ^b | Ref | 1.06 (0.82, 1.37) | 0.96 (0.74, 1.25) | 1.04 (0.80, 1.34) | 0.155 (0.660) |
| Model 2 ^c | Ref | 1.04 (0.80, 1.36) | 0.92 (0.70, 1.21) | 0.97 (0.74, 1.26) | 0.614 (0.819) |
| Mn | ≤10.79 | 10.80–13.96 | 13.97–17.99 | ≥18.00 | |
| <i>n</i> (case/total) | 263/758 | 296/757 | 261/757 | 270/757 | |
| Crude ^a | Ref | 1.22 (0.99, 1.51) | 1.00 (0.81, 1.23) | 1.05 (0.85, 1.30) | 0.885 (0.947) |
| Model 1 ^b | Ref | 1.46 (1.13, 1.90)* | 1.21 (0.93, 1.57) | 1.40 (1.07, 1.82)* | 0.286 (0.660) |
| Model 2 ^c | Ref | 1.48 (1.14, 1.93)* | 1.23 (0.94, 1.61) | 1.42 (1.08, 1.86)* | 0.053* (0.165) |
| Co | ≤0.14 | 0.15–0.28 | 0.29–0.39 | ≥0.40 | |
| <i>n</i> (case/total) | 314/881 | 260/665 | 276/742 | 240/741 | |
| Crude ^a | Ref | 1.16 (0.94, 1.43) | 1.07 (0.87, 1.31) | 0.87 (0.70, 1.06) | 0.009* (0.036*) |
| Model 1 ^b | Ref | 1.21 (0.94, 1.55) | 0.99 (0.77, 1.27) | 1.08 (0.84, 1.40) | 0.659 (0.820) |
| Model 2 ^c | Ref | 1.25 (0.97, 1.62) | 1.01 (0.78, 1.31) | 1.19 (0.92, 1.55) | 0.411 (0.705) |
| Ni | ≤1.26 | 1.27–2.24 | 2.25–3.96 | ≥3.97 | |
| <i>n</i> (case/total) | 283/758 | 262/764 | 282/753 | 263/754 | |
| Crude ^a | Ref | 0.88 (0.71, 1.08) | 1.01 (0.82, 1.24) | 0.90 (0.73, 1.11) | 0.208 (0.312) |
| Model 1 ^b | Ref | 0.96 (0.74, 1.24) | 1.01 (0.78, 1.31) | 0.89 (0.69, 1.15) | 0.330 (0.660) |
| Model 2 ^c | Ref | 0.97 (0.75, 1.26) | 1.02 (0.79, 1.33) | 0.89 (0.69, 1.16) | 0.477 (0.716) |
| Cu | ≤845.64 | 1845.65–945.24 | 945.25–1055.79 | ≥1055.80 | |
| <i>n</i> (case/total) | 238/758 | 248/757 | 296/757 | 308/757 | |
| Crude ^a | Ref | 1.07 (0.86, 1.32) | 1.40 (1.14, 1.73)* | 1.50 (1.21, 1.85)* | 0.168 (0.312) |
| Model 1 ^b | Ref | 0.96 (0.74, 1.25) | 1.13 (0.87, 1.46) | 1.11 (0.85, 1.44) | 0.482 (0.820) |
| Model 2 ^c | Ref | 0.93 (0.71, 1.22) | 1.10 (0.84, 1.43) | 1.13 (0.86, 1.48) | 0.245 (0.588) |
| Zn | ≤4778.14 | 4778.15–5578.83 | 5578.84–6481.80 | ≥6481.81 | |
| <i>n</i> (case/total) | 263/758 | 251/757 | 277/757 | 299/757 | |
| Crude ^a | Ref | 0.93 (0.76, 1.16) | 1.09 (0.88, 1.34) | 1.23 (1.00, 1.51)* | 0.188 (0.312) |
| Model 1 ^b | Ref | 0.89 (0.68, 1.16) | 1.02 (0.79, 1.33) | 1.16 (0.90, 1.51) | 0.569 (0.820) |
| Model 2 ^c | Ref | 0.83 (0.63, 1.08) | 1.01 (0.77, 1.32) | 1.07 (0.82, 1.39) | 0.330 (0.660) |
| As | ≤2.65 | 2.66–4.45 | 4.46–7.06 | ≥7.07 | |
| <i>n</i> (case/total) | 264/760 | 277/756 | 274/756 | 275/757 | |
| Crude ^a | Ref | 1.09 (0.88, 1.34) | 1.07 (0.87, 1.32) | 1.07 (0.87, 1.32) | 0.373 (0.448) |
| Model 1 ^b | Ref | 1.16 (0.90, 1.50) | 1.20 (0.92, 1.57) | 1.44 (1.08, 1.94)* | 0.020* (0.240) |

(Continued)

TABLE 2 (Continued)

| Whole blood metals | Quartiles of whole blood metals (μg/L) | | | | P trend (P_{FDR}^d) |
|---|--|--------------------|--------------------|--------------------|----------------------------|
| | Q1 | Q2 | Q3 | Q4 | |
| Model 2 ^c | Ref | 1.12 (0.86, 1.45) | 1.18 (0.90, 1.55) | 1.41 (1.04, 1.91)* | 0.028* (0.165) |
| Se | ≤138.60 | 138.61–165.50 | 165.51–195.45 | ≥195.46 | |
| n (case/total) | 282/759 | 266/756 | 253/757 | 289/757 | |
| Crude ^a | Ref | 0.92 (0.75, 1.13) | 0.85 (0.69, 1.05) | 1.05 (0.85, 1.29) | 0.947 (0.947) |
| Model 1 ^b | Ref | 1.03 (0.79, 1.33) | 0.90 (0.69, 1.17) | 1.23 (0.95, 1.59) | 0.756 (0.820) |
| Model 2 ^c | Ref | 1.00 (0.76, 1.30) | 0.86 (0.66, 1.13) | 1.06 (0.81, 1.39) | 0.933 (0.933) |
| Cd | ≤1.30 | 1.31–2.28 | 2.29–4.37 | ≥4.38 | |
| n (case/total) | 240/760 | 252/755 | 303/758 | 295/756 | |
| Crude ^a | Ref | 1.09 (0.88, 1.35) | 1.44 (1.17, 1.78)* | 1.39 (1.12, 1.71)* | 0.002* (0.012*) |
| Model 1 ^b | Ref | 1.04 (0.80, 1.37) | 1.08 (0.82, 1.42) | 1.25 (0.93, 1.66) | 0.273 (0.660) |
| Model 2 ^c | Ref | 1.14 (0.86, 1.51) | 1.21 (0.91, 1.61) | 1.42 (1.05, 1.91)* | 0.023* (0.165) |
| Pb | ≤26.39 | 26.40–39.50 | 39.51–59.19 | ≥59.20 | |
| n (case/total) | 191/758 | 269/757 | 303/757 | 327/757 | |
| Crude ^a | Ref | 1.64 (1.31, 2.04)* | 1.98 (1.59, 2.47)* | 2.26 (1.82, 2.81)* | 0.001* (0.012*) |
| Model 1 ^b | Ref | 1.29 (0.97, 1.71) | 1.27 (0.95, 1.69) | 1.32 (0.98, 1.76) | 0.279 (0.660) |
| Model 2 ^c | Ref | 1.26 (0.94, 1.68) | 1.27 (0.95, 1.70) | 1.38 (1.02, 1.86)* | 0.055 [†] (0.165) |
| Multiple-metal model^d | | | | | |
| Mn | Ref | 1.44 (1.11, 1.88)* | 1.18 (0.90, 1.55) | 1.35 (1.02, 1.78)* | 0.129 (0.172) |
| As | Ref | 1.11 (0.85, 1.44) | 1.18 (0.90, 1.54) | 1.34 (0.99, 1.81) | 0.062 [†] (0.156) |
| Cd | Ref | 1.12 (0.84, 1.49) | 1.15 (0.86, 1.54) | 1.33 (0.98, 1.81) | 0.078 [†] (0.156) |
| Pb | Ref | 1.21 (0.91, 1.63) | 1.19 (0.88, 1.60) | 1.24 (0.91, 1.68) | 0.263 (0.263) |

^aCrude Model was the crude odds ratios (95% CI).

^bModel 1 was adopted the general logistics regression with the region (coastal/inland) as fixed effect and adjusted for age, sex, region, education level, drinking status, smoking status, family history of hypertension and anti-hypertensive use.

^cModel 2 was additionally adjusted for BMI, TC, TG and FBG except for covariates in Model 1.

^dMetals that were significant in the single-metal model 2 were all included in multi-metal models, adjusted covariates were the same to single-metal model 2.

*P < 0.05, [†]P < 0.10 and >0.05.

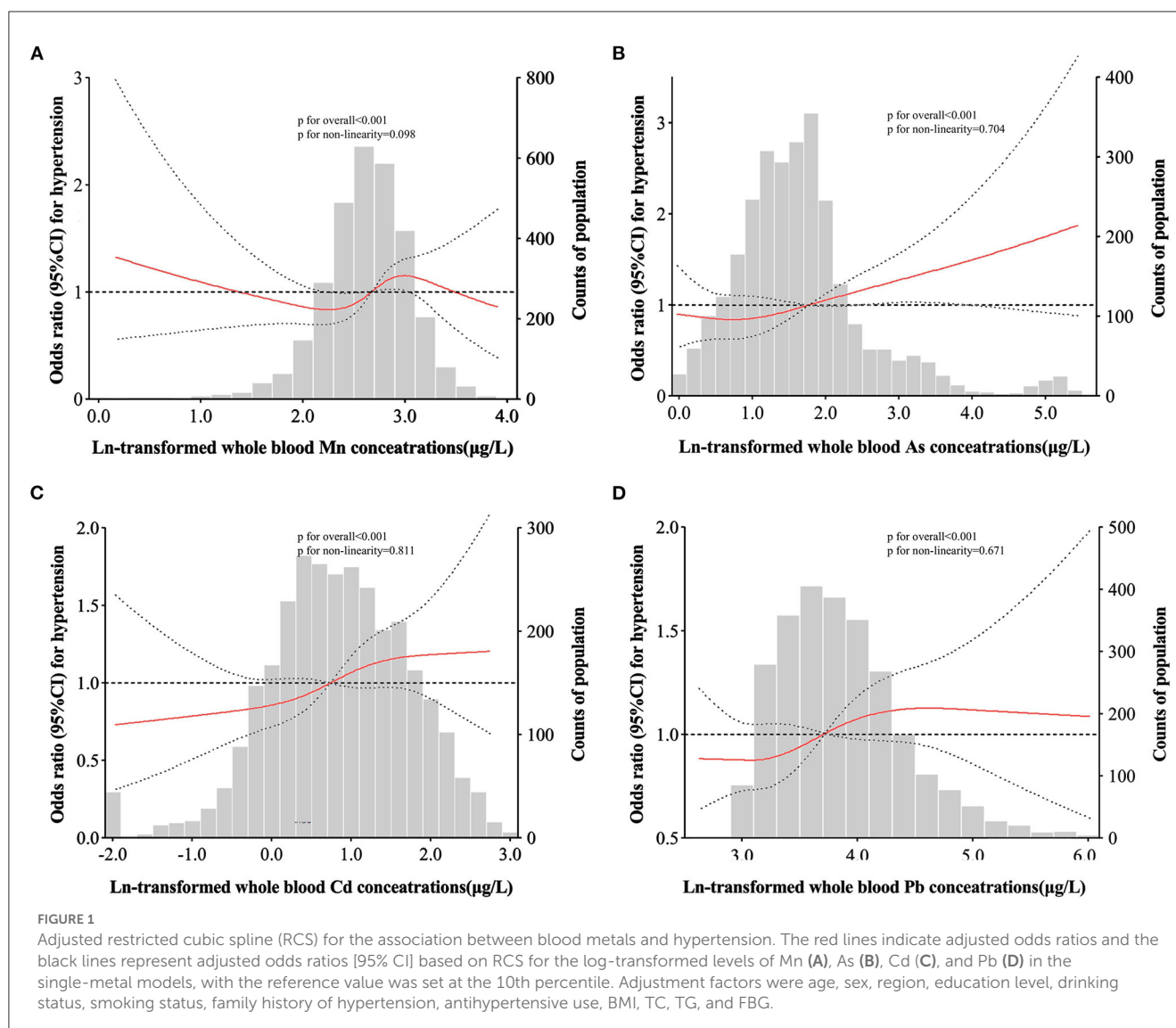
the human kidneys, liver, bones and respiratory system (11). The concentration of Cd in blood is regarded as internal exposure biomarkers, which can reflect cumulative exposure. And blood Cd with a half-life of 3–4 months is more appropriate to reflect short-term fluctuations in exposure (35, 36). Although the studies on the association of Cd exposure with hypertension are increasing, inconsistencies among studies still remain. The most common mechanisms associated with Cd exposure and the development of hypertension are similar to As (37). Cd may have direct vascular effects, it can inhibit endothelial nitric oxide synthase in blood vessels, thereby reducing endothelial relaxation by blocking cholinergic action and ultimately inducing hypertension (38). Cd may also lead to elevated vasoconstriction by increasing half-life of noradrenaline in vascular smooth muscle tissue (39, 40). Acute Cd exposure may activate the renin–angiotensin system (RAS), increase local release of angiotensin II and elevate activity of COX-2 and NADPH oxidase, and contribute to increased peripheral blood resistance with consequent genesis and maintenance of hypertension (39). Xu et al. (26) found no association between airborne Cd exposure and hypertension risk in the Sister Study.

However, a systematic review reported that a positive association between blood Cd levels and blood pressure and/or hypertension was identified, while the association of urinary Cd remained uncertain (41). In the present study, Cd in whole blood was also found to be related to increased odds of hypertension and elevated SBP and DBP level in the single-metal model, but these relationships disappeared in the multiple-metal model, which could be due to the significant correlation between whole blood Cd and Zn ($r = 0.146$). We also found a negative interaction between whole blood Cd and subjects with BMI ≥ 28 kg/m². High BMI has been reported to be associated with low level of Cd and other heavy metals (12, 18), but the relationships between Cd exposure and BMI are not consistent, which may be attributed to differences in exposure levels and the specific marker (42). Alexandre et al. (43) reported that no correlation of urine Cd level with blood pressure and hypertension in the general population of France, but a negative correlation of urine Cd with hypertension in obese subjects, chronic renal function and current smokers in the stratified model. The 1999–2014 NHANES suggested that the negative interaction between Cd exposure and

TABLE 3 Beta coefficients and 95% CI of blood pressures in generalized linear regression analysis.

| Whole blood metals | Quartiles of whole blood metals (μg/L) | | | | P trend (P ^a _{FDR}) |
|-----------------------------------|--|---------------------|---------------------|---------------------|--|
| | Q1 | Q2 | Q3 | Q4 | |
| SBP | | | | | |
| Single-metal model ^b | | | | | |
| V | Ref | 1.07 (−1.01, 3.15) | −0.65 (−2.84, 1.53) | 0.15 (−1.99, 2.29) | 0.768 (0.838) |
| Cr | Ref | −0.17 (−2.31, 1.96) | 0.40 (−1.71, 2.51) | 0.98 (0.49, 1.47)* | 0.971 (0.971) |
| Mn | Ref | 2.71 (0.66, 4.77)* | 3.52 (1.46, 5.58)* | 3.41 (1.32, 5.50)* | 0.001* (0.012*) |
| Co | Ref | 2.28 (0.22, 4.33)* | 0.28 (−1.72, 2.29) | 1.11 (−0.93, 3.15) | 0.546 (0.728) |
| Ni | Ref | 0.69 (−1.37, 2.75) | 1.05 (−1.02, 3.11) | −0.56 (−2.63, 1.50) | 0.752 (0.838) |
| Cu | Ref | −0.55 (−2.62, 1.51) | 1.65 (−0.44, 3.75) | 0.93 (−1.20, 3.06) | 0.233 (0.399) |
| Zn | Ref | −1.77 (−3.83, 0.29) | 1.33 (−0.76, 3.41) | 0.91 (−1.20, 3.03) | 0.089 [‡] (0.212) |
| As | Ref | 0.87 (−1.18, 2.93) | 0.42 (−1.67, 2.50) | 2.39 (0.09, 4.69)* | 0.060 [‡] (0.180) |
| Se | Ref | 0.38 (−1.68, 2.44) | 0.69 (−1.37, 2.76) | 1.67 (−0.42, 3.77) | 0.106 (0.212) |
| Cd | Ref | 1.71 (−0.36, 3.79) | 2.80 (0.64, 4.95)* | 2.73 (0.40, 5.05)* | 0.013* (0.064 [‡]) |
| Pb | Ref | 1.49 (−0.64, 3.61) | 2.28 (0.07, 4.50)* | 2.77 (0.48, 5.06)* | 0.016* (0.064 [‡]) |
| Multiple-metal model ^c | | | | | |
| Mn | Ref | 2.51 (0.45, 4.56)* | 3.11 (1.02, 5.19)* | 2.83 (0.71, 4.96)* | 0.008* (0.040*) |
| Zn | Ref | −2.06 (−4.13, 0.01) | 0.84 (−1.25, 2.94) | 0.18 (−1.96, 2.31) | 0.311 (0.311) |
| As | Ref | 0.76 (−1.29, 2.81) | 0.38 (−1.70, 2.47) | 1.71 (−0.61, 4.02) | 0.225 (0.281) |
| Cd | Ref | 1.29 (−0.80, 3.38) | 2.11 (−0.08, 4.31) | 1.79 (−0.62, 4.19) | 0.108 (0.180) |
| Pb | Ref | 1.11 (−1.00, 3.22) | 1.68 (−0.55, 3.92) | 1.90 (−0.45, 4.24) | 0.107 (0.180) |
| DBP | | | | | |
| Single-metal model ^b | | | | | |
| V | Ref | −0.08 (−1.37, 1.21) | −0.67 (−2.02, 0.69) | −1.00 (−2.33, 0.33) | 0.100 (0.200) |
| Cr | Ref | −0.53 (−1.85, 0.80) | −1.00 (−2.33, 0.33) | −0.08 (−1.40, 1.24) | 0.783 (0.899) |
| Mn | Ref | 1.37 (0.09, 2.65)* | 1.92 (0.63, 3.21)* | 1.92 (0.61, 3.23)* | 0.003* (0.009*) |
| Co | Ref | 0.91 (−0.38, 2.20) | −0.21 (−1.47, 1.05) | 0.33 (−0.94, 1.61) | 0.973 (0.973) |
| Ni | Ref | −0.28 (−1.57, 1.00) | 0.55 (−0.74, 1.85) | 0.19 (−1.10, 1.48) | 0.496 (0.744) |
| Cu | Ref | −0.17 (−1.45, 1.12) | 0.45 (−0.86, 1.76) | −0.42 (−1.75, 0.91) | 0.764 (0.899) |
| Zn | Ref | −0.14 (−1.43, 1.15) | 0.95 (−0.35, 2.24) | 2.09 (0.77, 3.41)* | 0.001* (0.004*) |
| As | Ref | 0.17 (−1.13, 1.46) | 0.06 (−1.25, 1.36) | 1.25 (−0.18, 2.69) | 0.144 (0.247) |
| Se | Ref | −0.20 (−1.48, 1.08) | 0.14 (−1.15, 1.42) | 1.17 (−0.14, 2.48) | 0.076 [‡] (0.182) |
| Cd | Ref | 0.98 (−0.31, 2.28) | 1.41 (0.06, 2.75)* | 2.38 (0.92, 3.83)* | 0.001* (0.004*) |
| Pb | Ref | 1.25 (−0.07, 2.56) | 2.33 (0.96, 3.70)* | 2.63 (1.21, 4.05)* | <0.001* (0.001*) |
| Multiple-metal model ^c | | | | | |
| Mn | Ref | 1.02 (−0.25, 2.30) | 1.46 (0.17, 2.76)* | 1.10 (−0.23, 2.42) | 0.081 [‡] (0.101) |
| Zn | Ref | −0.40 (−1.70, 0.89) | 0.62 (−0.70, 1.93) | 1.45 (0.10, 2.81)* | 0.013* (0.033*) |
| Se | Ref | −0.26 (−1.54, 1.02) | −0.40 (−1.70, 0.90) | 0.09 (−1.27, 1.44) | 0.979 (0.979) |
| Cd | Ref | 0.57 (−0.73, 1.87) | 0.63 (−0.74, 1.99) | 1.42 (−0.07, 2.91) | 0.079 [‡] (0.101) |
| Pb | Ref | 1.02 (−0.30, 2.33) | 1.85 (0.46, 3.25)* | 2.06 (0.59, 3.53)* | 0.004* (0.020*) |

^a False Discovery Rate (FDR) adjusted p-values.^b Model was adjusted for age, sex, region, education level, drinking status, smoking status, family history of hypertension, BMI, TC, TG, and FBG.^c Five whole blood metals were simultaneously included in generalized linear regression with adjustment for the covariates used in the single-metal models.* $P < 0.05$, [†] $P < 0.10$ and > 0.05 .

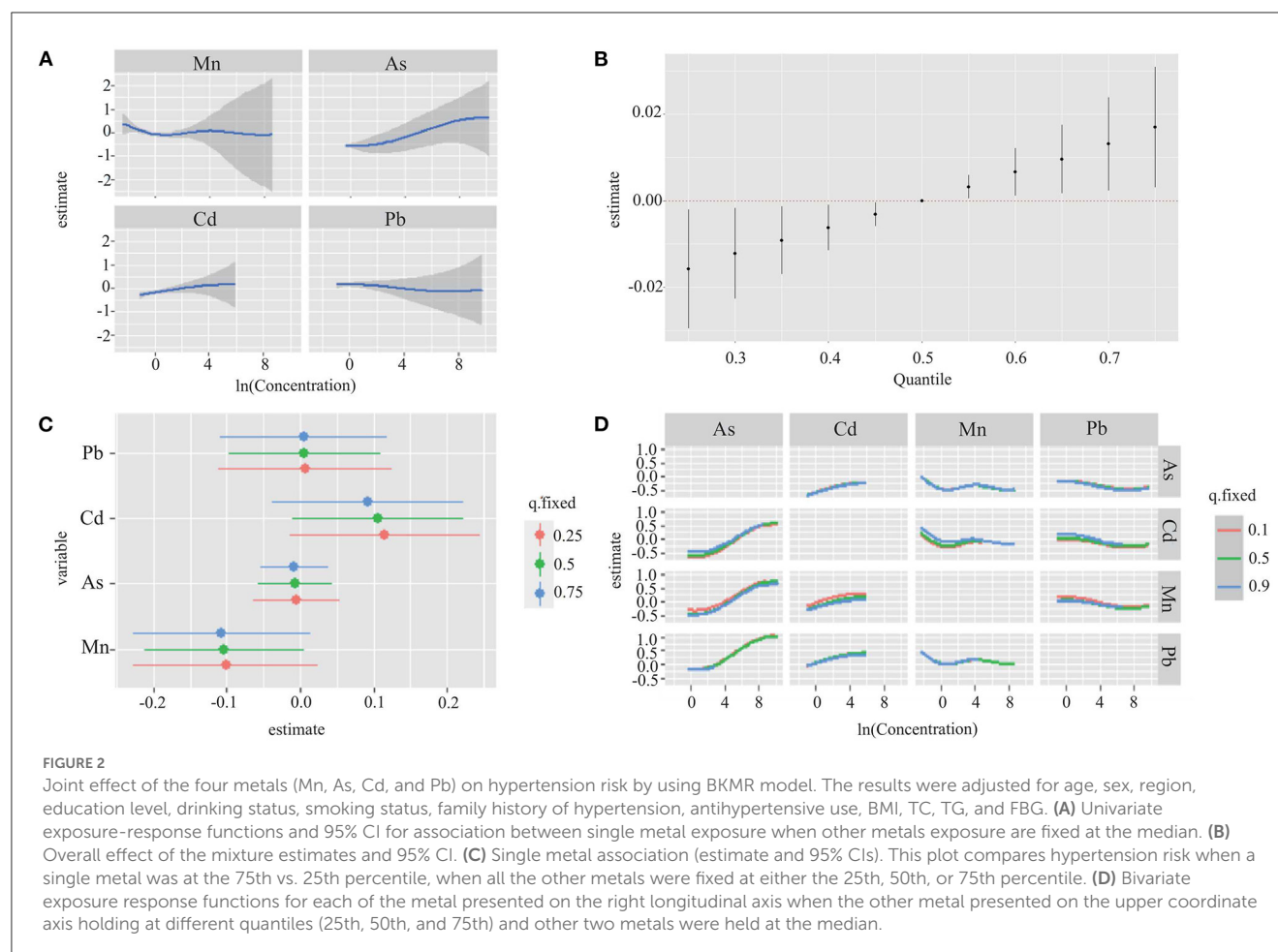


obesity influenced systolic hypertension risk (18). Therefore, the potential mechanisms underlying the interaction of obesity/BMI and Cd exposure on hypertension or blood pressure are needed to further investigation.

4.3. Lead (Pb)

Lead (Pb) is an important heavy metal that is widely used in several applications, especially in industry, but it has no biological role in humans. Exposure to Pb produces various deleterious effects on the hemopoietic, renal, reproductive and central nervous system (44). Oxidative stress is recognized as one of the key mechanism of Pb-induced toxicity. Exposure to Pb promotes oxidative stress by enhancing the production of reactive oxygen species (ROS) or reducing the activity of antioxidant enzymes (45). Beside, Pb can reduce the availability of NO, which is an endogenous catalyst of several biochemical processes and plays an key role in the regulation of cardiovascular system, and lead to

endothelial dysfunction (46). Epidemiological studies have shown that Pb exposure can increase the risk of hypertension (47–49). The 1999–2016 US NHANES demonstrated that blood lead level was not associated with hypertension [OR, 1.002 (0.983–1.021)], but positive associations of the blood Pb levels with SBP and DBP levels were found in non-Hispanic whites and non-Hispanic blacks who did not take antihypertensive, respectively (50). A cross-sectional study included 948 Brazilian adults with aged ≥ 40 showed that a positive trend and association of the blood lead level with the risk of hypertension or levels of SBP and DBP after adjusting the covariates (51). In this study, an increasing trend and association of whole blood Pb with the risk of hypertension and levels of SBP and DBP were found in single-metal model, and this trend and association of DBP level were maintained in multiple-metal model, similar to previous studies (52, 53). In subgroup analysis, compared with the Low Pb + BMI $< 28 \text{ kg/m}^2$ subgroup, the risk of hypertension in High Pb + BMI $< 28 \text{ kg/m}^2$ subgroup and High Pb + BMI $\geq 28 \text{ kg/m}^2$ subgroup increased by 173 and 77%, respectively. Significantly negative interaction between blood Pb and BMI (only



that of obesity but not overweight) was found. Conversely, Swayze et al. (54) found an interaction between high Pb levels and non-obese subjects ($\text{BMI} < 30 \text{ kg/m}^2$). This may be due to inconsistent results due to different definitions of obesity. There are several possible mechanisms by which lead exposure and obesity negatively interact with hypertension. One is that acute or chronic lead exposure does not destroy TC, but may reduce TC levels, which may be the reason why non obese individuals with high lead level have a higher risk of hypertension than obese individuals (55, 56). The Other is that Pb exposure may damage the gastrointestinal function and lead to weight loss (57). In addition, Pb exposure can reduce the basal level of cortisol and possibly reduce the risk of obesity by destroying the hypothalamic-pituitary-adrenal axis (58), which is consistent with the negative interaction between lead and obesity found in this study. However, the mechanism of interaction between lead and obesity on hypertension still needs more sufficient evidence to support.

4.4. Other metals

Arsenic (As) is a non-essential trace metal that has been classified as a human carcinogen (59). Long-term exposure to As by ingesting water, food and air pollutants may be associated with diabetes, diabetes, reproductive disorders, skin diseases, kidney

diseases, cardiovascular diseases and cancer (60). A meta-analysis included eight studies indicated a positive association of As exposure with the risk of hypertension (61). After adjusted for age, sex and smoking status, a significant relationship between As level in the hair and the increased risk of hypertension was observed [OR, 2.0 (1.2–3.3); $P < 0.05$] (62). A cohort study conducted in Spanish male adolescents suggested that urinary As was associated with a slight elevation in SBP [0.70 mmHg (0.11–1.29)], per each 50% increase in metal concentrations) and an increased risk of elevated SBP ($\geq 120 \text{ mmHg}$) [OR, 1.28 (1.04–1.56); $P < 0.05$] (63). Similarly, our findings showed that significant associations of blood As level with hypertension risk or SBP level in single-metal model after adjusting blood pressure-related covariates. In contrast, a null effect of As exposure in urinary on hypertension in a Chinese general population from a cross-sectional study was found (34). Blood As should be used as an internal exposure biomarker due to the metabolites of inorganic As could directly interact with target organs (64). The most common mechanisms underlying hypertension associated with As include oxidative stress, impaired nitric oxide (NO) signaling, inflammation, renal damage, altered vascular response to neurotransmitters and disturbed vascular muscle Ca^{2+} signaling, and interference with the renin-angiotensin system (37, 65, 66). Beside, As is a potential obesogen that adversely affect the basic metabolic functions of adipocytes by diminishing pre-adipocytes

TABLE 4 Effect of interaction between whole blood metal and BMI on hypertension risk.

| Whole blood metals (μg/L) | Odds ratios (95% CI) | | |
|---------------------------|----------------------|--------------------|-------------------------|
| | BMI | | Within strata of metals |
| | <28 | ≥28 | |
| Mn | | | |
| Lower 50% | 1.00 | 1.10 (0.90, 1.34) | 2.17 (1.56, 3.89)* |
| Upper 50% | 2.49 (1.57, 3.93)* | 1.83 (1.16, 2.90)* | 1.67 (1.04, 2.69)* |
| Within strata of BMI | 1.10 (0.90, 1.34) | 0.83 (0.44, 1.56) | |
| As | | | |
| Lower 50% | 1.00 | 1.14 (0.92, 1.41) | 1.67 (1.01, 2.74)* |
| Upper 50% | 1.69 (1.04, 2.74)* | 2.71 (1.73, 4.24)* | 2.44 (1.57, 3.80)* |
| Within strata of BMI | 1.14 (0.92, 1.42) | 1.78 (0.87, 3.67) | |
| Cd | | | |
| Lower 50% | 1.00 | 1.21 (0.97, 1.50) | 2.99 (1.87, 4.78)* |
| Upper 50% | 2.81 (1.79, 4.43)* | 1.75 (1.09, 2.82)* | 1.46 (0.91, 2.33) |
| Within strata of BMI | 1.21 (0.97, 1.51) | 0.49 (0.23, 1.02) | |
| Pb | | | |
| Lower 50% | 1.00 | 1.18 (0.95, 1.46) | 3.04 (1.91, 4.84)* |
| Upper 50% | 2.73 (1.74, 4.29)* | 1.77 (1.10, 2.84)* | 1.35 (0.85, 2.14) |
| Within strata of BMI | 1.17 (0.94, 1.45) | 0.78 (0.39, 1.56) | |

Measure of interaction on additive scale: Cd, RERI (95% CI) = -2.35 (-4.58 , -0.12); Pb, RERI (95% CI) = -1.17 (-2.28 , -0.06). Measure of interaction on multiplicative scale: Cd, ratio of ORs (95% CI) = 0.50 (0.26 , 0.95); Pb, ratio of ORs (95% CI) = 0.53 (0.28 , 1.00). Model was adjusted for age, sex, district, education level, drinking status, smoking status, family history of hypertension, anti-hypertensive use, TC, TG, FBG, and four metals in the multi-metal model.

* $P < 0.05$.

adipogenesis and increasing size of mature adipocytes (67). In our subgroup analysis, the risk of hypertension in High As + BMI ≥ 28 kg/m² subgroup was 2.71-folds higher comparing with the Low As + BMI < 28 kg/m² subgroup, additionally, whole blood As level and obesity had a synergistic effect on the risk of hypertension. A previous study has reported that measurements of body size, especially BMI, are associated with As metabolism biomarkers and this association may be related to obesity, fat free mass or body size (68). Accumulating evidence has presented that the risks of As-induced disease are significantly higher in obese individuals (69). Future epidemiological studies of As should consider BMI as a potential modifier for As-related diseases.

Zinc (Zn) is a basic chemical element for humans that widely involved in physiological processes, including protein, lipid, nucleic acid metabolism and gene transcription (70). Zn deficiency has been studied a lot, however, excess zinc may also exert toxic effects (71). Excessive Zn intake can cause oxidative stress and increase of ROS such as superoxide and peroxynitrite in the blood vessel wall, weaken the effect of vasodilator NO, which may play a role in inhibiting the increase of blood pressure by activating guanylate cyclase/cyclic guanosine monophosphate (CGMP) pathway, and ultimately lead to the increase of blood pressure (72). Currently, limited reports are available on the association between Zn and blood pressure, and the conclusions are still controversial. It may be due to differences in study subjects and internal exposure markers. A cross-sectional study included 40 obese Korean women aged 19–28 years (BMI ≥ 25 kg/m²) revealed that dietary Zn level was

negatively correlated with SBP level, but serum and urine Zn levels were not significantly related to blood pressure (73). A prospective cohort study of 1,303 adults from the Yangtze River region of China reported a significant increased trend of Zn with the risk of hypertension in multiple-metals models (10). A cross-sectional study conducted in 823 adults from the physical examination center of the Union Hospital in Wuhan found that the risk of hypertension in the highest urine Zn quartiles had a 4.2-fold (95% CI:1.7–10.0) higher compared with the lowest quartiles (34). In this study, whole blood Zn concentration of participants with hypertension was significantly higher than that of non-hypertension (5703.99 vs. 5534.83 $\mu\text{g/L}$). A positive trend and association of blood Zn with DBP levels in single- and multiple-metals models was observed, and this trend remained after FDR adjustments. In the future, large-scale clinical trials and longitudinal studies are demanded to investigate the possibility of causality between Zn exposure and blood pressure.

4.5. Advantages and limitations

This study has several strengths that could improve the robustness of our findings. First, this is a relatively large Chronic Disease and Nutrition Surveillance Survey dataset (3,029 subjects), which ensures the adequate statistical power to test the associations between multiple metals and the risk of hypertension. Secondly, the multipollutant-based statistical methods, including multivariate

logistic regression, RCS, BKMR and interaction analysis, were applied to explore the association of multiple metals with the risk of hypertension and their interaction with obesity, which could decrease the risk of misclassification bias. Third, the large sample size allowed us to adjust for many covariates including demographic characteristics, lifestyle behaviors, anthropometric features, socioeconomic status and other chronic diseases, which reduced the risk of confounding bias.

Several limitations should also be considered when interpreting the result. First, measurements of 13 metals in the same whole blood sample might increase measurement errors and the false positive rates, although FDR-method had been applied in our study. Secondly, we did not collect the information of dietary habit such as salt and sugar intake, while diet is an important factor that influences both levels of blood pressure and metals. However, the sensitivity analysis further confirmed the robustness of our findings. Third, this study was a cross-sectional study, the causal association between multiple metals and the risk of hypertension needs to be confirmed in further prospective studies.

5. Conclusions

In summary, this study indicated that higher levels of whole blood Mn, As, Cd, and Pb might be associated with the risk of hypertension, higher whole blood levels of Mn, Zn, As, Cd, and Pb might be related to elevated SBP levels, and higher whole blood levels of Mn, Zn, Se, Cd, and Pb might be associated with elevated DBP levels in the general population of South China. A significant joint effect of four metals (Mn, As, Cd, and Pb) were associated with the risk of hypertension. Moreover, the negative interactions between blood Cd, Pb levels and obesity influences the risk of hypertension. These findings extend our understanding of the relationship between exposure to multiple heavy metals and hypertension. The preventive strategies on heavy metal exposure may also contribute to the prevention of hypertension.

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 protocol of the present study was approved by the Institutional Review Board of Chinese Center for Disease Control

and Prevention (No. 201519-B), and all of subjects provided their written informed consent.

Author contributions

SW, LL, and GJ made a substantial contributions to conceptualization, methodology, formal analysis, and writing—original draft. XX was involved in data curation. JL, AM, YW, DZ, and HH completed the methodology and data collection. WM contributed to the review, editing, and supervision. BW made contributions to project and resource management. MD, TL, and QC have made considerable contributions to funding acquisition, supervision, writing review, editing, and project administration. 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.2023.1090935/full#supplementary-material>

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Association of blood cadmium with all-cause and cause-specific mortality in patients with hypertension

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Background: Cadmium is a commonly found heavy metal with a prolonged biological half-life, which results in long-term health burden for the population. Prior studies have demonstrated an association between blood cadmium and hypertension. However, few studies examined the relationship between blood cadmium and long-term health outcomes in patients with hypertension. This study aimed to investigate the association of blood cadmium with mortality in patients with hypertension.

Methods: This study analyzed data from the National Health and Nutrition Examination Survey 1999–2012. Complex sampling-weighted multivariate Cox proportional hazards models were used to evaluate the hazard ratios (HRs) of all-cause, cardiovascular, and Alzheimer's disease mortality in patients with hypertension classified by blood cadmium concentrations' quantiles.

Results: The study included 12,208 patients with hypertension with a median follow-up duration of 10.8 years. During this period, there were 4,485 all-cause deaths, including 1,520 cardiovascular deaths and 180 Alzheimer's disease deaths. Compared with the lowest quintile of blood cadmium ($\leq 0.25 \mu\text{g/L}$) group, the highest quintile of blood cadmium ($\geq 0.80 \mu\text{g/L}$) group's adjusted HRs were 1.85 (95% CI, 1.59–2.14) for all-cause mortality, 1.76 (95% CI, 1.33–2.34) for cardiovascular mortality, and 3.41 (95% CI, 1.54–7.51) for Alzheimer's disease mortality. Additionally, the adjusted HR for cardiovascular mortality was 2.12 (95% CI, 1.36–3.30) in never-smoking patients with hypertension.

Conclusion: Higher blood cadmium is associated with increased risks of all-cause, cardiovascular, and Alzheimer's disease mortality in patients with hypertension. The effect of blood cadmium on cardiovascular mortality may be more pronounced in never-smoking hypertensive patients.

KEYWORDS

blood cadmium, mortality, cardiovascular risk, hypertension, NHANES

Introduction

Cadmium is a common toxic heavy metal that is widely present in the environment. It has an extremely long biological half-life in both the environment (approximately 10–30 year) and the human body (greater than 20 years), leading to a long-term health burden (1–3). The exposure of cadmium mainly occurs through industrial production (e.g., mining, metallurgical

industries, and manufacturers of nickel-Cd batteries) (4), tobacco smoking, ambient air (5), and foods (6). Although exposure levels have decreased in recent years, cadmium still has a significant impact on various health status, including cardiovascular diseases, diabetes, cognitive dysfunction, Alzheimer's disease, kidney injury, arthritis, and cancer (3, 7–12). Since the 1950s, multiple studies have suggested an association between cadmium and blood pressure and hypertension (13–17). A recent meta-analysis reconfirmed the positive association between blood cadmium and hypertension (18). In addition, some studies have shown the effect of cadmium exposure on mortality (19–26). Previous studies mostly focus on the general population (20, 22–24). And several studies have focused on cadmium-contaminated areas (25, 26). However, few studies have investigated the association between blood cadmium and long-term health outcomes in high-risk populations who had already suffered from related diseases (e.g., patients with hypertension). There is an urgent need to generate more scientific evidence on the relationship between cadmium exposure and mortality in high-risk populations to raise public awareness of cadmium exposure reduction. Therefore, this study aimed to evaluate the association of blood cadmium with all-cause and cause-specific mortality in patients with hypertension, which may provide reference for the long-term adverse health effects of cadmium exposure, especially in high-risk population.

Methods

Study participants

The National Health and Nutrition Examination Survey (NHANES) is a program designed to assess the health and nutritional status of the US population (27). The US National Center for Health Statistics (NCHS) is responsible for producing vital and health statistics by using a stratified, multi-stage probability sampling design that ensures participants are representative of the civilian deinstitutionalized population of the United States (27). The NHANES protocol has been approved by the NCHS Research Ethics Review Board, and written informed consent has been obtained from each participant (28).

For this study, we obtained data on NHANES participants between 1999 and 2012. Since there were few mortality-related cases under the age of 40, we focused on participants aged 40 years or older with hypertension at baseline, resulting in a total of 14,664 participants. Hypertension was defined as measured systolic blood pressure (SBP) ≥ 140 mmHg or/and diastolic blood pressure (DBP) ≥ 90 mmHg, or/and previous diagnosis of hypertension, or/and taking antihypertensive prescription. We excluded patients with missing information on blood cadmium ($n = 1,354$), missing blood pressure data ($n = 428$), lacking follow-up data ($n = 11$), and missing information on potential confounding variables ($n = 663$). This left us with a final sample size of 12,208 participants for analysis (Figure 1).

Abbreviations: HR, Hazard ratio; CI, Confidence interval; NHANES, National Health and Nutrition Examination Survey; NCHS, National Center for Health Statistics; SBP, Systolic blood pressure; DBP, Diastolic blood pressure; ICD-10, International Classification of Diseases, 10th revision; BMI, Body mass index; eGFR, Estimated glomerular filtration rate; SE, Standard error; Ref, Reference.

Measurement of blood cadmium

Whole blood specimens are processed, stored, and shipped to the Division of Laboratory Sciences, National Center for Environmental Health, and Centers for Disease Control and Prevention for analysis. Blood cadmium was determined using a PerkinElmer Model SIMAA 6000 simultaneous multi-element atomic absorption spectrometer with Zeeman background correction in NHANES 1999–2002 and using inductively coupled plasma mass spectrometry in NHANES 2003–2012 (27).

The extremely high and low values were verified by NCHS staff carefully, and numerous consistency checks were performed. The limit of detection (LOD) for blood cadmium was 0.1–0.2 $\mu\text{g/L}$. Blood cadmium below LOD was replaced with a value equal to LOD divided by the square root of two. Blood cadmium below LOD were found in 12% of participants overall ($n = 1,488$), which did not affect grouping according to quintile.

Outcome ascertainment

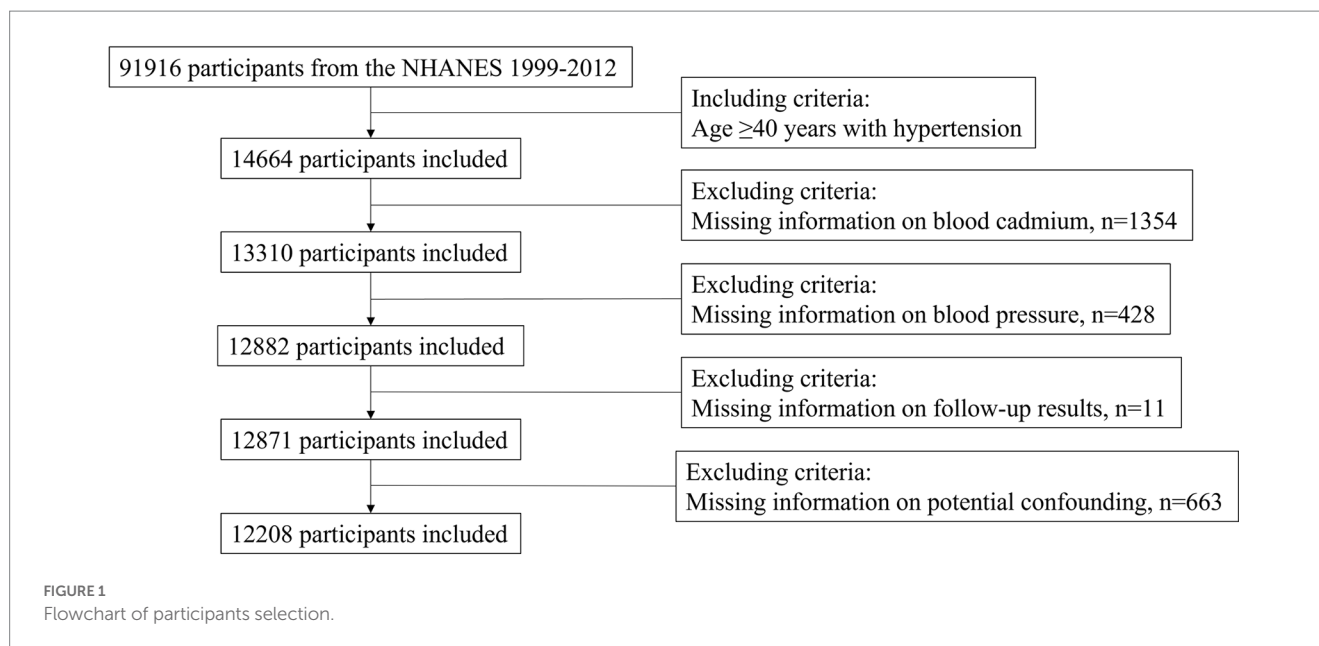
The NHANES linked mortality data used in this study were updated with follow-up data through December 31, 2019 (29). All-cause death was defined as death from any cause. According to the International Classification of Diseases, 10th revision (ICD-10), cardiovascular death was defined as death due to diseases of heart (I00–I09, I11, I13, and I20–I51) and cerebrovascular diseases (I60–I69) (30). Alzheimer's disease death was defined as death due to Alzheimer's disease (G30).

Covariate assessment

The following variables were obtained through the interview questionnaire: age, sex, race/ethnicity, educational level, smoking status, medical conditions (including hypertension, diabetes, asthma, heart failure, coronary heart disease, stroke, emphysema, chronic bronchitis, liver condition, and cancer), and antihypertensive medication use. The following variables were measured according to standard protocols: body mass index (BMI; calculated as weight in kilograms divided by height in meters squared), blood pressure, total cholesterol, triglycerides, high density lipoprotein, serum creatinine, uric acid, and cotinine. The estimated glomerular filtration rate (eGFR) was calculated by the Chronic Kidney Disease Epidemiology Collaboration equation (31). Obese was defined as BMI ≥ 30 kg/m². Optimal blood pressure was defined as SBP < 140 mmHg and DBP < 90 mmHg.

Statistical analysis

Given that the NHANES data used in this study were not simple random samples, appropriate weights were used for each analysis based on the selected variables, as recommended by the US Centers for Disease Control and Prevention (32). Continuous variables were expressed as mean \pm standard error (mean \pm SE), and comparisons between groups were made by analysis of variance. Categorical variables were expressed as percentages, and comparisons between



groups were made by the chi-square test. Complex sampling-weighted multivariate Cox models were applied to investigate the hazard ratio (HR) and 95% confidence interval (CI) between blood cadmium and mortality. Model 1 was adjusted for age, sex, race/ethnicity, and education level. Model 2 was further adjusted for BMI, eGFR, SBP, DBP, total cholesterol, triglycerides, high-density lipoprotein, and disease conditions (heart failure, coronary heart disease, stroke, diabetes, and cancer). Model 3 was further adjusted for serum cotinine and smoking status (never, former, or current smoker). Based on model 3, subgroup analyses were stratified by gender, age, BMI, blood pressure control, eGFR, and smoking status. Interactions between blood cadmium (lowest and highest quintiles) and stratification factors were assessed. Restricted cubic spline regression was used to explore the dose–response association of blood cadmium and mortality. Furthermore, we performed sensitivity analysis by using the random forest imputation method to interpolate values of blood cadmium below LOD and missing variables. All statistical analyses were performed using R version 4.1.2 (R Project for Statistical Computing). $p < 0.05$ was regarded as statistically significant for all tests.

Results

Baseline characteristics

In this study, a total of 12,208 patients with hypertension aged 40 years or older were included. During a median follow-up of 10.8 years (interquartile range [IQR], 7.9–14.4), 4,485 all-cause deaths occurred, including 1,520 cardiovascular deaths and 180 Alzheimer's disease deaths. The range and percentage of blood cadmium below the limit of detection were 0.1–0.2 $\mu\text{g/L}$ and 12.2%, respectively. The baseline characteristics of the patients grouped according to blood cadmium quintiles were listed in Table 1. Participants with higher blood cadmium levels were more likely to be older, female, non-Hispanic black, non-obese, and current smokers. They also had

lower education levels, lower triglycerides, lower eGFR, lower DBP, and higher SBP, higher total cholesterol, and higher high-density lipoprotein levels. In addition, participants with higher blood cadmium levels had a lower prevalence of diabetes but a higher prevalence of cardiovascular diseases (heart failure, coronary heart disease, and stroke).

Association of blood cadmium with mortality

Table 2 presented survey-weighted Cox regression results. The multivariate adjusted model 3 showed that blood cadmium was positively associated with all-cause, cardiovascular, and Alzheimer's disease mortality in participants. Compared with participants in the lowest quintile ($\leq 0.25 \mu\text{g/L}$) of blood cadmium, participants in the highest quintile ($\geq 0.8 \mu\text{g/L}$) had HRs of 1.85 (95% CI, 1.59–2.14) for all-cause mortality, 1.76 (95% CI, 1.33–2.34) for cardiovascular mortality, and 3.41 (95% CI, 1.54–7.51) for Alzheimer's disease mortality. Moreover, the effect of blood cadmium on mortality may be in a dose-dependent manner.

Dose–response curves for blood cadmium and mortality

The restricted cubic spline analysis showed a dose–response association between blood cadmium and all-cause, cardiovascular, and Alzheimer's disease mortality (Figure 2), which was consistent with the weighted Cox regression results. We observed a non-linear association for all-cause ($p < 0.001$, Nonlinear $p < 0.001$) and cardiovascular mortality ($p < 0.001$, Nonlinear $p = 0.027$), with adjusted HRs steeper at lower blood cadmium concentrations than at higher concentrations. For Alzheimer's disease mortality, the association was close to a linear correlation ($p = 0.055$, Nonlinear $p = 0.863$).

TABLE 1 Participants baseline demographic and clinical characteristics.

| Characteristic | Total | Blood cadmium ($\mu\text{g/L}$) | | | | | <i>p</i> value | <i>p</i> value for trend |
|------------------------------------|-------------------|-----------------------------------|------------------------|-----------------------|------------------------|---------------------------|----------------|--------------------------|
| | | Quintile 1 (≤ 0.25) | Quintile 2 (0.26–0.38) | Quintile 3 (0.39–0.5) | Quintile 4 (0.51–0.79) | Quintile 5 (≥ 0.8) | | |
| Age, years | 60.70 \pm 0.18 | 56.76 \pm 0.28 | 60.62 \pm 0.28 | 63.22 \pm 0.34 | 64.29 \pm 0.39 | 59.89 \pm 0.33 | <0.001 | <0.001 |
| Gender, % | | | | | | | | |
| Male | 47.4 | 60.4 | 47.3 | 40.6 | 39.2 | 46 | <0.001 | <0.001 |
| Female | 52.6 | 39.6 | 52.7 | 59.4 | 60.8 | 54 | <0.001 | <0.001 |
| Race, % | | | | | | | | |
| Mexican American | 4.6 | 5 | 4.8 | 4.9 | 4.6 | 3.2 | 0.001 | 0.003 |
| Other hispanic | 3.9 | 3.9 | 4.6 | 5.1 | 3.1 | 2.6 | 0.004 | 0.03 |
| Non-hispanic white | 75.3 | 76.5 | 76.2 | 75.9 | 76.9 | 70.6 | <0.001 | 0.006 |
| Non-hispanic black | 11.8 | 11.5 | 10.9 | 9.9 | 11 | 15.7 | <0.001 | <0.001 |
| Other race | 4.5 | 3 | 3.5 | 4.2 | 4.3 | 8 | <0.001 | <0.001 |
| Education level, % | | | | | | | | |
| Less than high school | 22.8 | 14.8 | 19.2 | 23.8 | 25 | 33.7 | <0.001 | <0.001 |
| At least high school | 77.2 | 85.2 | 80.8 | 76.2 | 75 | 66.3 | <0.001 | <0.001 |
| Smoking status, % | | | | | | | | |
| Current | 17.1 | 1.2 | 2.1 | 5.7 | 18.1 | 65.6 | <0.001 | <0.001 |
| Former | 34.8 | 29.9 | 36.5 | 42.5 | 46.1 | 21.3 | <0.001 | 0.08 |
| Never | 48 | 69 | 61.4 | 51.8 | 35.8 | 13.2 | <0.001 | <0.001 |
| Cotinine, ng/ml | 51.90 \pm 1.68 | 12.07 \pm 2.05 | 13.59 \pm 1.70 | 17.95 \pm 1.76 | 51.00 \pm 3.37 | 182.80 \pm 4.22 | <0.001 | <0.001 |
| Body mass index, kg/m ² | 30.00 \pm 0.09 | 31.75 \pm 0.19 | 30.63 \pm 0.18 | 29.77 \pm 0.16 | 29.28 \pm 0.17 | 28.00 \pm 0.15 | <0.001 | <0.001 |
| Body mass index, % | | | | | | | | |
| <30 | 56.9 | 47.1 | 52.5 | 58.7 | 61.1 | 68.2 | <0.001 | <0.001 |
| ≥ 30 | 43.1 | 52.9 | 47.5 | 41.3 | 38.9 | 31.8 | <0.001 | <0.001 |
| Systolic blood pressure, mmHg | 136.71 \pm 0.28 | 133.56 \pm 0.43 | 135.24 \pm 0.50 | 139.49 \pm 0.51 | 138.06 \pm 0.61 | 137.97 \pm 0.66 | <0.001 | <0.001 |
| Diastolic blood pressure, mmHg | 73.70 \pm 0.27 | 75.21 \pm 0.41 | 73.99 \pm 0.39 | 73.15 \pm 0.44 | 72.15 \pm 0.57 | 73.38 \pm 0.52 | <0.001 | <0.001 |
| Total Cholesterol, mmol/L | 5.27 \pm 0.02 | 5.19 \pm 0.03 | 5.23 \pm 0.03 | 5.33 \pm 0.03 | 5.31 \pm 0.03 | 5.31 \pm 0.04 | 0.015 | 0.005 |
| Triglycerides, mmol/L | 1.94 \pm 0.02 | 2.07 \pm 0.05 | 1.97 \pm 0.04 | 1.85 \pm 0.04 | 1.85 \pm 0.04 | 1.92 \pm 0.04 | 0.003 | <0.001 |
| High density lipoprotein, mmol/L | 1.37 \pm 0.43 | 1.28 \pm 0.38 | 1.36 \pm 0.40 | 1.40 \pm 0.43 | 1.44 \pm 0.45 | 1.39 \pm 0.48 | <0.001 | <0.001 |
| Creatinine, $\mu\text{mol/L}$ | 83.69 \pm 0.52 | 82.71 \pm 0.54 | 82.96 \pm 0.78 | 81.47 \pm 1.18 | 85.28 \pm 1.27 | 86.89 \pm 1.69 | 0.033 | 0.002 |
| eGFR, ml/min/1.73m ² | 81.94 \pm 0.34 | 85.26 \pm 0.50 | 81.11 \pm 0.56 | 80.64 \pm 0.64 | 78.28 \pm 0.70 | 83.19 \pm 0.67 | <0.001 | <0.001 |
| eGFR, % | | | | | | | | |
| <60 | 15.4 | 10 | 14.4 | 16.7 | 20.9 | 16.9 | <0.001 | <0.001 |
| ≥ 60 | 84.6 | 90 | 85.6 | 83.3 | 79.1 | 83.1 | <0.001 | <0.001 |
| Diabetes, % | 16.1 | 20.1 | 16.4 | 14.4 | 14.7 | 13.7 | <0.001 | <0.001 |
| Heart failure, % | 5.2 | 3.3 | 4.3 | 5.1 | 7.4 | 7 | <0.001 | <0.001 |
| Coronary heart disease, % | 7.6 | 5.7 | 6.9 | 8.6 | 8.8 | 8.8 | 0.001 | <0.001 |
| Stroke, % | 5.8 | 3.1 | 5.7 | 5.4 | 7.5 | 8.2 | <0.001 | <0.001 |
| Cancer, % | 15 | 13.3 | 13.8 | 16.3 | 18.5 | 13.9 | <0.001 | 0.045 |

eGFR, estimated glomerular filtration rate.

Continuous variables are expressed as means and standard error and categorical variables as percentages. Means and percentages are weighted.

Linear trends across categories of blood cadmium quintiles were assessed with linear regression for continuous variables and chi-square tests for categorical variables.

Subgroup analysis

The results of subgroup analysis according to multivariate adjusted model 3 showed that the significant association between

blood cadmium and mortality still existed in most subgroups ([Supplementary Table 1](#)). The adjusted HRs of the highest quintile of blood cadmium compared with the lowest quintile of blood cadmium and interactions in subgroups were shown in [Figure 3](#). For all-cause

TABLE 2 Hazard ratios for all-cause, cardiovascular and Alzheimer's disease mortality of all participants, stratified by blood cadmium.

| Outcomes | Blood cadmium (µg/L) | | | | | <i>p</i> value for trend |
|-------------------------------|----------------------|------------------------|-----------------------|------------------------|-------------------|--------------------------|
| | Quintile 1 (≤0.25) | Quintile 2 (0.26–0.38) | Quintile 3 (0.39–0.5) | Quintile 4 (0.51–0.79) | Quintile 5 (≥0.8) | |
| All-cause mortality | | | | | | |
| Unadjusted HR | 1 [Ref] | 1.41(1.22–1.63) | 1.85(1.59–2.15) | 2.55(2.22–2.93) | 2.86(2.48–3.30) | <0.001 |
| <i>p</i> value | | <0.001 | <0.001 | <0.001 | <0.001 | |
| Model 1 HR | 1 [Ref] | 1.10(0.95–1.28) | 1.19(1.04–1.35) | 1.44(1.26–1.65) | 2.37(2.07–2.70) | <0.001 |
| <i>p</i> value | | 0.192 | 0.009 | <0.001 | <0.001 | |
| Model 2 HR | 1 [Ref] | 1.12(0.97–1.30) | 1.25(1.11–1.41) | 1.50(1.31–1.72) | 2.41(2.11–2.75) | <0.001 |
| <i>p</i> value | | 0.119 | <0.001 | <0.001 | <0.001 | |
| Model 3 HR | 1 [Ref] | 1.09(0.94–1.26) | 1.18(1.05–1.34) | 1.33(1.16–1.53) | 1.85(1.59–2.14) | <0.001 |
| <i>p</i> value | | 0.259 | 0.006 | <0.001 | <0.001 | |
| Cardiovascular mortality | | | | | | |
| Unadjusted HR | 1 [Ref] | 1.47(1.10–1.98) | 1.93(1.50–2.49) | 2.48(1.92–3.21) | 2.52(1.96–3.25) | <0.001 |
| <i>p</i> value | | 0.01 | <0.001 | <0.001 | <0.001 | |
| Model 1 HR | 1 [Ref] | 1.11(0.82–1.49) | 1.15(0.92–1.45) | 1.27(0.99–1.64) | 1.97(1.55–2.52) | <0.001 |
| <i>p</i> value | | 0.507 | 0.224 | 0.064 | <0.001 | |
| Model 2 HR | 1 [Ref] | 1.11(0.83–1.50) | 1.23(0.98–1.53) | 1.35(1.05–1.73) | 2.06(1.61–2.64) | <0.001 |
| <i>p</i> value | | 0.473 | 0.071 | 0.019 | <0.001 | |
| Model 3 HR | 1 [Ref] | 1.09(0.81–1.48) | 1.19(0.96–1.49) | 1.26(0.97–1.63) | 1.76(1.33–2.34) | <0.001 |
| <i>p</i> value | | 0.556 | 0.119 | 0.083 | <0.001 | |
| Alzheimer’s disease mortality | | | | | | |
| Unadjusted HR | 1 [Ref] | 2.94(1.40–6.19) | 4.51(2.39–8.50) | 5.10(2.67–9.74) | 4.12(2.21–7.70) | <0.001 |
| <i>p</i> value | | 0.005 | <0.001 | <0.001 | <0.001 | |
| Model 1 HR | 1 [Ref] | 2.02(0.96–4.24) | 2.20(1.14–4.25) | 1.92(0.98–3.77) | 2.98(1.50–5.92) | 0.014 |
| <i>p</i> value | | 0.124 | 0.026 | 0.077 | 0.003 | |
| Model 2 HR | 1 [Ref] | 1.99(0.94–4.19) | 2.11(1.07–4.13) | 1.83(0.94–3.55) | 2.86(1.43–5.74) | 0.021 |
| <i>p</i> value | | 0.071 | 0.03 | 0.075 | 0.003 | |
| Model 3 HR | 1 [Ref] | 2.02(0.96–4.27) | 2.18(1.10–4.29) | 1.98(1.00–3.90) | 3.41(1.54–7.51) | 0.014 |
| <i>p</i> value | | 0.066 | 0.025 | 0.049 | 0.002 | |

HR, hazard ratio; Ref, reference; eGFR, estimated glomerular filtration rate; SBP, systolic blood pressure; and DBP, diastolic blood pressure.

Model 1: adjusted for age, sex, race/ethnicity, and education level.

Model 2: adjustments for model 1 plus BMI, eGFR, SBP, DBP, total cholesterol, triglycerides, high-density lipoprotein, and disease conditions (heart failure, coronary heart disease, stroke, diabetes, and cancer).

Model 3: adjustments for model 2 plus serum cotinine and smoking status (never, former, or current smoker).

p value for trend was obtained from Cox proportional hazards models with blood cadmium quintiles as a continuous variable.

Statistically significant HR and *p*-values were shown in bold.

mortality, there was a significant interaction between age and blood cadmium ($p = 0.048$). The HR of the highest blood cadmium quintile compared to the lowest blood cadmium quintile in middle-aged (40–59 years) participants was 2.64 (95% CI, 1.72–4.06), while the HR of older participants (≥ 60 years) was only 1.66 (95% CI, 1.44–1.90). For cardiovascular mortality, there was no statistical significance in the Cox regression results of the blood cadmium quintiles of the current smokers (the highest quintile of blood cadmium, HR 1.18 [95% CI, 0.21–6.76]) or former smokers (the highest quintile of blood cadmium, HR 1.31 [95% CI, 0.83–2.08]). But blood cadmium was significantly associated with an increased risk of cardiovascular mortality in never-smoking participants (the

highest quintile of blood cadmium, HR 2.12 [95% CI, 1.36–3.30]). For Alzheimer's disease mortality, the HR of the highest blood cadmium quintile was higher in non-obese participants (HR 3.91 [95% CI, 1.71–8.97]) compared to obese participants (HR 2.31 [95% CI, 0.31–17.14]).

Association of blood cadmium with mortality in sensitivity analysis

As shown in [Supplementary Figure 1](#), triglycerides exhibited the highest proportion of missing data (11.26%, $n = 1,650$). Therefore,

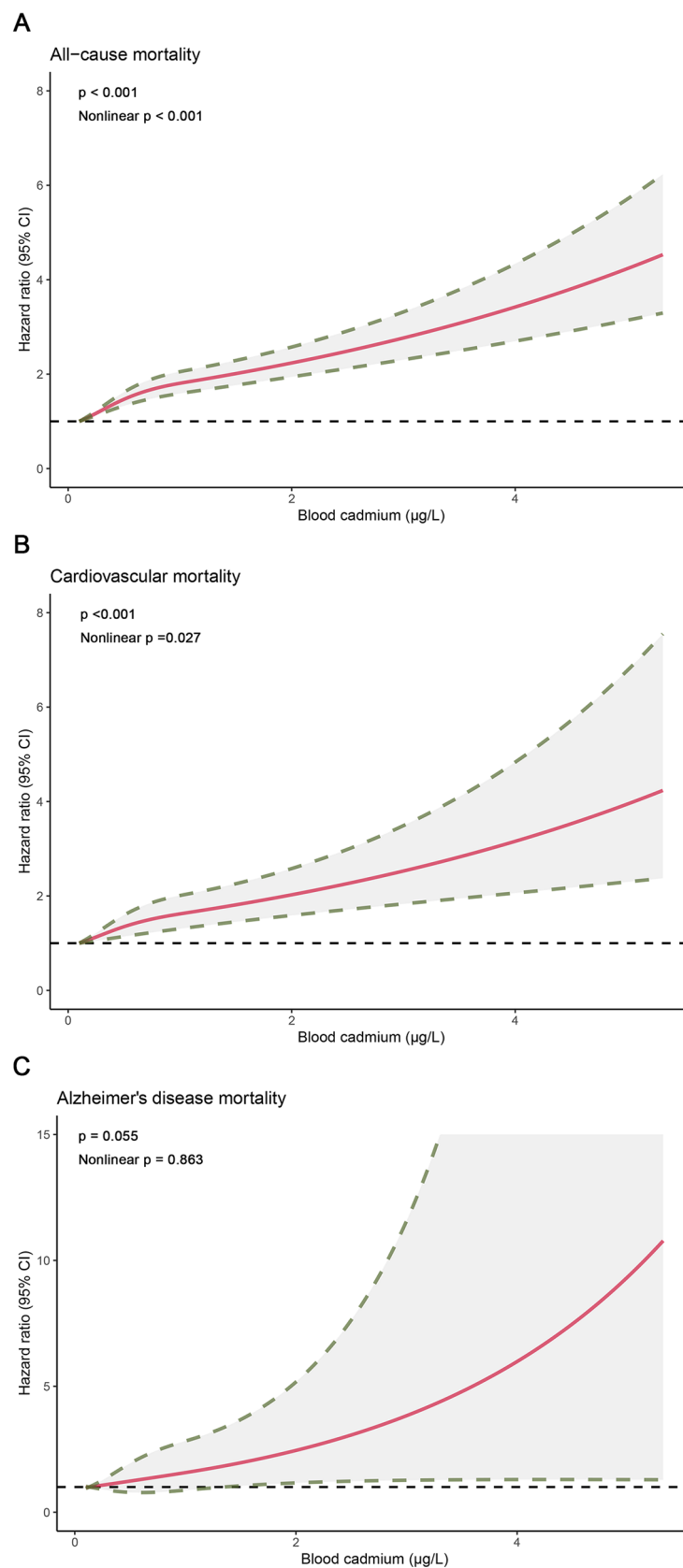


FIGURE 2
Dose-response curves for concentrations of blood cadmium and mortality. **(A)** All-cause mortality. **(B)** Cardiovascular mortality. **(C)** Alzheimer's disease mortality.

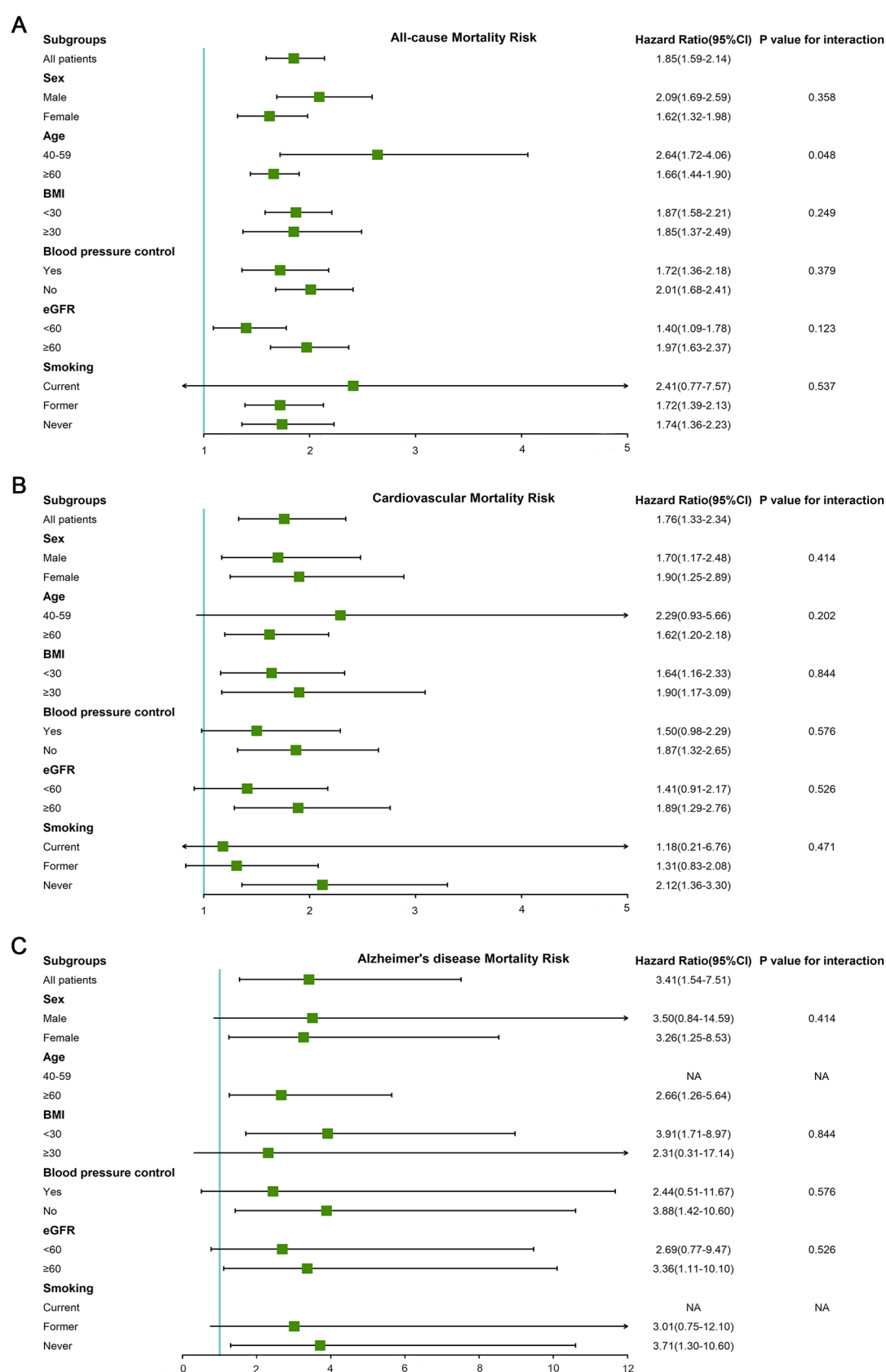


FIGURE 3

The adjusted HRs of the highest quintile of blood cadmium compared with the lowest quintile of blood cadmium in subgroups (gender, age, BMI, blood pressure control, eGFR, and smoking status). (A) All-cause mortality. (B) Cardiovascular mortality. (C) Alzheimer's disease mortality.

we conducted imputation for blood cadmium below LOD and missing variables. [Supplementary Table 2](#) presented a similar positive association of blood cadmium with all-cause, cardiovascular and Alzheimer's disease mortality. Compared to participants in the lowest quintile ($\leq 0.25 \mu\text{g/L}$) of blood cadmium, those in the highest quintile ($\geq 0.8 \mu\text{g/L}$) had the HRs of 1.76 (95%CI, 1.54–2.01) for all-cause mortality, 1.82 (95%CI, 1.44–2.31) for cardiovascular mortality and 2.57 (95%CI, 1.33–4.98) for Alzheimer's disease mortality. [Supplementary Figure 2](#) demonstrated the significant dose–response association of blood cadmium with all-cause and cardiovascular mortality after the interpolation.

Discussion

Our study suggests that blood cadmium levels are linked to higher risks of all-cause, cardiovascular, and Alzheimer's mortality in patients with hypertension, even after adjusting for smoking status and cotinine concentrations. Moreover, a positive dose–response curve was observed between blood cadmium and mortality. Notably, in patients with low blood cadmium levels, the risks associated with elevated blood cadmium were even more pronounced. Furthermore, the effect of blood cadmium on cardiovascular mortality was more significant in never-smoking patients with hypertension than in smoking patients with hypertension.

Many previous studies have investigated the association of blood cadmium and mortality. Nawrot et al. (33) found that blood cadmium was associated with all-cause and non-cardiovascular mortality in a population with relatively high cadmium exposure. Several NHANES studies have suggested that blood cadmium is associated with all-cause, cardiovascular, and cancer mortality in the US general population (19, 34, 35). Similar results were also observed among patients with type 2 diabetes (36). Moreover, a few studies have suggested that higher blood cadmium is associated with higher mortality from influenza and pneumonia, as well as Alzheimer's disease (37, 38). Our study also found a positive correlation between blood cadmium and mortality in a hypertensive population. Studies among the US general population suggest that the effect of blood cadmium in increasing the risk of cardiovascular death is more significant in smokers than in non-smokers (19, 34). However, our study among a hypertensive population found that the effect of blood cadmium in increasing the risk of cardiovascular death was more pronounced in participants who never smoked. This may be related to the different effects of blood cadmium on blood pressure in patients with different smoking status. Based on the 1999–2004 NHANES, Tellez-Plaza et al. proposed that blood cadmium is associated with increased blood pressure, and this association is mainly reflected in never-smokers.

Additionally, the dose–response curves in our study showed that the increased risk of cardiovascular mortality was more pronounced with the increase in blood cadmium at lower levels of exposure. Furthermore, the blood cadmium level of never-smokers was significantly lower than that of smokers. This finding suggests that the cardiovascular mortality risk of never-smoking patients with hypertension is more susceptible to the influence of blood cadmium than smoking patients with hypertension. However, more precisely designed prospective studies are needed to confirm this finding. It is recommended that patients with hypertension should reduce

cadmium exposure as much as possible, and even non-smokers may benefit from reducing cadmium exposure from various dietary and environmental sources.

Our results also showed that higher blood cadmium had lower prevalence of diabetes, which are contrary to previous study (8). Possible explanations are as follows: Urban residents have a higher burden of cadmium pollution compared to rural residents, but they have a lower incidence of diabetes than rural residents (39, 40). The mean value \pm SE of blood cadmium in our study was $0.57 \pm 0.01 \mu\text{g/L}$, which was much lower than the blood cadmium biological threshold limit value for non-occupational exposure recommended by the World Health Organization.¹ The cadmium exposure in our study may not be high enough to induce overt diabetes (41).

Blood cadmium has been linked to increased risk of cardiovascular mortality, possibly due to its effects on blood pressure and atherosclerosis. Studies have suggested that cadmium may cause oxidative stress (42), activate calcium channels (43), inhibit nitric oxide (44), and vascular tissue damage by leading to endothelial dysfunction (45). Additionally, there is evidence to suggest that cadmium-induced oxidative stress may lead to renal injury, which in turn indirectly affects the cardiovascular system (10). Moreover, cadmium may play a role in the formation of amyloid plaques and neuronal damage, potentially contributing to Alzheimer's disease mortality risk. Given that hypertension is a risk factor for Alzheimer's disease, blood cadmium may indirectly affect Alzheimer's disease through its impact on blood pressure. However, the mechanism behind the increased mortality risk associated with blood cadmium exposure requires further investigation. It is recommended that further studies be conducted to clarify the precise mechanisms involved and identify effective preventive measures.

Although this is the first study to investigate the effect of blood cadmium on the mortality of patients with hypertension, we acknowledge several limitations. Blood cadmium was measured only once, which may not accurately reflect long-term blood cadmium levels. Moreover, the low proportion of blood cadmium below the detection limit still affects the accuracy of the dose–response curve. Additionally, outcome variables identified by death certificates may not fully reflect the exact cause of death. Finally, this study is an observational study, and despite correction for multiple confounding variables, there is still a risk of confounding bias. Nonetheless, this study has several strengths, including a large sample size and a long follow-up time. The Cox model fully adjusted for many traditional risk factors, as well as smoking status and cotinine concentration.

Conclusion

Our study found that higher blood cadmium levels are associated with an increased risk of all-cause, cardiovascular, and Alzheimer's disease mortality in patients with hypertension. The association remained significant after adjusting for demographic characteristics, serum cotinine, smoking status, BMI, eGFR, blood

¹ <http://www.inchem.org/documents/ehc/ehc/ehc134.htm>

pressure, lipids, and various disease conditions. Notably, the effect of blood cadmium on cardiovascular mortality may be more pronounced in never-smoking patients with hypertension than in smoking patients with hypertension. Considering the risk of poor long-term health outcomes, cadmium exposure is recommended to be reduced as much as possible in patients with hypertension, even non-smokers.

Data availability statement

Publicly available datasets were analyzed in this study. This data can be found here: The NHANES website: NHANES Questionnaires, Datasets, and Related Documentation (<https://wwwn.cdc.gov/nchs/nhanes/Default.aspx>).

Ethics statement

The studies involving human participants were reviewed and approved by The NCHS Research Ethics Review Board approved the NHANES protocol (<https://www.cdc.gov/nchs/nhanes/irba98.htm>). The patients/participants provided their written informed consent to participate in this study.

Author contributions

TW, LL, and SC conceived and designed research. RS and JS processed data and performed statistical analysis. SC and RS wrote the initial paper. TW and LL reviewed and corrected the article. 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.2023.1106732/full#supplementary-material>

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