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Nutritional toxicity: physiology, nutrition, and human health

Edited by
Tingting Ju, Siddika Yalcin and Misha Vrolijk

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Nutritional toxicity: physiology, nutrition, and human health

Topic editors

Tingting Ju — University of Alberta, Canada

Siddika Yalcin — Hacettepe University, Türkiye

Misha Vrolijk — Maastricht University, Netherlands

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EDITED BY
Misha Vrolijk,
Maastricht University, Netherlands

REVIEWED BY
Marco Iammarino,
Experimental Zooprophylactic Institute of Puglia and Basilicata (IZSPB), Italy

*CORRESPONDENCE
Lihchyun Joseph Su
✉ Lihchyun.Su@UTSouthwestern.edu

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Arsenic in brown rice: do the benefits outweigh the risks?

Lihchyun Joseph Su[1]*, Tung-Chin Chiang[2] and Sarah N. O'Connor[1]

[1]Peter O'Donnell Jr. School of Public Health, University of Texas Southwestern Medical Center, Dallas, TX, United States, [2]Fay W. Boozman College of Public Health, University of Arkansas for Medical Sciences, Little Rock, AR, United States

Brown rice has been advocated for as a healthier alternative to white rice. However, the concentration of arsenic and other pesticide contaminants is greater in brown rice than in white. The potential health risks and benefits of consuming more brown rice than white rice remain unclear; thus, mainstream nutritional messaging should not advocate for brown rice over white rice. This mini-review aims to summarize the most salient concepts related to dietary arsenic exposure with emphasis on more recent findings and provide consumers with evidence of both risks and benefits of consuming more brown rice than white rice. Despite risk-benefit assessments being a challenging new frontier in nutrition, researchers should pursue an assessment to validate findings and solidify evidence. In the interim, consumers should be cognizant that the dose of arsenic exposure determines its toxicity, and brown rice contains a greater concentration of arsenic than white rice.

KEYWORDS

arsenic, exposure, brown rice, cancer, obesity, cardiovascular health

Introduction

In recent years, gluten-free, dairy-free, and plant-based dieting has increased in popularity, and rice is a common substitute. The potential health risks and benefits of consuming more brown rice than white rice remain unclear. Despite this, brown rice is often advocated as a healthier alternative to white rice in mainstream diet and nutritional messaging. Evidence of any protective effect of consuming more brown rice than white is limited.

Even though *in-vitro* and animal studies using nutrients and fiber extracted from brown rice have demonstrated improved cardiovascular function and prevention of heart diseases (1–5), these studies fail to utilize the whole grain of brown rice. Additionally, Sun et al. (6) found that when compared to white rice intake, brown rice intake reduced the risk of type 2 diabetes by 16%. The study, however, lacked diversity and included a homogenous population of European descent health professionals.

Current literature widely includes animal studies and primarily examines the benefits of consuming whole grains, not specifically brown rice. Other study limitations fail to provide solid evidence for the health benefits of consuming brown rice over white rice. There is a clear lack of research focusing on human consumption of brown rice that includes a risk-benefit approach. Risk-benefit assessment of foods is a challenging new frontier in food safety research. The assessment estimates human health benefits and risks following exposure (or lack thereof) to a particular food or food component and integrates them into comparable measures (7–9).

What might continue diminishing the evidence between brown rice and its human health benefits? Brown rice contains a greater arsenic concentration than white rice, and the human health risks associated with dietary arsenic exposure are well-established.

Dietary exposure to arsenic

Arsenic is ubiquitous in the environment and is a global public health concern. Arsenic is a well-known carcinogenic, mutagenic, and toxic environmental element that occurs as inorganic arsenic and organoarsenical compounds (10). Arsenic can be found in food, water, soil, and airborne particles. Inorganic forms of arsenic are found in the environment dissolved in water, and human exposure occurs through drinking water. Additionally, diet is an alternative source of exposure through the consumption of plant-based foods such as wheat, rice, and vegetables grown in contaminated soil and animal products such as dairy, milk, and fish exposed to contaminated feed. Even soil from organic farms can have remnants of arsenic due to historical pesticide use.

Because of the rapid globalization in the food trade, the ingestion of arsenic through rice consumption is not limited to a regional issue but a worldwide health concern (11, 12). A study of more than 204 rice samples sold in the U.S. found that rice grown in certain Southern states, which accounts for more than 47% of the U.S. market, had the highest arsenic content compared to the rice imported from Asia or grown in California (13). A 2017 study estimated Americans' inorganic arsenic exposures from drinking water and rice. It concluded that rice consumption might account for as much inorganic arsenic exposure as drinking water in some U.S. populations (14).

The U.S. Environmental Protection Agency set a limit for total arsenic in drinking water at 10 parts per billion (ppb). However, no such limit exists for food or other beverages. Thus, rice can contain levels of inorganic arsenic that surpass the limit set for arsenic in drinking water. In 2014, a Consumer Reports analysis of the U.S. Food and Drug Administration (FDA) data on 656 rice products confirmed the worrisome levels of arsenic exposure from white and brown rice. In rice, inorganic arsenic is found in the two outer layers of the grain (i.e., bran and germ), and the bran and germ are removed to refine the grain into white rice. Thus, a greater concentration of arsenic is found in brown rice than in white rice. In the previously cited Consumer Reports study, brown rice contained 80 percent more inorganic arsenic on average than white rice of the same type (15).

In response to concerns raised by the public, the FDA Center for Food Safety and Applied Nutrition conducted an assessment based on the existing evidence of health risks from inorganic arsenic in rice and products that contain rice (16). The investigation concluded that the average concentrations of inorganic arsenic are 92 ppb in white rice, 154 ppb in brown rice, 104 ppb in infants' dry white rice cereal, and 119 ppb in infants' dry-brown rice cereal. The data demonstrated that inorganic arsenic concentration is 1.5 times higher in brown rice than in white rice. The expert panel concluded that the risk of exposure and associated health condition(s) increases proportionally with consumption and depends on the type of rice consumed. Notably, the FDA assessment focused on lung and bladder cancer. The expert panel concluded that cancer cases would have increased by 148.6% if rice consumption increased from less than one serving per day, the current level, to precisely one serving per day (16). Although none of the products analyzed in the Consumer Reports study reached the acute toxicity level, the health effects of long-term low-dose exposure are unclear. According to the U.S. FDA, the adverse health effects of arsenic exposure depend on various factors, such as the type of arsenic (organic or inorganic), the level of exposure, and the age of the person exposed to the arsenic. Many studies have linked arsenic exposure to cancers, cardiovascular disease, diabetes mellitus, hypertension, and obesity (17, 18).

Arsenic exposure and disease

Various studies have demonstrated that pregnant females, fetuses, and neonates suffer adverse pregnancy outcomes when exposed to arsenic (19–23). *In utero*, inorganic arsenic exposure was positively associated with DNA damage in offspring (24). Recent health risk assessments reported that the consumption of arsenic-containing rice and rice-based foods (e.g., cereals, cakes, and crackers) led to increased cancer risks, especially in subpopulations of infants and children (25–29).

In humans, inorganic arsenic compounds are converted to trivalent arsenic (As^{III}) and pentavalent arsenate (As^{V}). As^{V} is rapidly converted to As^{III}. As^{III} species are more toxic and bioactive than As^{V} species, both because of the greater chemical reactivity of As^{III} and because As^{III} enters cells more easily (30). Both arsenic species coexist in drinking water with varying toxicity (31). According to the International Agency for Research on Cancer (IARC), there is sufficient evidence in humans for the carcinogenicity of mixed exposure to inorganic arsenic compounds, including arsenic trioxide, arsenite, and arsenate. Exposure to arsenic stimulates epigenetic disruption in various cellular processes, which can cause cancer. Presently, three modes (i.e., chromosomal abnormality, oxidative stress, and altered growth factors) of arsenic carcinogenesis have a degree of positive evidence, both in experimental systems (animal and human cells) and in human tissues (32–34). The IARC concludes that different inorganic arsenic species should be considered carcinogenic independent of the mechanisms of carcinogenic action and independent of which metabolites are the ultimate carcinogen (30).

Chronic arsenic exposure through consuming certain foods and contaminated water has been associated with an increased risk of prostate, lung, bladder, pancreatic, and skin cancer (31, 35–40). According to the U.S. National Cancer Institute, cancers of the digestive tract, liver, kidney, and lymphatic and hematopoietic systems have also been linked to arsenic exposure. Additionally, arsenic trioxide (As_2O_3) treatment in human fibroblasts was shown to disrupt the normal function of the DNA repair pathway and increase genomic instability (24), such that women carrying specific BRCA-1 mutations (e.g., 5382insC, C61G, and 4153delA) were found to be at higher risk of breast cancer with increased arsenic exposure (41).

Arsenic is one of many environmental pollutants linked to metabolic syndrome development (17, 18, 42). Metabolic risk factors that lead to a diagnosis of arsenic-induced metabolic syndrome include having a large waistline, high blood pressure, elevated fasting blood sugar, high triglyceride level, and low HDL cholesterol. Those risk factors are identical to the ones for cardiovascular diseases.

Epidemiological studies have shown that the cardiovascular system is susceptible to long-term ingestion of arsenic (43). Noticeable effects include hypertension and increased cardiovascular disease mortality (43). A growing body of literature suggests that DNA

Abbreviations: As^{III}, Trivalent arsenic; As_2O_3, Arsenic trioxide; As^{V}, Pentavalent arsenate; FDA U.S., Food and Drug Administration; GLUT4, Glucose transporter 4 protein; HDL, High-density lipoprotein; U.S., The United States of America.

methylation, one of the most frequently researched epigenetic mechanisms, is associated with various outcomes in response to exposure to heavy metals like arsenic (44–47). A recent study proposed epigenetic dysregulation as a critical arsenic-related cardiovascular disease (CVD) mechanism. Researchers conducted a mediation analysis to assess the potential role of DNA methylation on arsenic-related CVD. Results supported that blood DNA methylation influences arsenic-related CVD, and the results were replicated in a mouse model and three independent and diverse human cohorts (48).

In addition, arsenic exposure is also suspected to be related to obesity (24, 49). We recently found a significant dose–response relationship between arsenic concentration and obesity among 270 postmenopausal women randomly selected from a study cohort where most rice is produced (50). Furthermore, we also found a significantly positive association between weight gain velocity and salivary arsenic concentration in the same study. The mechanism for arsenic exposure and obesity is unclear. Arsenic upregulates the cytokine IL-6 expression in various cell types (51). IL-6, a pro-inflammatory cytokine and an anti-inflammatory myokine, is hypothesized to increase the amount of free fatty acids in the body, thus increasing obesity. Another explanation involves regulating glucose uptake by the glucose transporter 4 protein (GLUT4) in adipose and skeletal muscles (52). A recent study determined that low-dose exposure to arsenic for 8 weeks decreased GLUT4 expression (53). When GLUT4 is silenced, the glucose is not effectively transported into the cell. Research has further identified that steady-state glucose homeostasis dysregulations are due to arsenic exposure (54). Although the mechanism remained largely unknown, patterns of dyslipidemia influenced by arsenic have been identified (55).

Conclusion

There is a clear lack of research focusing on human consumption of brown rice that includes a risk-benefit approach. The fact that brown rice contains more arsenic than white rice cannot be denied, and the human health risks associated with dietary arsenic exposure are well-established. Health effects of arsenic exposure depend on various factors, such as the type of arsenic (organic or inorganic), the level of exposure, and the age of the person exposed to the arsenic. Arsenic exposure has been associated with cancers, cardiovascular disease, diabetes mellitus, hypertension, and obesity. Despite risk-benefit assessment of foods being a challenging new frontier in food safety research, future studies should include an assessment to validate findings and solidify evidence. In the interim, consumers should be cognizant that the dose of arsenic exposure determines its toxicity, and brown rice contains a greater concentration of arsenic than white rice.

Author contributions

LS drafted the manuscript. LS, T-CC, and SO'C reviewed and completed the manuscript. All authors contributed to the article and approved the submitted version.

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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References

1. Kim JY, Do MH, Lee SS. The effects of a mixture of brown and black rice on lipid profiles and antioxidant status in rats. *Ann Nutr Metab*. (2006) 50:347–53. doi: 10.1159/000094298

2. Petchdee S, Laosripaiboon W, Jarussophon N. Cardiac protection of germinated brown rice extract in rabbit model of chronic myocardial infarction. Translational. *Anim Sci*. (2020) 4:txaa067. doi: 10.1093/tas/txaa067

3. Yen H-W, Lin H-L, Hao C-L, Chen F-C, Chen C-Y, Chen J-H, et al. Effects of pre-germinated brown rice treatment high-fat diet-induced metabolic syndrome in C57BL/6J mice. *Biosci Biotechnol Biochem*. (2017) 81:979–86. doi: 10.1080/09168451.2017.1279848

4. Zhao R, Ghazzawi N, Wu J, Le K, Li C, Moghadasian MH, et al. Germinated brown rice attenuates atherosclerosis and vascular inflammation in low-density lipoprotein receptor-knockout mice. *J Agric Food Chem*. (2018) 66:4512–20. doi: 10.1021/acs.jafc.8b00005

5. Petchdee S, Laosripaiboon W, Jarussophon N, Kumphune S. Cardio-protective effects of germinated Brown Rice extract against myocardial ischemia reperfusion injury. *High Blood Pressure Cardiovasc Prevent Off J Italian Soc Hypertension*. (2020) 27:251–8. doi: 10.1007/s40292-020-00378-x

6. Sun Q, Spiegelman D, van Dam RM, et al. White Rice, Brown Rice, and risk of type 2 diabetes in U.S. men and women. *Arch Intern Med*. (2010) 170:961–9. doi: 10.1001/archinternmed.2010.109

7. Assunção R, Pires S, Nauta M. Risk-benefit assessment of foods. *EFSA J*. (2019) 17:e170917. doi: 10.2903/j.efsa.2019.e170917

8. Boué G, Guillou S, Antignac JP, Bizec B, Membré JM. Public health risk-benefit assessment associated with food consumption – a review. *Eur J Nutr Food Safety*. (2015) 5:32–58. doi: 10.9734/ejnfs/2015/12285

9. Pires SM, Boué G, Boobis A, Eneroth H, Hoekstra J, Membré JM, et al. Risk-benefit assessment of foods: key findings from an international workshop. *Food Res Int*. (2019) 116:859–69. doi: 10.1016/j.foodres.2018.09.021

10. D'Amore T, Miedico O, Pompa C, Preite C, Iammarino M, Nardelli V. Characterization and quantification of arsenic species in foodstuffs of plant origin by HPLC/ICP-MS. *Life*. (2023) 13:511. doi: 10.3390/life13020511

11. Islam S, Rahman MM, Rahman MA, Naidu R. Inorganic arsenic in rice and rice-based diets: health risk assessment. *Food Control*. (2017) 82:196–202. doi: 10.1016/j.foodcont.2017.06.030

12. Kumarathilaka P, Seneweera S, Meharg A, Bundschuh J. Arsenic accumulation in rice (Oryza sativa L.) is influenced by environment and genetic factors. *Sci Total Environ*. (2018) 642:485–96. doi: 10.1016/j.scitotenv.2018.06.030

13. Zavala YJ, Duxbury JM. Arsenic in rice: I. estimating normal levels of total arsenic in rice grain. *Environ Sci Technol*. (2008) 42:3856–60. doi: 10.1021/es702747y

14. Mantha M, Yeary E, Trent J, Creed PA, Kubachka K, Hanley T, et al. Estimating inorganic arsenic exposure from U.S. Rice and Total water intakes. *Environ Health Perspect*. (2017) 125:057005. doi: 10.1289/EHP418

15. Consumer Reports Food Safety and Sustainability Center. (2014). Report: analysis of arsenic in Rice and other grains. Available at: https://advocacy.consumerreports.org/wp-content/uploads/2019/05/CR_FSASC_Arsenic_Analysis_Nov2014.pdf

16. Center for Food Safety and Applied Nutrition—U.S. Food and Drug Administration. (2016). Risk Assessment Report: Arsenic in Rice and Rice Products.

Available at: http://www.fda.gov/Food/FoodScienceResearch/RiskSafetyAssessment/default.htm

17. Jeon JY, Ha KH, Kim DJ. New risk factors for obesity and diabetes: environmental chemicals. *J Diabetes Investig*. (2015) 6:109–11. doi: 10.1111/jdi.12318

18. Lin H-C, Huang Y-K, Shiue H-S, Chen L-S, Choy C-S, Huang S-R, et al. Arsenic methylation capacity and obesity are associated with insulin resistance in obese children and adolescents. *Food Chem Toxicol*. (2014) 74:60–7. doi: 10.1016/j.fct.2014.08.018

19. Farzan SF, Karagas MR, Chen Y. In utero and early life arsenic exposure in relation to long-term health and disease. *Toxicol Appl Pharmacol*. (2013) 272:384–90. doi: 10.1016/j.taap.2013.06.030

20. Milton AH, Hussain S, Akter S, Rahman M, Mouly TA, Mitchell K. A review of the effects of chronic arsenic exposure on adverse pregnancy outcomes. *Int J Environ Res Public Health*. (2017) 14:556. doi: 10.3390/ijerph14060556

21. Milton AH, Smith W, Rahman B, Hasan Z, Kulsum U, Dear K, et al. Chronic arsenic exposure and adverse pregnancy outcomes in Bangladesh. *Epidemiology*. (2005) 16:82–6. doi: 10.1097/01.ede.0000147105.94041.e6

22. Rahman A, Vahter M, Smith AH, Nermell B, Yunus M, El Arifeen S, et al. Arsenic exposure during pregnancy and size at birth: a prospective cohort study in Bangladesh. *Am J Epidemiol*. (2009) 169:304–12. doi: 10.1093/aje/kwn332

23. Ronco AM, Gutierrez Y, Gras N, Muñoz L, Salazar G, Llanos MN. Lead and arsenic levels in women with different body mass composition. *Biol Trace Elem Res*. (2010) 136:269–78. doi: 10.1007/s12011-009-8546-z

24. Peremartí J, Ramos F, Marcos R, Hernández A. Arsenic exposure disrupts the normal function of the FA/BRCA repair pathway. *Toxicol Sci Off J Soc Toxicol*. (2014) 142:93–104. doi: 10.1093/toxsci/kfu159

25. Fakhri Y, Bjørklund G, Bandpei AM, Chirumbolo S, Keramati H, Hosseini Pouya R, et al. Concentrations of arsenic and lead in rice (Oryza sativa L.) in Iran: a systematic review and carcinogenic risk assessment. *Food Chem Toxicol*. (2018) 113:267–77. doi: 10.1016/j.fct.2018.01.018

26. Islam S, Rahman MM, Islam MR, Naidu R. Arsenic accumulation in rice: consequences of rice genotypes and management practices to reduce human health risk. *Environ Int*. (2016) 96:139–55. doi: 10.1016/j.envint.2016.09.006

27. Lin K, Lu S, Wang J, Yang Y. The arsenic contamination of rice in Guangdong Province, the most economically dynamic provinces of China: arsenic speciation and its potential health risk. *Environ Geochem Health*. (2015) 37:353–61. doi: 10.1007/s10653-014-9652-1

28. Rahman MA, Rahman MM, Reichman SM, Lim RP, Naidu R. Arsenic speciation in Australian-grown and imported rice on sale in Australia: implications for human health risk. *J Agric Food Chem*. (2014) 62:6016–24. doi: 10.1021/jf501077w

29. Sofuoglu SC, Güzelkaya H, Akgül Ö, Kavcar P, Kurucaovalı F, Sofuoglu A. Speciated arsenic concentrations, exposure, and associated health risks for rice and bulgur. *Food Chem Toxicol*. (2014) 64:184–91. doi: 10.1016/j.fct.2013.11.029

30. IARC—International Agency for Research on Cancer (2012). Arsenic, metals, fibres, and dusts. *IARC monographs on the evaluation of carcinogenic risks to humans*; World Health Organization WHO-Press: Lyon, France; *100C*

31. Sorg TJ, Chen ASC, Wang L. Arsenic species in drinking water wells in the USA with high arsenic concentrations. *Water Res*. (2014) 48:156–69. doi: 10.1016/j.watres.2013.09.016

32. Bhattacharjee P, Banerjee M, Giri AK. Role of genomic instability in arsenic-induced carcinogenicity. *Rev Environ Int*. (2013) 53:29–40. doi: 10.1016/j.envint.2012.12.004

33. Kitchin KT. Recent advances in arsenic carcinogenesis: modes of action, animal model systems, and methylated arsenic metabolites. *Toxicol Appl Pharmacol*. (2001) 172:249–61. doi: 10.1006/taap.2001.9157

34. Tong D, Ortega J, Kim C, Huang J, Gu L, Li G-M. Arsenic inhibits DNA mismatch repair by promoting EGFR expression and PCNA phosphorylation. *J Biol Chem*. (2015) 290:14536–41. doi: 10.1074/jbc.M115.641399

35. Ayotte JD, Belaval M, Olson SA, Burow KR, Flanagan SM, Hinkle SR, et al. Factors affecting temporal variability of arsenic in groundwater used for drinking water supply in the United States. *Sci Total Environ*. (2015) 505:1370–9. doi: 10.1016/j.scitotenv.2014.02.057

36. Brambila EM, Achanzar WE, Qu W, Webber MM, Waalkes MP. Chronic arsenic-exposed human prostate epithelial cells exhibit stable arsenic tolerance: mechanistic implications of altered cellular glutathione and glutathione S-transferase. *Toxicol Appl Pharmacol*. (2002) 183:99–107. doi: 10.1006/taap.2002.9468

37. García-Esquinas E, Pollán M, Umans JG, Francesconi KA, Goessler W, Guallar E, et al. Arsenic exposure and cancer mortality in a U.S.-based prospective cohort: the strong heart study. Cancer epidemiology, Biomarkers & Prevention: a publication of the American Association for Cancer Research, cosponsored by the American Society of Preventive. *Oncology*. (2013) 22:1944–53. doi: 10.1158/1055-9965.EPI-13-0234-T

38. Lewis DR, Southwick JW, Ouellet-Hellstrom R, Rench J, Calderon RL. Drinking water arsenic in Utah: a cohort mortality study. *Environ Health Perspect*. (1999) 107:359–65. doi: 10.1289/ehp.99107359

39. Sanchez TR, Perzanowski M, Graziano JH. Inorganic arsenic and respiratory health, from early life exposure to sex-specific effects: a systematic review. *Environ Res*. (2016) 147:537–55. doi: 10.1016/j.envres.2016.02.009

40. Yang Y, McDonald AC, Wang X, Pan Y, Wang M. Arsenic exposures and prostate cancer risk: a multilevel meta-analysis. *J Trace Elem Med Biol Organ Soc Miner Trace Elements*. (2022) 72:126992. doi: 10.1016/j.jtemb.2022.126992

41. Muszyńska M, Jaworska-Bieniek K, Durda K, Sukiennicki G, Gromowski T, Jakubowska A. Arsenic (as) and breast cancer risk. *Hereditary Cancer Clin Practice*. (2012) 10:A8. doi: 10.1186/1897-4287-10-S4-A8

42. Steinmaus C, Castriota F, Ferreccio C, Smith AH, Yuan Y, Liaw J, et al. Obesity and excess weight in early adulthood and high risks of arsenic-related cancer in later life. *Environ Res*. (2015) 142:594–601. doi: 10.1016/j.envres.2015.07.021

43. National Research Council Subcommittee to Update the Arsenic in Drinking Water, R. *Arsenic in drinking water: 2001 update*. U.S.: National Academies Press (2001).

44. Bozack AK, Domingo-Relloso A, Haack K, Gamble MV, Tellez-Plaza M, Umans JG, et al. Locus-specific differential DNA methylation and urinary arsenic: an epigenome-wide association study in blood among adults with low-to-moderate arsenic exposure. *Environ Health Perspect*. (2020) 128:67015. doi: 10.1289/EHP6263

45. Cardenas A, Koestler DC, Houseman EA, Jackson BP, Kile ML, Karagas MR, et al. Differential DNA methylation in umbilical cord blood of infants exposed to mercury and arsenic in utero. *Epigenetics*. (2015) 10:508–15. doi: 10.1080/15592294.2015.1046026

46. Green BB, Karagas MR, Punshon T, Jackson BP, Robbins DJ, Houseman EA, et al. Epigenome-wide assessment of DNA methylation in the placenta and arsenic exposure in the New Hampshire birth cohort study (USA). *Environ Health Perspect*. (2016) 124:1253–60. doi: 10.1289/ehp.1510437

47. Koestler DC, Avissar-Whiting M, Houseman EA, Karagas MR, Marsit CJ. Differential DNA methylation in umbilical cord blood of infants exposed to low levels of arsenic in utero. *Environ Health Perspect*. (2013) 121:971–7. doi: 10.1289/ehp.1205925

48. Domingo-Relloso A, Makhani K, Riffo-Campos AL, Tellez-Plaza M, Klein KO, Subedi P, et al. Arsenic exposure, blood DNA methylation, and cardiovascular disease. *Circ Res*. (2022) 131:e51–69. doi: 10.1161/CIRCRESAHA.122.320991

49. Chou W-C, Chung Y-T, Chen H-Y, Wang C-J, Ying T-H, Chuang C-Y, et al. Maternal arsenic exposure and DNA damage biomarkers, and the associations with birth outcomes in a general population from Taiwan. *PLoS One*. (2014) 9:e86398. doi: 10.1371/journal.pone.0086398

50. Stahr S, Chiang T, Bauer MA, Runnells GA, Rogers LJ, Joseph Su L. Low-level environmental heavy metals are associated with obesity among postmenopausal women in a southern state. *Exposure Health*. (2021) 13:269–80. doi: 10.1007/s12403-020-00381-6

51. Agrawal S, Bhatnagar P, Flora SJS. Changes in tissue oxidative stress, brain biogenic amines and acetylcholinesterase following co-exposure to lead, arsenic and mercury in rats. *Food Chem Toxicol*. (2015) 86:208–16. doi: 10.1016/j.fct.2015.10.013

52. Rüegg J, Cai W, Karimi M, Kiss NB, Swedenborg E, Larsson C, et al. Epigenetic regulation of glucose transporter 4 by estrogen receptor β. *Molecul Endocrinol Baltimore*. (2011) 25:2017–28. doi: 10.1210/me.2011-1054

53. Farkhondeh T, Samarghandian S, Azimi-Nezhad M. The role of arsenic in obesity and diabetes. *J Cell Physiol*. (2019) 234:12516–29. doi: 10.1002/jcp.28112

54. Kirkley AG, Carmean CM, Ruiz D, Ye H, Regnier SM, Poudel A, et al. Arsenic exposure induces glucose intolerance and alters global energy metabolism. *Am J Physiol Regul Integr Comp Physiol*. (2018) 314:R294–303. doi: 10.1152/ajpregu.00522.2016

55. Afolabi OK, Wusu AD, Ogunrinola OO, Abam EO, Babayemi DO, Dosumu OA, et al. Arsenic-induced dyslipidemia in male albino rats: comparison between trivalent and pentavalent inorganic arsenic in drinking water. *BMC Pharmacol Toxicol*. (2015) 16:15. doi: 10.1186/s40360-015-0015-z

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EDITED BY
Siddika Yalcin,
Hacettepe University, Türkiye

REVIEWED BY
Ganime Ayar,
Ankara City Hospital, Türkiye
Meda Kondolot,
Erciyes University, Türkiye
İzzet Erdal,
Ankara Etlik City Hospital, Türkiye
Misha Vrolijk,
Maastricht University, Netherlands

*CORRESPONDENCE
Lourdes Rodrigo
✉ lourdesr@ugr.es

†These authors have contributed equally to this work

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Levels of Bisphenol A and its analogs in nails, saliva, and urine of children: a case control study

Yolanda Gálvez-Ontiveros[1,2,3†], Inmaculada Moscoso-Ruiz[2,3,4†], Vega Almazán Fernández de Bobadilla[5], Celia Monteagudo[1,2,3], Rafael Giménez-Martínez[1,2], Lourdes Rodrigo[2,3,6*], Alberto Zafra-Gómez[2,3,4] and Ana Rivas[1,2,3†]

[1]Department of Nutrition and Food Science, University of Granada, Granada, Spain, [2]Instituto de Investigación Biosanitaria ibs. GRANADA, Granada, Spain, [3]"José Mataix Verdú" Institute of Nutrition and Food Technology (INYTA), Biomedical Research Centre (CIBM), University of Granada, Granada, Spain, [4]Department of Analytical Chemistry, University of Granada, Granada, Spain, [5]Heath Center of Maracena, Granada, Spain, [6]Department of Legal Medicine and Toxicology, University of Granada, Granada, Spain

Introduction: A growing number of studies link the increase in overweight/obesity worldwide to exposure to certain environmental chemical pollutants that display obesogenic activity (obesogens). Since exposure to obesogens during the first stages of life has been shown to have a more intense and pronounced effect at lower doses, it is imperative to study their possible effects in childhood. The objective here was to study the association of Bisphenol A (BPA) and 11 BPA analogs in children, using three biological matrices (nails, saliva and urine), and overweight and obesity (n = 160).

Methods: In this case–control study, 59 overweight/obese children and 101 controls were included. The measuring of Bisphenols in the matrices was carried out by ultra-high performance liquid chromatography coupled with triple quadrupole tandem mass spectrometry (UHPLC-MS/MS). Logistic regression was used to study the association between overweight/obesity and Bisphenol exposure.

Results: The results suggested that BPF in nails is associated with overweight/obesity in children (OR:4.87; p = 0.020). In saliva, however, the highest detected concentrations of BPAF presented an inverse association (OR: 0.06; p = 0.010) with overweight/obesity. No associations of statistical significance were detected between exposure to BPA or its other analogs and overweight/obesity in any of the biological matrices.

KEYWORDS

overweight and obesity, childhood obesity, obesogens, BPA and analogs, biological samples

1. Introduction

Overweight and obesity are defined by the World Health Organization (WHO) as "an abnormal or excessive accumulation of fat that may be harmful to health." The prevalence of overweight and obesity has tripled in most of the world's population since 1975 (1). Therefore, according to the WHO, obesity is one of the most important public health problems in the world today (2). Obesity is known to be a major risk factor in the development of cardiovascular disease, several types of cancer, diabetes and premature death, among other associated problems (1, 3, 4).

All the triggers and mechanisms involved in the development of obesity are not yet fully understood (5). The main cause of the onset of obesity is an imbalance between consumed and expended calories (1). Obesity has also been linked to genetic factors (6, 7). However, in these last few decades evidence has mounted linking the increase of obesity worldwide to exposure to obesogens (8, 9). Obesogens are understood to be environmental chemicals that promote inadequate fat storage through their interference with adipogenesis, as well as interfering with mechanisms controlling satiety, appetite and food preferences, among others (10–13). Bisphenol A (BPA) is among the most studied obesogens.

Bisphenols are produced in large quantities worldwide (>5 million tons per year) and their use has been increasing in the last decades. Due to this ubiquity, bisphenols have been detected in food, dust, sludge, drinking water, etc. "(14)" and the main route of human exposure to them is diet (15, 16). Bisphenols are constituents of polycarbonate plastics and epoxy resins, used to make varnishes, lacquers, adhesives, plastics, dental sealants, water pipes and food packaging. However, their presence in the latter is not stable and over time can migrate from the packaging to the food (14, 17). In 2011, the European Commission banned the use of BPA in the manufacture of polycarbonate infant feeding bottles (18). This is because the early stages of development and childhood are the most vulnerable to exposure to environmental chemical contaminants, as it has been shown that the effect is more intense and pronounced in children at lower doses. This is largely because certain protective mechanisms present in adulthood, such as detoxifying liver enzymes and the blood–brain barrier, are not fully developed in the fetal and postnatal stage. Additionally, metabolism is higher in these early stages of development than in the later stages, enhancing the effects of these environmental chemical pollutants (19). In 2015, the European Food Safety Authority (EFSA) reduced the tolerable daily intake (TDI) of BPA from 50 to 4 micrograms per kilogram of body weight per day. In 2018, the EU Regulation on the use of BPAs in varnishes and coatings in contact with food was adopted, prohibiting those specifically intended to come into contact with food for infants. Additionally, a tolerable limit of 0.05 mg BPA per kilogram of food was set for plastic materials in contact with food, and further guidelines were established to ensure that exposure to BPAs remains below the TDI (20). Currently, EFSA has re-evaluated the risks of BPA and has lowered the TDI from 4 micrograms per kilogram of body weight per day to 0.2 nanograms per kilogram of body weight. As a result of the alarm generated by the adverse effects associated with BPA exposure, BPAs have begun to be replaced by their analogs. Information on the toxic potential of these analogs is still very deficient, though since they have a very similar chemical structure to BPAs, they are expected to exhibit similar endocrine disrupting and obesogenic activity as BPA, as several studies have shown (12, 21–24).

In vitro studies have shown that BPA and analogs [Bisphenol S (BPS) and Bisphenol F (BPF)] promote adipocyte differentiation, leading to excessive fat accumulation. This effect was observed from concentrations of 10 nM to 50 μM (22, 23, 25). Animal studies, at concentrations of 2.4 and 25 μg BPA per kilogram of body weight, have found that BPA exposure increases adipose tissue mass and promotes weight gain (26, 27). Numerous epidemiological studies have focused on BPA as an obesogen, showing that BPA could exert effects on all organs involved in the regulation of energy homeostasis, such as adipose tissue and the brain, among others (8). In these studies, exposure to low-dose BPA and its analogs was associated with weight gain, disruption of carbohydrate and lipid homeostasis, as well as having an effect on brain regions involved in food intake (8, 22, 23).

Few epidemiological studies focused on the effects of BPA and its analogs on obesity/overweight in children, and all of said studies were restricted to urine as the biological matrix. The focus of this research was thus to study the association between the presence of BPA and its analogs and obesity/overweight in children, using three distinct biological matrices (nails, saliva, and urine).

2. Materials and methods

2.1. Study design and setting

This case–control study was designed to evaluate environmental factors affecting the overweight and obesity in Spanish children and adolescents, funded by 'FEDER-Consejería de Salud y Familias' of the Junta de Andalucía PE-0250-2019. Recruitment of the study population was carried out between January 2020 and January 2022 in various health and educational centers in the province of Granada, Spain. The parents or legal guardians of the participants gave their written informed consent. Confidentiality was guaranteed with the deletion of participants personal data. The study was approved by the Ethics Committee of the University of Granada.

2.2. The study population

Eligible cases met the following inclusion criteria: overweight or obesity diagnosis; between the ages of 6 and 12 years-old; having resided continuously in the study areas for at least 6 months. The same inclusion criteria was applied to the controls, with the exception of a diagnosis of overweight or obesity. Exclusion criteria included obesity as a symptom of other pathologies, or as a side effect of pharmacological treatment.

Of the 231 who agreed to participate, the selected subjects were those that correctly collected and submitted biological samples (saliva, urine and nails), amounting to 160 participants (53.5% male). After comparing total population with selected sample (subjects with the three biological samples correctly collected), non-significant differences were observed for gender, age, weight, height and urinary creatinine level, both for cases and for controls groups (Supplementary Table S1).

2.3. Data collection

The variables taken into account in the study were anthropometry (weight and height), sociodemographic variables (gender and age), urine creatinine levels and levels of Bisphenols in biological matrices.

Anthropometric measurements were taken by qualified personnel. Height was taken with a measuring rod [model SECA 214 (20–207 cm)], while weight was measured with a portable Tanita scale (model MC 780-S MA). Body mass index (BMI) was calculated as weight in kg divided by height squared, in meters. Participants were classified as underweight, normal weight, overweight and obese using

the standards proposed by the International Obesity Task Force, as described by Cole et al. (28, 29).

The determination of creatinine levels in the urine samples was analyzed by The Ángel Méndez Soto Clinical Analysis Laboratory. The method used was the classical Jaffé method, based on photometric measurement of the reaction kinetics of creatinine with picric acid at 37°C (30, 31). Biosystems provided a reagent kit (Barcelona, Spain).

2.4. Determination of bisphenols in biological samples

The biological samples used were saliva, urine and nails. For saliva collection, each subject was given a wide-mouth glass bottle, and for the duration of a week, they had to passively collect saliva on an empty stomach until the bottle was approximately half-full. Urine was collected in a polypropylene bottle. A single urine sample is taken from the study subjects, this should be the first urine of the day. Saliva and urine were stored in the participants' homes under frozen conditions until collection. For the nail samples the participants were given a bottle to collect both finger and toenails, over a 3-month period. The nails are collected without nail polish. After collection, all samples were stored at −80°C until their laboratory analysis, with the exception of the nails that were kept at room temperature.

A total of 12 Bisphenols (BPA; BPF; BPS; Bisphenol AP, BPAP; Bisphenol AF, BPAF; Bisphenol B, BPB; Bisphenol E, BPE; Bisphenol C, BPC; Bisphenol FL, BPFL; Bisphenol M, BPM; Bisphenol P, BPP; Bisphenol Z, BPZ) were analyzed for.

Following the completion of the questionnaire, all samples were collected within a 1 to 4 month-period. The validation parameters, LOQ, LOD, calibration range, recovery, etc., can be corroborated in our research group's previously published studies (32–34).

2.4.1. Determination of bisphenols in saliva

The method followed for the determination of Bisphenols in saliva ($n = 89$) was developed by members of the research group (33). 1 g of saliva was deposited in a 10 mL glass tube. Subsequently, 2 mL of acetonitrile and 150 µL of acetic acid solution (0.1 M) were added. This mixture was vortexed and centrifuged for 5 min. The supernatant was recovered and transferred to a 10 mL glass tube, then evaporated to dryness. Subsequently, the first extraction was carried out by adding 1.5 mL of acetone extraction solvent to the dry residue. Then ultrasound-assisted extraction was carried out for 30 min at a power setting of 35%. The mixture was then centrifuged for 5 min, and the supernatant was recovered by transferring it to another glass tube. Extraction was performed a second time, using 1.5 mL ethanol as the extraction solvent under the same conditions. The supernatant was evaporated to achieve complete dryness, then it was reconstituted with 20 µL of methanol (MeOH) and 80 µL of ultrapure water. Finally it was centrifuged and analyzed using ultra-high performance liquid chromatography coupled with a triple quadrupole tandem mass spectrometry (UHPLC-MS/MS) system (31).

2.4.2. Determination of bisphenols in urine

The urine samples were submitted previously to an enzymatic treatment (β-glucuronidase and β-glucuronidase/arylsulfatase), the enzymatic treatment followed is described in the work of Moscoso-Ruiz et al. (34) and Vela-Soria et al. (35).

For the determination of Bisphenols in the urine (n = 149) we used the improved optimization of the extraction method for endocrine disrupting chemicals (EDCs) as described in Vela-Soria et al. (35) and further developed as per Moscoso-Ruiz et al. (34). 4 mL of 10% (w/v) NaCl and 100 µL of HCl (6 N) were added to 4 mL of urine until pH 2 was reached. Subsequently, dispersive microliquid-liquid extraction was performed, with the addition of a mixture of 600 µL of chloroform and 400 µL of acetone, injected directly into the urine sample using a Hamilton syringe. The mixture was then vortexed gently and centrifuged for 5 min. The sedimented phase was recovered, then transferred to another 10 mL glass tube. This extraction process was repeated three times, and the resulting organic phase was then evaporated to dryness (sedimented phase). The dried residue was reconstituted with 20 µL of MeOH and 80 µL of ultrapure water, centrifuged and analyzed on a UHPLC-MS/MS system (34).

2.4.3. Determination of bisphenols in nails

The method for the determination of Bisphenols in nails ($n = 74$) was also developed by members of this research group as described in the article Martín-Pozo et al. (32). Firstly, the nails were washed following the protocol described in the work of Martin-Pozo et al. (32) so that any external contamination was removed. Then 0.1 g of lyophilized and shredded nails were weighed and 1 mL of sodium hydroxide/MeOH (0.04 mol L^{-1}) was added, shaken in a vortex for 2 min and incubated at 30°C for 15 h. After incubation, the digested nails were cooled to room temperature. Subsequently, they were centrifuged for 10 min, the organic phase was then recovered and evaporated to dryness. The residue was reconstituted, using 20 µL of MeOH and 80 µL of ultrapure water, then it was centrifuged and analyzed on a UHPLC-MS/MS system (32).

2.5. Statistical analysis

The distribution of the continuous and parametric variables (height) was summarized via mean and standard deviation (SD), while the distribution of the continuous and non-parametric variables (weight, urinary creatinine levels and Bisphenol concentrations in the biological samples) was summarized via the median and interquartile range (IQR). Frequency distributions were calculated for the categorical variables (sex and age).

To evaluate the differences between cases and controls for all variables, the Student's *t*-test (for parametric variables), Mann–Whitney *U*-test (for non-parametric variables) and Pearson's Chi-square test (for categorical variables) were used.

A logistic regression model was used to analyze the influence of Bisphenol concentrations (ng g^{-1} or ng mL^{-1}) in the three biological matrices as the independent variable, and on overweight and obesity as the dependent variable. The independent variables were dichotomized according to the median value (reference category: concentration ≤ median value). When the % of undetected concentrations of an analyte was >30%, the cut-off point for the dichotomisation was the limit of detection (LOD)/$\sqrt{2}$ (reference category: concentration ≤ LOD/$\sqrt{2}$) (36). LOD value for total Bisphenol in urine was considered as the sum of each Bisphenol LOD separately.

Odds ratios (OR) and standard error (S.E.) were calculated for the initial and adjusted models. Gender, age (for nails, urine and saliva)

and creatinine levels (for urine analysis) were considered confusion factors (CF) in the adjusted models.

SPSS v.23 (version 23, IBM® SPSS® Statistics, Armonk, NY, United States) was employed for all the statistical analyses. The significance was set to $p < 0.05$.

3. Results

Table 1 displays the study population's characteristics. Significant differences were observed for anthropometric variables, the values being higher for cases than for controls ($p < 0.001$) for all three parameters (weight, height, and BMI). The population distribution according to gender and age, and median values for urinary creatinine levels, did not show significant differences between cases and controls.

Table 2 shows the concentrations of Bisphenols determined in the nails, urine and saliva. BPA and BPAF were detected in all three matrices, followed by BPF which was detected in the nails and urine, BPS in the urine and saliva, and BPAP in the nails and saliva. The analogs BPE, BPB, BPC, BPZ, BPM, BPP and BPFL were only detected in the saliva. One of the most important findings of the study was that the nails were the biological matrix with the highest total concentrations of Bisphenol, BPA and BPF (149.22 ng g^{-1}, 136.26 ng g^{-1}, and 23.06 ng g^{-1}). The highest concentration was found for overweight and obese subjects, with significant differences for BPA and total Bisphenols. In the urine, BPA and total Bisphenol were found in higher concentrations in the control group than in the cases, but without significant differences. However, only BPA in the nails showed significant differences ($p = 0.005$). In the saliva the highest detected value of BPA was determined in subjects with a BMI ≥ 25 kg/m^2. For total Bisphenols determined in the nails and saliva, the highest values were detected in the cases. Nevertheless, significant differences were found only for total Bisphenols ($p = 0.011$) in the nails. In the urine, the highest concentration of total Bisphenols was found in the control group, but no significant differences were observed.

Tables 3–5 show the influence of BPF, BPA, BPS, BPAF, and the total Bisphenol concentrations determined in the three biological matrices, on overweight and obesity in the study population. Crude and adjusted OR values were significant for BPF in the nails and BPAF in the saliva. Participants with higher BPF concentrations than the median value for nails had a higher likelihood of excess weight (OR = 3.64, $p = 0.020$; OR = 4.87, $p = 0.012$, crude and adjusted values, respectively; Table 3). On the other hand, an inverse association was observed between BPAF and overweight and obesity. Subjects with concentration of BPAF higher than LOD in the saliva had a lower likelihood to be overweight or obese (OR = 0.06, $p = 0.010$; OR = 0.06, $p = 0.009$, crude and adjusted values, respectively; Table 5). A non-significant association was observed between Bisphenol concentrations in urine and overweight/obesity (Table 4).

TABLE 1 General characteristics of study population ($N = 160$).

		n	Controls ($n = 101$)	Cases ($n = 59$)	*p*
Gender (%)	Male	85	58.80	41.20	0.505[b]
	Female	76	67.10	32.90	
Age, categorized (%)	≤10 years	125	64.00	36.00	0.280[b]
	>10 years	36	58.30	41.70	
Weight, kg	Median		25.45	53.30	**<0.001**[c]
	IQR		12.58	21.90	
Height, cm	Mean		127.79	140.37	**<0.001**[a]
	SD		20.68	12.93	
BMI, kg/m^2	Mean		16.14	24.45	**<0.001**[a]
	SD		2.03	3.84	
Urinary creatinine, g L^{-1}	Median		0.87	0.90	0.439[c]
	IQR		0.60	0.77	

IQR, interquartile range; SD, standard deviation; *p*-values < 0.05 are highlighted in bold; [a]Student's *t*-test; [b]Chi-square test; [c]*U* Mann–Whitney test.

4. Discussion

The aim of this study was to determine the presence of BPA and its analogs in nails, urine and saliva and to analyze the association between their concentrations and overweight and obesity in children. The findings suggest that a high concentration of BPF in nails is associated with an increased likelihood of overweight/obesity. However, the concentration of BPAF in saliva is inversely related to body weight. The most relevant results obtained in nails for BPF and in saliva for BPAF cannot be compared with previous studies, since the association in these two biological samples has not been studied to date, to the best of our knowledge.

The results of this study show that children who have a higher concentration of BPF, BPA, BPAF and total Bisphenols in urine have a lower likelihood of overweight/obesity, yet without statistically significant results. However, the highest detected concentrations of BPS in urine were associated with a higher likelihood of overweight/obesity, though also without statistically significant results. Previous similar studies show the following findings: Jacobson et al. (37) observed in children and adolescents that urine BPS concentrations were associated with an increased likelihood of obesity (OR = 1.16; 95% confidence intervals (CI): 1.02–1.32); the findings obtained in the work of Liu et al. show that children in the highest quartile of urine BPS concentrations had a 1.36-fold increased risk (95% CI, 0.53–3.51) of obesity compared to children in lower quartiles (38); Gajjar et al. also showed that urinary BPS concentrations were directly associated with higher % body fat in children at 8 years of age (OR = 1.1; 95% CI: −0.6–2.7) (39); as for urinary BPA concentrations, no association was found with obesity in work by Seo et al. (40), Jacobson et al. (37), Xue et al. (41), and Okubo et al. (42). Regarding the concentrations of bisphenols detected in urine of schoolchildren and adolescents in previous studies, it is observed that the concentration range detected for BPA, BPS and BPF is higher than that detected in the present study, being 1.2–1.6 ng mL^{-1} BPA, 0.3–0.4 ng mL^{-1} BPS and 0.2–0.3 ng mL^{-1} BPA (37–39). Whereas for the present study it is 0.58–0.70 ng mL^{-1} BPA and <LOD BPS and BPF.

Conversely, other previous studies found a positive association between high urinary BPA concentrations and overweight/obesity. In the work of Amin et al. a statistically significant direct association between in urine BPA and the risk of obesity was observed, being 12.48 times higher in children found in the third tercile of BPA vs. children in the first and second terciles (95% CI: 3.36–46.39, $p < 0.001$) (5). Liu et al. also found that for children in the highest quartile of Bisphenols (BPA and BPF) in urine, their risk of obesity was 1.74

TABLE 2 Bisphenol concentrations in nails, urine, and saliva (ng g^{-1} or ng mL^{-1}).

Nails (ng g^{-1})									
	Controls (n = 52)				Cases (n = 22)				
	% detected	Median	P_{25}	P_{75}	% detected	Median	P_{25}	P_{75}	p
BPF	92.31	7.78	5.50	17.02	81.82	12.10	8.37	23.06	0.062
BPE	0	<LOD	<LOD	<LOD	4.55	<LOD	<LOD	<LOD	-
BPA	100	21.18	13.17	32.73	100	43.67	18.47	136.26	**0.005**
BPS	0	<LOD	<LOD	<LOD	4.55	<LOD	<LOD	<LOD	-
BPB	0	<LOD	<LOD	<LOD	0	<LOD	<LOD	<LOD	-
BPC	0	<LOD	<LOD	<LOD	0	<LOD	<LOD	<LOD	-
BPZ	0	<LOD	<LOD	<LOD	0	<LOD	<LOD	<LOD	-
BPAP	1.92	<LOD	<LOD	<LOD	9.10	<LOD	<LOD	<LOD	-
BPAF	3.85	<LOD	<LOD	<LOD	0	<LOD	<LOD	<LOD	-
BPM	0	<LOD	<LOD	<LOD	0	<LOD	<LOD	<LOD	-
BPP	0	<LOD	<LOD	<LOD	0	<LOD	<LOD	<LOD	-
BPFL	0	<LOD	<LOD	<LOD	0	<LOD	<LOD	<LOD	-
Bisphenols total	100	36.20	24.42	55.60	100	70.71	33.61	149.22	**0.011**

Urine (ng mL^{-1})									
	Controls (n = 97)				Cases (n = 52)				
	% detected	Median	P_{25}	P_{75}	% detected	Median	P_{25}	P_{75}	p
BPF	2.06	<LOD	<LOD	<LOD	3.85	<LOD	<LOD	<LOD	0.522
BPE	0	<LOD	<LOD	<LOD	0	<LOD	<LOD	<LOD	-
BPA	58.76	0.70	<LOD	2.61	71.15	0.58	0.20	1.98	0.969
BPS	9.28	<LOD	<LOD	<LOD	7.69	<LOD	<LOD	<LOD	0.262
BPB	0	<LOD	<LOD	<LOD	0	<LOD	<LOD	<LOD	-
BPC	0	<LOD	<LOD	<LOD	0	<LOD	<LOD	<LOD	-
BPZ	0	<LOD	<LOD	<LOD	0	<LOD	<LOD	<LOD	-
BPAP	0	<LOD	<LOD	<LOD	0	<LOD	<LOD	<LOD	-
BPAF	5.15	<LOD	<LOD	<LOD	7.69	<LOD	<LOD	<LOD	0.882
BPM	0	<LOD	<LOD	<LOD	0	<LOD	<LOD	<LOD	-
BPP	0	<LOD	<LOD	<LOD	0	<LOD	<LOD	<LOD	-
BPFL	0	<LOD	<LOD	<LOD	0	<LOD	<LOD	<LOD	-
Bisphenols total	59.79	1.95	<LOD	4.10	69.23	1.73	<LOD	3.12	0.594

Saliva (ng g^{-1})									
	Controls (n = 58)				Cases (n = 31)				
	% detected	Median	P_{25}	P_{75}	% detected	Median	P_{25}	P_{75}	p
BPF	0	<LOD	<LOD	<LOD	0	<LOD	<LOD	<LOD	-
BPE	3.45	<LOD	<LOD	<LOD	6.45	<LOD	<LOD	<LOD	0.650
BPA	34.48	<LOD	<LOD	0.71	41.94	<LOD	<LOD	0.75	0.333
BPS	6.90	<LOD	<LOD	<LOD	19.35	<LOD	<LOD	<LOD	0.499
BPB	1.72	<LOD	<LOD	<LOD	16.13	<LOD	<LOD	<LOD	-
BPC	1.72	<LOD	<LOD	<LOD	9.68	<LOD	<LOD	<LOD	-
BPZ	3.45	<LOD	<LOD	<LOD	3.22	<LOD	<LOD	<LOD	0.755
BPAP	12.07	<LOD	<LOD	<LOD	3.23	<LOD	<LOD	<LOD	0.106
BPAF	93.1	0.24	0.19	0.50	87.10	<LOD	<LOD	0.24	0.073
BPM	39.65	<LOD	<LOD	0.71	51.61	<LOD	<LOD	<LOD	0.913
BPP	27.59	<LOD	<LOD	0.71	16.13	<LOD	<LOD	<LOD	0.174
BPFL	8.62	<LOD	<LOD	<LOD	3.23	<LOD	<LOD	<LOD	0.313
Bisphenols total	100	2.66	2.33	2.97	100	2.68	2.19	3.18	0.455

LOD, limit of detection; p-values < 0.050 are highlighted in bold; U de Mann–Whitney test.

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TABLE 3 Bisphenol levels in nails as influencing factors on overweight/obesity (logistic regression analysis).

	Crude			Adjusted		
	p	OR	S. E.	*p*	OR	S. E.
BPF (Ref. BPF concentration ≤ median)	**0.020**	3.64	0.56	**0.012**	4.87	0.63
BPA (Ref. BPA concentration ≤ median)	0.131	2.21	0.52	0.157	2.14	0.54
Bisphenols Total (Ref. Bisphenols total concentration ≤ median)	0.131	2.21	0.52	0.107	2.44	0.55

Ref., reference category; OR, odds ratio; S. E., Standard error; *p*-values < 0.050 are highlighted in bold. All analytes were adjusted for age and gender.

TABLE 4 Bisphenol levels in urine as influencing factors on overweight/obesity (logistic regression analysis).

	Crude			Adjusted		
	p	OR	S. E.	*p*	OR	S. E.
BPF (Ref. BPF concentration ≤ LOD)	0.527	0.53	1.02	0.424	0.43	1.04
BPA (Ref. BPA concentration ≤ LOD)	0.653	0.85	0.35	0.551	0.78	0.42
BPS (Ref. BPS concentration ≤ LOD)	0.225	2.38	0.72	0.268	3.76	1.20
BPAF (Ref. BPAF concentration ≤ LOD)	0.886	0.90	0.75	0.616	0.62	0.94
Bisphenols total (Ref. Bisphenols total concentration ≤ LOD)	0.267	0.66	0.38	0.602	0.79	0.46

Ref., reference category; OR, odds ratio; S. E., Standard error; *p*-values < 0.050 are highlighted in bold. All analytes were adjusted for age, gender and urinary creatinine level.

TABLE 5 Bisphenol levels in saliva as influencing factors on overweight/obesity (logistic regression analysis).

	Crude			Adjusted		
	p	OR	S. E.	*p*	OR	S. E.
BPA (Ref. BPA concentration ≤ LOD)	0.527	1.34	0.46	0.601	1.28	0.47
BPAF (Ref. BPAF concentration ≤ LOD)	**0.010**	0.06	1.10	**0.009**	0.06	1.10
Bisphenols total (Ref. Bisphenols total concentration ≤ median)	0.823	1.11	0.45	0.884	1.07	0.45

Ref., reference category; OR, odds ratio; S. E., Standard error; *p*-values < 0.050 are highlighted in bold. All analytes were adjusted for age and gender.

times higher (95% CI: 0.92–3.31) for BPA and 1.54 times higher for BPF (95% CI, 1.02–2.32) (38). A study in 63 prepubertal children with exogenous obesity found that obese children with metabolic syndrome had significantly higher urinary BPA levels than obese children without metabolic syndrome, and both obese groups had significantly higher urinary BPA levels than the control group (43). Five other studies also found that higher urinary BPA concentrations were directly associated with a greater likelihood of higher BMI in school-aged children (44–48).

Most related Bisphenol studies only focused on urinary BPA and reported that, in childhood, higher BPA concentrations were associated with higher body fat percentages (5, 38, 43–48). However, this work observed that higher detected concentrations of urinary BPA, BPF, BPAF and total Bisphenols show a trend toward a lower BMI (OR: 0.78; OR: 0.43; OR: 0.62; OR: 0.79, respectively), this association was not statistically significant ($p = 0.551$; $p = 0.424$; $p = 0.616$; $p = 0.602$, respectively). Other recent studies on urinary BPA show the following results. Gajjar et al. observed an inverse association between urinary BPA concentrations and body fat % in children 8 years of age (OR = −1.2; 95% CI: −3.4–1.0) (39). Silva et al. observed in children 6 to 10 years of age that higher concentrations of BPA and total Bisphenols in urine were associated with lower BMI (49). In the case of the work carried out by Malik et al., it was observed that the presence of BPA in urine was associated with both obesity and low weight in children, i.e., children who were found to have the highest BPA concentrations in urine (fourth quartile) were associated with both obese and underweight children (50). In relation to the results obtained in saliva in this study, it was observed that the highest BPAF concentrations were associated with lower BMI, the association being statistically significant (OR = 0.06; $p = 0.009$). In the case of nails, BPF showed a direct and statistically significant association (OR = 4.87; $p = 0.012$).

In this study biological samples analyses were used to measure bisphenols exposure. Although multiple sources of bisphenols exposure exist in children, the diet is considered to be one of the main sources of exposure as we have been demonstrated in other studies (51, 52). Food contamination with these chemicals typically occurs during food processing, packaging, transportation, and storage (53).

The use of urine as the only indicator of exposure to Bisphenols may be responsible for the lack of consistency between the associations of Bisphenols and adiposity in the previous studies, given that there is a large within-subject variation in the concentrations of BPA and its analogs in urine due to the short half-life of Bisphenols; even within the same subject there are variations in urinary concentrations from day to day and even over the course of the same day. A key issue is that some BPA and BPA analogs are metabolized very rapidly in the body (21), therefore, spot urine samples are limited in their ability to reflect long-term exposure levels. Urine is commonly used in studies because of the ease and non-invasive nature of its collection in the child population. Nails, however, share both these advantages in addition to being a better biomarker, since they display long-term exposure without fluctuations in concentration levels within the same day, or from 1 day to another. Therefore, the use of nails as a bioindicator is very promising since, by their nature they reflect long-term cumulative exposure, given their relatively slow growth rate (54). In fact, nails have been widely used in forensic analysis and as a bioindicator of the ingestion of drugs and heavy metals (55–57). Moreover, nail samples offer other advantages over other commonly used biological samples such as blood and urine, namely that their collection is simple and non-invasive, and do not require specialized personnel, transport or storage conditions (58). On the other hand, saliva samples are a good alternative to blood samples for assessing human exposure to toxicants because of their non-invasive nature and because they do not require qualified personnel to collect them (59). Another reason why saliva is an adequate alternative to blood is because saliva is secreted by glands

surrounded by blood capillaries that allow the passage of toxicants from blood to saliva (60). Therefore, the concentration of toxicants detected in saliva are an accurate reflection of their concentrations in the blood.

Obesity in general is of great concern worldwide. Since increased dietary intake and sedentary lifestyles alone do not explain its increase globally, particular attention is being paid to a wide variety of environmental chemicals that may play an important role. Experimental models and epidemiological evidence suggest that BPA and some of its analogs (BPS and BPF) may act as environmental obesogens (12, 22–24). It is true that various potential mechanisms of action of Bisphenols during adipogenesis have been reported, but there is no common consensus. Some of these mechanisms of action described in the scientific literature is the action of BPA on the modulation of key regulators of adipogenesis [peroxisome proliferator-activated receptor gamma of preadipocyte (PPARγ), CCAAT/enhancer-binding protein Alpha (C/EBPα), dual leucine zipper-bearing kinase (DLK), lipoprotein lipase (LPL)] through interference with receptor signaling (61–63). In one *in vitro* study, it was observed that BPA enters adipose stem cells and interacts with the estrogen receptor (ER), then translocating to the nucleus, where it increases transcription of key adipogenic genes such as DLK, C/EBPα, PPARγ and LPL, which in turn enhances and accelerates the pathway from human adipose stromal/stem cells to mature adipocyte (61). Another adipogenic effect resulting from BPA exposure in 3 T3-L1 cells may also be mediated by increased glucocorticoid receptor and C/EBPδ transcriptional activity (64). Boucher et al. performed an *in vitro* study in which they demonstrated that BPA exposure induced differentiation of primary human preadipocytes through a non-classical ER pathway rather than via glucocorticoid activation (65). The results showed that BPA induced the differentiation of primary preadipocytes through increased expression of adipogenic markers at the mRNA level and increases the expression levels of factors involved in the transcriptional cascade responsible for the differentiation of primary preadipocytes to adipocytes (65). Another mechanism of action that has been studied is the effect of exposure to BPA and its analogs on perturbations in the synthesis and signaling of peripheral serotonin, especially in the intestine, that may contribute to obesity since serotonin also plays an important role in the energy balance of mammals (66). Thus, Barra et al. proposed that BPA and its analogs could increase the intestinal production of peripheral serotonin in the human organism and could contribute to its obesogenic effects (66). The study by Barra et al. (66) is based on additional experiments to support this hypothesis, in one of those studies it was observed that the exposure of mice with genetic or pharmacological inhibition of ryptophan hydroxylase 1 (Tph1) to BPA, evaluated whether the metabolic deficits induced by BPA, such as obesity, depend on the production of peripheral serotonin, since peripheral serotonin in adipose tissue functions as an obesity hormone that reduces energy expenditure and increases lipid accumulation (67).

The importance of bisphenols derives from their ubiquity, as these compounds are present in most commonly used plastic packaging (polycarbonates) and epoxy resins used in the coating of cans in contact with food (14, 17). Exposure to bisphenols is daily. Bisphenols usually enters the bloodstream via the oral route. Its absorption is immediate and with a bioavailability of more than 70% (68). These are conjugated with glucuronic acid and almost entirely eliminated in the urine (69). Bisphenols are also lipophilic, with studies showing that they bioaccumulate in adipose tissue (12). It is true that, if adipose tissue were taken from an obese person, a higher load of these contaminants would be found than in a non-obese person. However, in this study, biological matrices that are not fatty in nature (urine, nails and saliva) have been taken. Therefore, it is not to be expected that in these matrices there will be a greater bioaccumulation behavior in obese people, as they do not have this lipophilic character. Thus, an important question to raise is the relationship between bisphenol exposure and obesity. As mentioned previously, obese people can be expected to have higher concentrations of pollutants in their organism (70). For this reason, statistical models are adjusted for % fat and other predisposing factors (energy intake, physical inactivity, etc.) to obtain the probability of obesity due to bisphenols rather than other factors.

There were several limitations to this study. Firstly, the relatively small sample size could be a contributing factor to the non-significant findings for most of the Bisphenols. Also, it has prevented us to split the sample in order to analyze influencing factors on overweight and obesity separately. Secondly, complication in the collection of saliva and nail samples resulted in fewer samples being submitted of these two matrices. In the case of nails, many of the children participating in the study tended to bite their nails, being a considerable drawback in their acquisition. In relation to saliva, some children refused to collect saliva because they were disgusted by it. However, the main strength of this study is that, to the best of our knowledge, this case–control study in Spanish children is the first to evaluate the possible association between BPA and 11 of its analogs, and overweight and obesity, analyzed in three distinct biological matrices (urine, nails and saliva). By not exclusively using a single biological sample, such as urine, to assess exposure to BPA and its analogs, we eliminate the risk of having one measurement that can bias our results via a misclassification of exposure due to, for example, the great variability of urine composition within a single day, or from 1 day to another.

The adverse health effects of BPA analogs should continue to be monitored, since BPA has already begun to be replaced by them, and human exposure to these alternatives will continue to increase. In addition, the limited evidence on the association between Bisphenols and overweight/obesity calls for further epidemiological and toxicological studies to assess whether human exposure to BPA substitutes increases the risk of overweight/obesity in children.

5. Conclusion

The current study is the first to report on the association between BPA and 11 of its analogs, and childhood overweight and obesity, analyzed in three distinct biological matrices (urine, nails, and saliva). The results suggest a positive association between BPF exposure in nails and a negative association between BPAF exposure in saliva. No associations of statistical significance were found between BPA and its analogs and overweight/obesity.

However, the contradictions between the associations of Bisphenols and adiposity in the previous studies require further epidemiological and toxicological studies, ideally of a longitudinal design and including Bisphenol measurements in different biological matrices that show long-term exposure, assuring that the

concentration of Bisphenols in the samples does not fluctuate within the same day, or from day to day.

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.

Ethics statement

This study was approved by the Ethics Committees of the University of Granada, the Provincial Biomedical Research of Granada (CEI), Spain (reference 1939-M1–22, Andalusian Biomedical Research Ethics Portal). The study was performed in accordance with the corresponding ethical standards. The studies were conducted in accordance with the local legislation and institutional requirements. Written informed consent for participation in this study was provided by the participants' legal guardians/next of kin.

Author contributions

YG-O and IM-R collected the data, performed the analyses, interpreted the data, and wrote the manuscript. VA provided the means for recruitment of the study population, collected the data, critically reviewed the manuscript, and gave final approval of the version to be published. CM collected the data, interpreted the data, revised the manuscript, critically reviewed the manuscript, and gave final approval of the version to be published. LR collected the data, supervised the writing, reviewed the manuscript, and gave final approval of the version to be published. AZ-G: conceptualized and designed the study, critically reviewed the manuscript, and gave final approval of the version to be published. RG-M provided project administration and funding acquisition. AR: conceptualized and designed the study, coordinated, supervised data collection, reviewed the manuscript critically, and gave final approval of the version to be published, as well provided project administration and funding acquisition. 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/fnut.2023.1226820/full#supplementary-material

References

1. World Health Organization (WHO) Obesidad y Sobrepeso (2021). Available at: https://www.who.int/es/news-room/fact-sheets/detail/obesity-and-overweight (Accessed 29 March 2023).

2. World Health Organization (WHO). Obesity (2023). Available at: https://www.who.int/westernpacific/health-topics/obesity (Accessed 29 March 2023).

3. Jarolimova J, Tagoni J, Stern TA. Obesity: its epidemiology, comorbidities, and management. *Prim Care Companion CNS Disord*. (2013) 15:PCC.12f01475. doi: 10.4088/PCC.12F01475

4. Gallagher EJ, LeRoith D. Obesity and diabetes: the increased risk of cancer and cancer-related mortality. *Physiol Rev*. (2015) 95:727–48. doi: 10.1152/PHYSREV.00030.2014

5. Amin MM, Ebrahim K, Hashemi M, Shoshtari-Yeganeh B, Rafiei N, Mansourian M, et al. Association of Exposure to bisphenol a with obesity and cardiometabolic risk factors in children and adolescents. *Int J Environ Health Res*. (2019) 29:94–106. doi: 10.1080/09603123.2018.1515896

6. Goodarzi MO. Genetics of obesity: what genetic association studies have taught us about the biology of obesity and its complications. *Lancet Diabetes Endocrinol*. (2018) 6:223–36. doi: 10.1016/S2213-8587(17)30200-0

7. Dubern B, Mosbah H, Pigeyre M, Clément K, Poitou C. Rare genetic causes of obesity: diagnosis and Management in Clinical. *Care Ann Endocrinol*. (2022) 83:63–72. doi: 10.1016/J.ANDO.2021.12.003

8. Heindel JJ, Blumberg B. Environmental Obesogens: mechanisms and controversies. *Annu Rev Pharmacol Toxicol*. (2019) 59:89–106. doi: 10.1146/ANNUREV-PHARMTOX-010818-021304

9. Naomi R, Yazid MD, Bahari H, Keong YY, Rajandram R, Embong H, et al. Bisphenol a (BPA) leading to obesity and cardiovascular complications: a compilation of current in vivo study. *Int J Mol Sci*. (2022) 23:2969. doi: 10.3390/IJMS23062969

10. Grün F, Blumberg B. Endocrine Disrupters as Obesogens. *Mol Cell Endocrinol*. (2009) 304:19–29. doi: 10.1016/J.MCE.2009.02.018

11. Arrebola JP, Alzaga BG. Exposición a Contaminantes Ambientales Por Vía Alimentaria y Repercusiones Metabólicas Relacionadas Con La Obesidad. *Nutr Clin Med*. (2016) X:164–74. doi: 10.7400/NCM.2016.10.3.5045

12. Darbre PD. Endocrine disruptors and obesity. *Curr Obes Rep*. (2017) 6:18–27. doi: 10.1007/S13679-017-0240-4

13. Muscogiuri G, Barrea L, Laudisio D, Savastano S, Colao A. Obesogenic endocrine disruptors and obesity: myths and truths. *Arch Toxicol*. (2017) 91:3469–75. doi: 10.1007/S00204-017-2071-1

14. ELIKA (Food Security). Bisfenol A (2023). Available at: https://seguridadalimentaria.elika.eus/fichas-de-peligros/bisfenol_a/#quees (Accessed April 2023).

15. Liu J, Wattar N, Field CJ, Dinu I, Dewey D, Martin JW. Exposure and dietary sources of bisphenol a (BPA) and BPA-alternatives among mothers in the APrON cohort study. *Environ Int*. (2018) 119:319–26. doi: 10.1016/J.ENVINT.2018.07.001

16. Martínez MA, Rovira J, Prasad Sharma R, Nadal M, Schuhmacher M, Kumar V. Comparing dietary and non-dietary source contribution of BPA and DEHP to prenatal exposure: a Catalonia (Spain) case study. *Environ Res*. (2018) 166:25–34. doi: 10.1016/J.ENVRES.2018.05.008

17. Apau J, Acheampong A, Adua E. Exposure to bisphenol a, bisphenol F, and bisphenol S can result in obesity in human body. *Cogent Chem*. (2018) 4:1506601. doi: 10.1080/23312009.2018.1506601

18. European Commission. COMMISSION DIRECTIVE 2011/8/EU of 28 January 2011 amending Directive 2002/72/EC as regards the restriction of use of Bisphenol A in plastic infant feeding bottles. OJEU. (2011). Available at: https://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2011:026:0011:0014:EN:PDF (Accessed April 2023).

19. García-Mayor RV, Larrañaga Vidal A, Docet Caamaño MF, Lafuente Giménez A. Endocrine disruptors and obesity: Obesogens. *Endocrinol Nutr*. (2012) 59:261–7. doi: 10.1016/J.ENDONU.2011.11.008

20. European Commission. COMMISSION REGULATION (EU) 2018/213—of 12 February 2018 on the Use of Bisphenol A in Varnishes and Coatings Intended to Come into Contact with Food and Amending Regulation (EU) No 10 / 2011 as Regards the Use of That Substance in Plastic Food Contact materials. OJEU. (2018). Available at: https://eur-lex.europa.eu/legal-content/EN/TXT/PDF/?uri=CELEX:32018R0213&from=CS (Accessed April 2023).

21. Rochester JR, Bolden AL. Bisphenol S and F: a systematic review and comparison of the hormonal activity of bisphenol a substitutes. *Environ Health Perspect*. (2015) 123:643–50. doi: 10.1289/EHP.1408989

22. Boucher JG, Ahmed S, Atlas E. Bisphenol S induces adipogenesis in primary human preadipocytes from female donors. *Endocrinology*. (2016) 157:1397–407. doi: 10.1210/EN.2015-1872

23. Verbanck M, Canouil M, Leloire A, Dhennin V, Coumoul X, Yengo L, et al. Low-dose exposure to bisphenols a, F and S of human primary adipocyte impacts coding and non-coding RNA profiles. *PLoS One*. (2017) 12:e0179583. doi: 10.1371/JOURNAL.PONE.0179583

24. Andújar N, Gálvez-Ontiveros Y, Zafra-Gómez A, Rodrigo L, Álvarez-Cubero MJ, Aguilera M, et al. Bisphenol a analogues in food and their hormonal and obesogenic effects: a review. *Nutrients*. (2019) 11:2136. doi: 10.3390/NU11092136

25. Ahmed S, Atlas E. Bisphenol S- and bisphenol A-induced adipogenesis of murine preadipocytes occurs through direct peroxisome proliferator-activated receptor gamma activation. *Int J Obes*. (2016) 40:1566–73. doi: 10.1038/IJO.2016.95

26. Rubin BS, Murray MK, Damassa DA, King JC, Soto AM. Perinatal exposure to low doses of bisphenol a affects body weight, patterns of Estrous cyclicity, and plasma LH levels. *Environ Health Perspect*. (2001) 109:675–80. doi: 10.1289/EHP.01109675

27. Rubin BS, Paranjpe M, DaFonte T, Schaeberle C, Soto AM, Obin M, et al. Perinatal BPA exposure alters body weight and composition in a dose specific and sex specific manner: the addition of peripubertal exposure exacerbates adverse effects in female mice. *Reprod Toxicol*. (2017) 68:130–44. doi: 10.1016/J.REPROTOX.2016.07.020

28. Cole TJ, Bellizzi MC, Flegal KM, Dietz WH. Establishing a standard definition for child overweight and obesity worldwide: international survey. *BMJ*. (2000) 320:1240–3. doi: 10.1136/BMJ.320.7244.1240

29. Cole TJ, Flegal KM, Nicholls D, Jackson AA. Body mass index cut offs to define thinness in children and adolescents: international survey. *BMJ*. (2007) 335:194–7. doi: 10.1136/BMJ.39238.399444.55

30. Weber JA, Van Zanten AP. Interferences in current methods for measurements of creatinine. *Clin Chem*. (1991) 37:695–700. doi: 10.1093/clinchem/37.5.695

31. Peake M, Whiting M. Measurement of serum creatinine--current status and future goals. *Clin Biochem Rev*. (2006) 27:173–84.

32. Martín-Pozo L, Cantarero-Malagón S, Hidalgo F, Navalón A, Zafra-Gómez A. Determination of endocrine disrupting Chemicals in Human Nails Using an alkaline digestion prior to ultra-high performance liquid chromatography-tandem mass spectrometry. *Talanta*. (2020) 208:120429. doi: 10.1016/J.TALANTA.2019.120429

33. Moscoso-Ruiz I, Gálvez-Ontiveros Y, Cantarero-Malagón S, Rivas A, Zafra A. Optimization of an ultrasound-assisted extraction method for the determination of parabens and bisphenol homologues in human saliva by liquid chromatography-tandem mass spectrometry. *Microchem J*. (2022) 175:107122. doi: 10.1016/J.MICROC.2021.107122

34. Moscoso-Ruiz I, Gálvez-Ontiveros Y, Giles-Mancilla M, del Carmen G-RM, Rivas A, Zafra-Gómez A. Improved method for the determination of endocrine-disrupting Chemicals in Urine of school-age children using microliquid-liquid extraction and UHPLC-MS/MS. *Anal Bioanal Chem*. (2022) 414:6681–94. doi: 10.1007/S00216-022-04231-Z

35. Vela-Soria F, Ballesteros O, Zafra-Gómez A, Ballesteros L, Navalón A. A multiclass method for the analysis of endocrine disrupting Chemicals in Human Urine Samples. Sample treatment by dispersive liquid-liquid microextraction. *Talanta*. (2014) 129:209–18. doi: 10.1016/J.TALANTA.2014.05.016

36. CDC. Fourth National Report on Human Exposure to Environmental Chemicals Updated Tables. (2015). Available at: https://www.cdc.gov/biomonitoring/pdf/fourthreport_updatedtables_feb2015.pdf (Accessed 30 March 2023).

37. Jacobson MH, Woodward M, Bao W, Liu B, Trasande L. Urinary bisphenols and obesity prevalence among U.S. children and adolescents. *J Endocr Soc*. (2019) 3:1715–26. doi: 10.1210/JS.2019-00201

38. Liu B, Lehmler HJ, Sun Y, Xu G, Sun Q, Snetselaar LG, et al. Association of Bisphenol a and its Substitutes, bisphenol F and bisphenol S, with obesity in United States children and adolescents. *Diabetes Metab J*. (2019) 43:59–75. doi: 10.4093/DMJ.2018.0045

39. Gajjar P, Liu Y, Li N, Buckley JP, Chen A, Lanphear BP, et al. Associations of mid-childhood bisphenol a and bisphenol S exposure with mid-childhood and adolescent obesity. *Environ Epidemiol*. (2021) 6:e187. doi: 10.1097/EE9.0000000000000187

40. Seo MY, Moon S, Kim SH, Park MJ. Associations of phthalate metabolites and bisphenol a levels with obesity in children: the Korean National Environmental Health Survey (KoNEHS) 2015 to 2017. *Endocrinol Metab*. (2022) 37:249–60. doi: 10.3803/ENM.2021.1235

41. Xue J, Wu Q, Sakthivel S, Pavithran PV, Vasukutty JR, Kannan K. Urinary levels of endocrine-disrupting chemicals, including bisphenols, bisphenol a Diglycidyl ethers, benzophenones, parabens, and triclosan in obese and non-obese Indian children. *Environ Res*. (2015) 137:120–8. doi: 10.1016/J.ENVRES.2014.12.007

42. Okubo Y, Handa A, Belin T. Serial cross-sectional study for the association between urinary bisphenol a and paediatric obesity: recent updates using NHANES 2003-2014. *Pediatr Obes*. (2019) 14:e12566. doi: 10.1111/IJPO.12566

43. Aktağ E, Yurdakök K, Yalçın SS, Kandemir N. Urinary bisphenol a levels in prepubertal children with exogenous obesity according to presence of metabolic syndrome. *J Pediatr Endocrinol Metab*. (2021) 34:495–502. doi: 10.1515/jpem-2020-0371

44. Trasande L, Attina TM, Blustein J. Association between urinary bisphenol a concentration and obesity prevalence in children and adolescents. *JAMA*. (2012) 308:1113–21. doi: 10.1001/2012.JAMA.11461

45. Wang HX, Zhou Y, Tang CX, Wu JG, Chen Y, Jiang QW. Association between bisphenol a exposure and body mass index in Chinese school children: a cross-sectional study. *Environ Health*. (2012) 11:79. doi: 10.1186/1476-069X-11-79

46. Bhandari R, Xiao J, Shankar A. Urinary bisphenol a and obesity in U.S. children. *Am J Epidemiol*. (2013) 177:1263–70. doi: 10.1093/AJE/KWS391

47. Eng DS, Lee JM, Gebremariam A, Meeker JD, Peterson K, Padmanabhan V. Bisphenol a and chronic disease risk factors in US children. *Pediatrics*. (2013) 132:e637–45. doi: 10.1542/PEDS.2013-0106

48. Li DK, Miao M, Zhou ZJ, Wu C, Shi H, Liu X, et al. Urine bisphenol-a level in relation to obesity and overweight in school-age children. *PLoS One*. (2013) 8:e65399. doi: 10.1371/JOURNAL.PONE.0065399

49. Silva CCV, Jaddoe VWV, Sol CM, El Marroun H, Martinez-Moral MP, Kannan K, et al. Phthalate and bisphenol urinary concentrations, body fat measures, and cardiovascular risk factors in Dutch school-age children. *Obesity*. (2021) 29:409–17. doi: 10.1002/OBY.23082

50. Malik VS, Ravindra K, Rattan P, Dayal D, Attri SV. Environmental exposure to urinary bisphenol-a in north Indian children aged between 6 and 16 years and its association with body mass index. *Environ Sci Pollut Res Int*. (2021) 28:29085–95. doi: 10.1007/S11356-021-12555-Z

51. Gálvez-Ontiveros Y, Moscoso-Ruiz I, Rodrigo L, Aguilera M, Rivas A, Zafra-Gómez A. Presence of parabens and bisphenols in food commonly consumed in Spain. *Foods*. (2021) 10:92. doi: 10.3390/foods10010092

52. Robles-Aguilera V, Gálvez-Ontiveros Y, Rodrigo L, Salcedo-Bellido I, Aguilera M, Zafra-Gómez A, et al. Factors associated with exposure to dietary bisphenols in adolescents. *Nutrients*. (2021) 13:1553. doi: 10.3390/nu13051553

53. Adeyi AA, Babalola BA. Bisphenol-a (BPA) in foods commonly consumed in Southwest Nigeria and its human health risk. *Sci Rep*. (2019) 9:17458. doi: 10.1038/s41598-019-53790-2

54. Alves A, Kucharska A, Erratico C, Xu F, Hond ED, Koppen G, et al. Human biomonitoring of emerging pollutants through non-invasive matrices: state of the art and future potential. *Anal Bioanal Chem*. (2014) 406:4063–88. doi: 10.1007/s00216-014-7748-1

55. Patteet L, Cappelle D, Maudens KE, Crunelle CL, Sabbe B, Neels H. Advances in detection of antipsychotics in biological matrices. *Clin Chim Acta*. (2015) 441:11–22. doi: 10.1016/J.CCA.2014.12.008

56. Alves A, Covaci A, Voorspoels S. Method development for assessing the human exposure to organophosphate flame retardants in hair and nails. *Chemosphere*. (2017) 168:692–8. doi: 10.1016/J.CHEMOSPHERE.2016.11.006

57. Cappelle D, De Doncker M, Gys C, Krysiak K, De Keukeleire S, Maho W, et al. A straightforward, validated liquid chromatography coupled to tandem mass spectrometry method for the simultaneous detection of nine drugs of abuse and their metabolites in hair and nails. *Anal Chim Acta*. (2017) 960:101–9. doi: 10.1016/J.ACA.2017.01.022

58. Sukumar A. Human nails as a biomarker of element exposure. *Rev Environ Contam Toxicol*. (2005) 185:141–77. doi: 10.1007/0-387-30638-2_5

59. Gröschl M. Saliva: a reliable sample matrix in bioanalytics. *Bioanalysis*. (2017) 9:655–68. doi: 10.4155/BIO-2017-0010

60. Langman LJ. The use of Oral fluid for therapeutic drug management: clinical and forensic toxicology. *Ann N Y Acad Sci*. (2007) 1098:145–66. doi: 10.1196/ANNALS.1384.001

61. Ohlstein JF, Strong AL, McLachlan JA, Gimble JM, Burow ME, Bunnell BA. Bisphenol a enhances Adipogenic differentiation of human adipose stromal/stem cells. *J Mol Endocrinol*. (2014) 53:345–53. doi: 10.1530/JME-14-0052

62. Hoepner LA. Bisphenol a: A narrative review of prenatal exposure effects on adipogenesis and childhood obesity via peroxisome proliferator-activated receptor gamma. *Environ Res*. (2019) 173:54–68. doi: 10.1016/J.ENVRES.2019.03.012

63. Aaseth J, Javorac D, Djordjevic AB, Bulat Z, Skalny AV, Zaitseva IP, et al. The role of persistent organic pollutants in obesity: a review of laboratory and epidemiological studies. *Toxics*. (2022) 10:65. doi: 10.3390/TOXICS10020065

64. Atlas E, Pope L, Wade MG, Kawata A, Boudreau A, Boucher JG. Bisphenol a increases AP2 expression in 3T3L1 by enhancing the transcriptional activity of nuclear receptors at the promoter. *Adipocytes*. (2014) 3:170–9. doi: 10.4161/ADIP.28436

65. Boucher JG, Boudreau A, Atlas E. Bisphenol a induces differentiation of human preadipocytes in the absence of glucocorticoid and is inhibited by an Estrogen-receptor antagonist. *Nutr Diabetes*. (2014) 4:e102. doi: 10.1038/NUTD.2013.43

66. Barra NG, Kwon YH, Morrison KM, Steinberg GR, Wade MG, Khan WI, et al. Increased gut serotonin production in response to bisphenol a structural Analogs may contribute to their obesogenic effects. *Am J Physiol Endocrinol Metab*. (2022) 323:E80–E091. doi: 10.1152/AJPENDO.00049.2022

67. Kim H, Kim YG, Choi W, Moon JH, Hwang I, Kim K, et al. Generation of a highly efficient and tissue-specific tryptophan hydroxylase 1 knockout mouse model. *Sci Rep*. (2018) 8:17642. doi: 10.1038/S41598-018-36097-6

68. Thayer KA, Doerge DR, Hunt D, Schurman SH, Twaddle NC, Churchwell MI, et al. Pharmacokinetics of bisphenol a in humans following a single oral administration. *Environ Int*. (2015) 83:107–15. doi: 10.1016/j.envint.2015.06.008

69. Dekant W, Völkel W. Human exposure to bisphenol a by biomonitoring: methods, results and assessment of environmental exposures. *Toxicol Appl Pharmacol*. (2008) 228:114–34. doi: 10.1016/j.taap.2007.12.008

70. Doadrio-Villarejo A. Role of obesogens in adipogenicity and obesity. *An Real Acad Farm*. (2016) 82:206–14.

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EDITED BY
Liliana Silva,
LAQV Network of Chemistry and Technology, Portugal

REVIEWED BY
André Pereira,
University of Coimbra, Portugal
Tingting Ju,
University of Alberta, Canada
Marco Iammarino,
Experimental Zooprophylactic Institute of Puglia and Basilicata (IZSPB), Italy
Yong-Hyeon Yim,
Korea Research Institute of Standards and Science, Republic of Korea

*CORRESPONDENCE
Xun Sun
✉ sunxun@ouc.edu.cn
Limin Cao
✉ caolimin@ouc.edu.cn

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Transformation of arsenic species from seafood consumption during *in vitro* digestion

Bei Liu, Jianxin Sui, Ruixue Feng, Hong Lin, Xiangning Han, Xun Sun* and Limin Cao*

Food Safety Laboratory, College of Food Science and Engineering, Ocean University of China, Qingdao, China

Arsenic (As) species analysis is important for the risk evaluation of seafood. Until now, there has been limited information on the change of As species during digestion. Here, the As species in different types of seafood before and after *in vitro* digestion were investigated. Although inorganic As was not detected in digested fish samples, As(V) contents in digested crabs and scallops were 17.12 ± 1.76 and 138.69 ± 7.53, respectively, which were approximately 2–3 times greater than those of the pre-digestion samples. In further experiments, arsenocholine, dimethylarsinate, arsenobetaine, and monomethylarsonate were all convertible to As(V) during *in vitro* digestions with different rates. The transformation demonstrates a complex process and could be affected by many factors, such as pH, time, and digestion juice composition, of which pH seemed to be particularly important. Free radicals were responsible for the oxidation in the transformation reactions. Unlike arsenobetaine, arsenocholine seemed to be able to directly transform to monomethylarsonate without the intermediate dimethylarsinate. This study reveals and validates the potential of other species (oAs or/and unknown species) to convert to iAs, identifies the main factors affecting this process, and proposes a reaction pathway. There is an important implication for promoting a more accurate risk assessment of arsenic in foodstuffs.

KEYWORDS

arsenic species, seafood, *in vitro* digestion, transformation, risk assessment

1. Introduction

Arsenic (As) is widely present in nature in the form of organic As (oAs) and inorganic As (iAs) (1). Human As exposure typically occurs through oral ingestion, skin contact, and respiratory ingestion, among which oral ingestion of food is the most important (2). Seafood is usually rich in As and is now considered the main source of As exposure for humans (3). Until now, the oAs in fishes, shellfishes, and crustaceans has been identified mainly as arsenocholine (AsC), arsenobetaine (AsB), dimethylarsinate (DMA), and monomethylarsonate (MMA), while iAs usually present as arsenious acid [As(III)] and arsenic acid [As(V)] (4). OAs is traditionally considered as not significantly hazardous but some recent studies have indicated that MMA and DMA, as well as some metabolites of oAs, may be also highly poisonous (5). IAs has been verified to exhibit strong biotoxicity (4). For example, As(III) interferes with enzyme catalysis in the human body and leads to slow cell decay, and As(V) competes with phosphate and disrupts the production of adenosine triphosphate (ATP) (6). Therefore, iAs is now used to assess the risk of As, and the maximal residue level (MRL) has been established in China, the European Union, the United States, and other countries (7–9). For the risk assessment of

chemical hazards in foodstuffs, the bio-accessibility analysis is gaining increasing attention. After oral intake, these chemicals are transferred to the gastrointestinal tract; however, only a proportion of them can be absorbed by the human body and cause toxic effects. Information on the real absorption of different As species in the human gastrointestinal tract is very limited, but according to some studies with mice, AsB has a significantly higher absorption efficiency (over 96%) than As(III) and As(V) (approximately 81 and 86%, respectively), and significant biotransformation of AsB to [As(V)] following oral administration has been indicated (10). Therefore, fully considering bio-accessibility (the proportion of these chemicals that have undergone possible biotransformation during digestion and can be released from the food matrix for human body absorption) in toxicity evaluation seems to be more accurate than just analyzing the undigested food sample itself. It can not only reflect the influence of gastrointestinal conditions on the physicochemical properties of the chemicals but also indicate the possible interconversion among different species. So far, many *in vitro* methods have been developed for this purpose, such as the physiologically based extraction test (PBET), *in vitro* gastrointestinal (IVG) method, and European Unified Bio-accessibility Research Group (BARGE) method (UBM) (11–13), of which the UBM method is widely recognized and used for different environmental and food samples (14–16). Now there is also an emerging trend to develop dynamic models to simulate more effectively the complex human gastrointestinal tract, such as the regular secretion of digestion juice, the dynamic pH values, and the influence of gut microbes (17, 18).

Previous studies have also found that the bio-accessibility of iAs in foodstuffs can be elevated during digestion. For example, Chavez-Capilla et al. evaluated the bio-accessibility of As in some food samples (rice, seaweed, shellfish, etc.) and found a significantly increased iAs content after *in vitro* digestion, suggesting that oAs had been converted to the more hazardous iAs during digestion (19–21). Wiele et al. (22) found that human colonic microbes also have the potential to actively metabolize arsenic into methylated arsenate and thioarsenate. Some chemical reactions can also mediate the interconversion among different As species, e.g., water-soluble compounds (such as glutathione) with antioxidant capacity in vegetables have the reducing activity to convert pentavalent arsenic into trivalent arsenic (23) and induce the demethylation of DMA (20). In addition, oxidative conversion has been studied by Chávez-Capilla and coworkers, who found that arsenosugars could be converted to inorganic arsenic species in simulated gastric juices (19). These results provide important clues that the content of iAs in foods may become higher after digestion, which increases the bio-accessibility of iAs, potentially resulting in higher toxicity. However, detailed and convincing information about the bio-accessibility and interconversion among different As species in food is still very limited, especially in seafood.

To help solve the question, here, the bio-accessibility of oAs (AsB, AsC, DMA, and MMA) and iAs [As(III) and As(V)] in some seafood samples (swimming crab, *Portunus trituberculatus*; scallop, *Azumapecten farreri*; turbot, *Scophthalmus maximus*; and flounder, *Paralichthys olivaceus*) was investigated using the UBM method. Then, the potential of other species (oAs or/and unknown ones) to transform to iAs was revealed, the main factors that influence the process were identified, and the reaction pathway was proposed. These findings contribute to a broader comprehension of the potential hazards associated with arsenic in seafood. They help to offer a more objective and scientific assessment of the health risks posed by heavy metal contamination in foodstuffs.

2. Materials and methods

2.1. Chemicals and samples

Ultrapure Water (purified by a Milli-Q system, 18 MΩ cm, Millipore-Q, Burlington, MA, United States) was used to prepare all solutions. Nitric acid (65%, v/v, GR grade) was used for the wet digestion of food samples and extraction of different As species. Standard solutions of AsC (0.374 μmol·g^{-1}, GBW08671), AsB (0.518 μmol·g^{-1}, GBW08670), DMA (0.706 μmol·g^{-1}, GBW08669), MMA (0.335 μmol·g^{-1}, GBW08668), As(III) (1.011 μmol·g^{-1}, GBW08666), and As(V) (0.233 μmol·g^{-1}, GBW08667) were purchased from the National Standards Substance Center (Beijing, China).

Commercial grade swimming crab was obtained from Qingdao West Coast New Area, scallop was obtained from Muping District, Yantai City, and turbot and flounder were obtained from Nanshan Market of Qingdao City in China. The crude samples were fully washed with water and steamed for a certain time according to a Chinese consumption custom (swimming crab was placed into a pot with hot water and steamed for approximately 20 min, and the body completely turned red; scallop was placed in boiling water for 5 min; and turbot and flounder were placed in boiling water for 10 min). Then the edible tissues of each kind of sample were dissected out, combined, and homogenized with a small meat mincer, packed in plastic valve bags with detailed markers, and stored at −40°C for further use.

2.2. Methods

2.2.1. *In vitro* digestion

The *in vitro* digestion was performed referring to the UBM method described by Wragg et al. (13). The digestion juice contents are summarized in Supplementary Tables 1–4. Briefly, 0.5 g of food samples (denoted as pre-digestion sample, PDS) were placed into 100 mL sample bottles. At first, 7.5 mL of saliva was added and the samples stood for 5 min. Then, 11.5 mL of gastric juice was added and the samples were shaken at 100 rpm for 60 min at 37°C (Constant Shaking Incubator, ZWY-2102C, Zhicheng). Then, 22.5 mL of intestinal juice and 7.5 mL of bile were added and shaken at 100 rpm for 4 h at 37°C. The digestion of food samples was performed using a NutriScan GI 20 Glycemic Index Analyzer at the College of Food Science, Qingdao Agricultural University. At the end of the gastric (G) phase and gastrointestinal (GI) phase, 19 and 49 mL of digestive samples were collected, respectively, and immediately cooled at −20°C for 10–15 min to stop the enzymatic reactions (13). Then, the digestive samples were centrifuged at 8,778 × *g* at 25°C for 10 min (High Speed Centrifuge, Neo 15, Heal Force), and the supernatants were collected and filtered through a 0.22 μm Cellulose Acetate (CA) membrane (Membrane, China) to obtain the digested samples (DS) for the analysis of As content. The *in vitro* digestion of standards was performed similarly, except that the seafood sample was replaced with a certain amount of arsenic standard solution to reach the required concentration (Supplementary Table 5). The effect of the radical

scavengers was evaluated in the *in vitro* gastric digestion of AsB. Radical scavengers (a mixture of 1 mL of 0.1 μg·ml^{-1} vitamin C solution, 1 mL of 0.1 μg ml^{-1} catechin solution, and 1 mL of 0.1 μg ml^{-1} cysteine solution) were added to the gastric juice containing AsB and then reacted in argon for 1 h. The digestion juice as the blank demonstrated no significant interference to the high-performance liquid chromatography with inductively coupled mass spectrometry (HPLC-ICP-MS) analysis of As species in digested samples (Supplementary Figure 1).

2.2.2. Determination of As content

The total As (tAs) in both PDS and DS were determined by inductively coupled plasma mass spectrometry (ICP-MS), as previously described, in which the reliability of the technique was verified by analyzing certified reference materials GBW 10024 (scallop, Institute of Geophysical and Geochemical Exploration) and SRM 1566b (oyster tissue, National Institute of Standards and Technology) (24). Briefly, 1.0 g of PDS or 5 mL of DS were placed into quartz digestion tubes, mixed with 4 mL of HNO_3 and 1 mL of $HClO_4$, and incubated overnight at room temperature. The mixture was heated at 140°C for 2 h, followed by heating up to 185°C until approximately 1 mL of solution remained in a digestion system (EHD-24). Then, the solution was transferred to microcentrifuge tubes, where the volume was maintained at a constant level of 5 mL with ultra-pure water. The samples were filtered through a 0.22 μm membrane and analyzed by inductively coupled plasma-mass spectrometry (ICP-MS, Agilent 8800, United States). Three parallel experiments were conducted for each sample. A blank was prepared according to the same operation without the addition of samples.

Different As species were determined by HPLC-ICP-MS (1260 Infinity HPLC, Agilent, United States; ICP-MS, Agilent 8800, United States) according to the method described by Chen (25), which can effectively separate and detect six As species (Supplementary Figure 2). DS was not treated, and PDS was pretreated by HNO_3 according to the following method: in brief, 1.0 g of PDS was put in a 50 mL centrifuge tube and mixed with 20 mL of 1% HNO_3 solution. The mixture was incubated at 90°C for 2.5 h, and during the incubation, the sample was shaken for 1 min every 0.5 h. After cooling to room temperature, the solution was centrifuged at 6,225 × *g* at 25°C for 15 min and filtered through a 0.22 μm membrane. The crab paste and crab roe were defatted with hexane after centrifugation: 5 mL of supernatant was added to a 50 mL centrifuge tube, followed by 5 mL of hexane, and then the solution was shaken for 1 min. Then, the mixture was centrifuged at 6,225 × *g* for 15 min, the upper hexane layer was discarded, and the remaining solution was treated with hexane again according to the same procedure. Finally, the aqueous layer was collected and filtered through a 0.22 μm membrane. Three parallel experiments were conducted for each sample. A blank was prepared according to the same operation without the addition of samples. The instrumental conditions for ICP-MS and HPLC-ICP-MS are summarized in Supplementary Table 6.

2.2.3. Statistical analyses

Bio-accessibility was calculated according to the following formula (26):

$$\text{Bio-accessibility}\ (\%) = \left(C_{IV} \times V_{IV}\right) / \left(T_S \times M_S\right),$$

where C_{IV} is the As concentration (μg·ml^{-1}) in DS, V_{IV} is the volume of the DS (ml), T_S is the As concentration in PDS (μg·g^{-1}), and M_S is the mass of PDS (g).

Differences among different groups were tested by Student's *t*-test, in which the significant difference was considered as $p < 0.05$. Unless otherwise stated, the results were analyzed based on triplicates, and all data were expressed as mean ± standard deviation (SD).

3. Results and discussion

3.1. Content of different As species in the four seafoods

The tAs content of the PDS was found within the range from 1.8 to 8.3 μg·g^{-1}, with the highest content in swimming crab and the lowest in flounder (Table 1). Similar to previous studies (3, 4), oAs (mainly AsB) was evaluated over 75–90% of the tAs. Toxic As(III) and As(V) were detected in all samples but the concentrations were far lower than those of oAs. The tAs was approximately 10–25% higher than the sum of six different forms of arsenic, and the difference should be due to some unknown As species, which could not be fully extracted or/and could not be identified with the available techniques. After the *in vitro* digestion, the As content changed significantly in all samples (Table 2). Owing to the possible loss by precipitation, complexation, and other physical/chemical interactions, the bio-accessibility of the target is usually considered below 100% (27). However, here the content of As(V) in crabs and scallops was found to be 17.12–138.69 ng·g^{-1}, which was approximately 2–3 times greater than those in the PDSs of the same samples (Table 1). Similarly, significant increases in iAs during digestion were also reported by previous studies on shellfish, rice, seaweed, etc. (19, 20). These results suggest that the real risk of As in these foodstuffs might have been underestimated if the bio-accessibility was not considered (28–30). Notably, for the two fish samples, iAs was not detected after *in vitro* digestion but the AsB content significantly increased ($p < 0.05$). This trend was totally different from that of crabs and scallops, indicating that the change of As species in fish samples might be significantly different from that of other seafood.

In principle the increased As(V) bio-accessibility could be attributed to the following reasons:

1. Release of bound-As species: for the extraction of As species, the PDSs were treated with HNO_3 and heated at 90°C for 2.5 h. Some previous studies have indicated the affinity of oAs and iAs with proteins or peptides, and the interaction was demonstrated to be unstable at high temperatures in the presence of strong acids (31–33). Therefore, such an HNO_3 pretreatment may effectively degrade the protein-based complex in the PDS and therefore release the bound chemicals (34), but it seems very difficult to elucidate the dissociation rate and the amount of remaining bound-As complex. Considering that the extraction recovery of tAs was only approximately 75%, there was still a significant amount of As compounds unable to be effectively extracted and detected in PDS, and they may be effectively released under the digestion conditions in

TABLE 1 Contents of different As species (ng·g^{-1}) in pre-digestion samples ($n = 3$).

Samples		tAs	AsB	AsC	DMA	MMA	As(III)	As(V)
Swimming crab	Muscle	6042.67 ± 179.72	4388.99 ± 155.28	69.68 ± 35.68	311.53 ± 75.60	< DL	4.17 ± 0.00	7.52 ± 0.89
	Crab Paste	4234.76 ± 902.12	3515.81 ± 711.20	50.45 ± 10.08	231.33 ± 57.01	23.45 ± 1.36	5.59 ± 1.00	8.26 ± 1.36
	Crab roe	5816.90 ± 684.93	4294.83 ± 817.52	49.98 ± 30.19	165.07 ± 19.90	29.25 ± 4.18	6.30 ± 0.34	9.92 ± 0.75
Scallop		1892.26 ± 134.48	1583.09 ± 147.02	208.13 ± 4.05	24.80 ± 0.70	48.09 ± 0.15	8.13 ± 0.69	67.79 ± 3.03
Turbot		3821.17 ± 89.99	2996.16 ± 98.72	91.13 ± 4.100	78.21 ± 3.68	< DL	< DL	88.72 ± 6.26
Flounder		1798.99 ± 77.45	1607.02 ± 380.81	111.78 ± 16.60	94.84 ± 6.59	< DL	< DL	111.7 ± 15.59

DL, the lowest detected level.

TABLE 2 Content of As (ng·g^{-1}) in the digested seafood samples ($n = 3$).

Samples			tAs	AsC	AsB	DMA	MMA	As(III)	As(V)
Swimming crab	Muscle	G	4078.90* ± 239.08	< DL	2943.99* ± 249.83	89.73* ± 13.65	< DL	1.98* ± 0.07	17.12* ± 1.76
		GI	5435.43* ± 53.78	< DL	4085.57* ±154.67	150.44* ± 27.27	< DL	< DL	24.57* ± 2.63
	Crab Paste	G	2532.07* ± 168.87	< DL	2115.23* ± 246.66	80.47* ± 11.13	10.36* ± 1.24	1.89* ± 0.15	22.26* ± 1.79
		GI	3754.97 ± 83.02	< DL	3033.31 ± 348.75	76.05* ± 12.10	< DL	< DL	26.73* ± 0.91
	Crab roe	G	4045.87* ± 177.16	< DL	2180.68* ± 54.09	112.85* ± 12.69	19.92* ± 3.38	2.19* ± 0.34	20.83* ± 2.32
		GI	4799.51* ± 6.66	< DL	3324.44 ± 343.43	86.95* ± 8.23	< DL	< DL	28.24* ± 2.21
Scallop		G	1241.61* ± 126.06	134.22* ± 4.50	1091.89* ± 162.09	14.79* ± 2.26	32.23* ± 1.81	< DL	94.65* ± 3.48
		GI	1450.79* ± 221.32	106.79* ± 14.80	1372.36* ± 21.00	12.62* ± 0.88	28.96* ± 0.32	< DL	138.69* ± 7.53
Turbot		G	2540.97* ± 151.44	58.85* ± 0.40	3687.15* ± 93.24	< DL	< DL	< DL	< DL
		GI	2746.91* ± 420.30	< DL	5549.26* ± 237.18	< DL	< DL	< DL	< DL
Flounder		G	1483.27* ± 64.58	51.61* ± 1.53	2121.13* ± 84.08	< DL	< DL	< DL	< DL
		GI	1547.93* ± 221.70	< DL	3132.90* ± 190.39	< DL	< DL	< DL	< DL

DL, the lowest detection limit. The asterisk (*) indicates that there is a significant difference between the sample after digestion and the pre-digestion sample (Table 1) with Student's *t*-test ($p < 0.05$).

TABLE 3 The content of As species in different seafoods (spiked with AsB and DMA) after *in vitro* gastric digestion.

Spiking concentration	AsB (421.35)	DMA (108.70)	AsV (0.00)
AsB and DMA standards	389.12* ± 5.87	91.64* ± 0.87	30.82* ± 1.35
Swimming crab (muscle)	381.85* ± 25.82	84.67* ± 9.75	36.28* ± 4.88
Scallop	386.48* ± 25.82	87.77* ± 7.50	35.01* ± 3.72
Turbot	415.76* ± 9.40	106.81* ± 2.87	< DL

DL, the lowest detection limit. The asterisk (*) indicates that there is a significant difference between the sample after digestion and the pre-digestion sample (Table 1) with Student's *t*-test ($p < 0.05$). The background was tared (calculated for As, ng; $n = 3$).

the form of detectable As(V) (with or without chemical transformation), therefore resulting in the increase in bio-accessibility. Additionally, some experiments using food samples have proposed the release of bound-As during *in vitro* digestions (20).

2. Direct transformation of free oAs or unknown As species to As(V): when further validated with AsB and DMA standards, significant As(V) was observed after the *in vitro* digestion ($p < 0.05$), which clearly confirmed the possible transformation among different As species, especially from oAs to iAs (Table 3). Moreover, the rate of such interconversion varied significantly among seafood samples. This result was in good agreement with Table 2. In crab and scallop, the spiked AsB and DMA also effectively transformed; in particular, the transformation rate of As(V) was even higher than that of the standards.

On the other hand, no interconversion was observed in fish samples, which again showed a different trend from other samples. The result indicates that the fish matrix may have a unique impact on the interconversion pathways of different As species. Similar to this, Chavez-Capilla et al. (19) demonstrated that the dimethylarsinoylpropionic acid in fish samples does not significantly change during gastric and intestinal digestions. However, until now, the unique characteristics of As species in fish samples has not been paid full attention to, and more detailed investigation would be an interesting study.

Owing to the uncertainty of the solubility/stability of different As species during the *in vitro* digestion (35), along with their possible interaction with other components, it seemed very difficult to pin down the contribution of each As species to the increased iAs in the seafood samples. However, the transformation seemed to have

TABLE 4 Content of As ($ng \cdot g^{-1}$) in the supernatants of gastric-digested muscle of swimming crab and turbot and in supernatants further treated with gastrointestinal digestion ($n = 3$).

Samples		tAs	AsC	AsB	DMA	MMA	As(III)	As(V)
Swimming crab (muscle)	G	4110.46* ± 40.87	< DL	2694.88* ± 87.22	128.88* ± 4.38	19.11* ± 0.00	0.02* ± 1.26	26.75* ± 0.02
	GI	4134.25* ± 10.75	< DL	2639.99* ± 159.71	98.47* ± 1.56	< DL	< DL	38.62* ± 0.06
Turbot	G	2574.47* ± 176.90	13.74* ± 0.10	2495.60* ±73.44	< DL	< DL	< DL	< DL
	GI	2602.69* ± 119.09	< DL	2496.54* ± 26.15	< DL	< DL	< DL	< DL

DL, the lowest detection limit. The asterisk (*) indicates that there is a significant difference between the sample after digestion and the pre-digestion sample (Table 1) with Student's *t*-test ($p < 0.05$).

TABLE 5 Transformation of oAs to other As species during *in vitro* digestions (calculated for As, ng; $n = 3$).

Samples		Spiking concentration	AsB	AsC	DMA	MMA	As(III)	As(V)
AsB	G	421.35	388.90 ± 11.38	< DL	11.41 ± 1.63	< DL	< DL	< DL
		1686.39	1500.20 ± 15.23	< DL	116.60 ± 4.01	< DL	< DL	38.04 ± 0.50
	GI	421.35	371.21 ± 21.07	< DL	28.89 ± 0.54	< DL	< DL	< DL
	1% HNO_3	421.35	406.08 ± 11.10	< DL	8.70 ± 1.17	< DL	< DL	6.66 ± 0.33
AsC	G	45.45	< DL	34.13 ± 0.14	< DL	0.04 ~ 0.16	< DL	6.17 ± 0.69
	GI	45.45	< DL	31.77 ± 2.77	< DL	0.04 ~ 0.16	< DL	7.92 ± 0.42
	1% HNO_3	45.45	< DL	41.47 ± 1.70	< DL	0.04 ~ 0.16	< DL	3.10 ± 0.44
DMA	G	108.70	< DL	< DL	84.24 ± 6.96	< DL	< DL	10.35 ± 0.85
		27.17	< DL	< DL	23.54 ± 0.46	< DL	< DL	< DL
	GI	108.70	< DL	< DL	75.56 ± 3.37	< DL	< DL	14.47 ± 1.27
	1% HNO_3	108.70	< DL	< DL	100.10 ± 4.12	< DL	< DL	7.18 ± 2.37
MMA	G	26.79	< DL	< DL	< DL	16.64 ± 2.49	< DL	6.29 ± 0.74
	GI	26.79	< DL	< DL	< DL	13.70 ± 0.83	< DL	8.08 ± 0.58
	1% HNO_3	26.79	< DL	< DL	< DL	20.70 ± 1.20	< DL	4.62 ± 0.73

DL, the lowest detection limit. Experiments were performed with standards of different As species.

occurred during both the G and GI phases, and the increase of As(V) during the G phase was higher than that during the GI phase (Table 4). These details have indicated the importance of G digestion for the proposed release or transformation to oAs.

3.2. Transformation tendency

Various reports have confirmed that the As in seafood is predominately oAs (36), and their potential to transform to As(V) seemed of greater importance. Although several studies have proposed the interconversion by demethylation or other pathways (20), there is still a vacancy for detailed information on the transformation mechanisms among different As species. Therefore, in this study, the possibility and rate of different oAs to transform to iAs were investigated through the *in vitro* digestion of standards (with the same concentration of seafood samples). Such a proposal was confirmed by the significant difference in the peak area of the As species standard before and after *in vitro* digestions (Supplementary Figure 3). As(V) was detected in digested AsC, DMA, and MMA samples, among which the transformation rate of MMA was highest, followed by that of AsC and DMA (Table 5). Similar to experiments with food samples, the G phase contributed most of the increase of As(V), while the increase was much smaller during the GI phase (Supplementary Table 8). AsB has been considered stable enough to endure common chemical and biological treatments (37), and its transformation is usually demonstrated under relatively harsh conditions, such as photo-oxidation or a long reaction time (38, 39). The transformation of AsB to DMA during *in vitro* digestion was observed at a rate of approximately 5.5%. Significant conversion to As(V) was also detected when investigated with a higher concentration of AsB (Table 5). The conversion rate of MMA was the highest, followed by DMA, AsC, and AsB. The conversion rate of AsB was the lowest, which may be determined by the structure of the compound. The simpler the structure, the higher the conversion rate. Considering the abundance of AsB in some seafoods (accounting for more than 70% in PDSs here) and the potential of this compound to be transformed to As(V), the real risk of AsB in these food samples should be more carefully and comprehensively re-evaluated in future studies.

The influence of time, pH value, and enzyme on the transformation from oAs to iAs was further investigated (Tables 6, 7). Using a DMA standard as the reaction substrate, the pH value was proved to be an important parameter for the reaction: when the pH was above 2.0, no As(V) was detected; on the other hand, at pH 1.2, a significant amount of As(V) was observed in both digestive juice and glycine buffers (Table 7). This pH-dependent reaction was even observed in the 1% HNO_3 solution (pH 0.8–0.9), which is the actual condition used for

TABLE 6 Transformation rate of the DMA standard to other As species in the G phase (calculated for As, %; $n = 3$).

Digestion conditions		AsB	AsC	DMA	MMA	As(III)	As(V)
pH	Time (h)						
1.2	0.5	< DL	< DL	93.48 ± 0.03	< DL	< DL	6.65 ± 0.42
	1.0	< DL	< DL	97.71 ± 0.11	< DL	< DL	9.40 ± 0.01
2	0.5	< DL	< DL	95.66 ± 1.20	< DL	< DL	< DL
	1.0	< DL	< DL	97.10 ± 0.22	< DL	< DL	< DL
4	0.5	< DL	< DL	98.26 ± 2.17	< DL	< DL	< DL
	1.0	< DL	< DL	93.26 ± 0.33	< DL	< DL	< DL
6	0.5	< DL	< DL	99.68 ± 2.39	< DL	< DL	< DL
	1.0	< DL	< DL	101.09 ± 1.52	< DL	< DL	< DL

DL, the lowest detection limit. Adjusted by 12 M HCl and without pepsin.

TABLE 7 The effect of digestion juice composition on As species transformation after the G and GI phases (calculated for As, ng; glycine buffer, pH 1.2; $n = 3$).

Samples	Pepsin	Solution	Time / h	AsB	AsC	DMA	MMA	As(III)	As(V)
DMA (108.70 ng)	√	Digestive juices	0.5	< DL	< DL	83.16 ± 1.96	< DL	< DL	9.40 ± 0.11
			1.0	< DL	< DL	72.83 ± 1.30	< DL	< DL	13.63 ± 0.95
	√	Glycine buffer	0.5	< DL	< DL	90.84 ± 2.85	< DL	< DL	6.30 ± 0.44
			1.0	< DL	< DL	77.75 ± 0.85	< DL	< DL	6.63 ± 0.18
	×	Glycine buffer	0.5	< DL	< DL	91.70 ± 1.25	< DL	< DL	3.92 ± 0.30
			1.0	< DL	< DL	85.72 ± 2.21	< DL	< DL	4.98 ± 0.46
MMA (26.79 ng)	√	Digestive juices	0.5	< DL	< DL	< DL	18.32 ± 1.88	< DL	6.68 ± 0.55
			1.0	< DL	< DL	< DL	17.04 ± 1.29	< DL	7.18 ± 0.01
	√	Glycine buffer	0.5	< DL	< DL	< DL	20.63 ± 1.13	< DL	5.28 ± 0.21
			1.0	< DL	< DL	< DL	20.87 ± 0.13	< DL	5.70 ± 0.61
	×	Glycine buffer	0.5	< DL	< DL	< DL	21.14 ± 0.54	< DL	5.10 ± 0.32
			1.0	< DL	< DL	< DL	19.15 ± 0.21	< DL	5.31 ± 0.79
AsC (45.45 ng)	√	Digestive juices	0.5	< DL	37.91 ± 0.54	< DL	< DL	< DL	4.96 ± 0.21
			1.0	< DL	37.77 ± 1.55	< DL	< DL	< DL	5.12 ± 0.02
	√	Glycine buffer	0.5	< DL	37.18 ± 3.00	< DL	< DL	< DL	4.54 ± 0.05
			1.0	< DL	38.59 ± 1.73	< DL	< DL	< DL	4.60 ± 0.05
	×	Glycine buffer	0.5	< DL	38.36 ± 0.55	< DL	< DL	< DL	4.23 ± 0.05
			1.0	< DL	38.00 ± 1.18	< DL	< DL	< DL	4.28 ± 1.16

DL, the lowest detection limit; √, in the presence of pepsin; and ×, without pepsin.

As extraction from food samples (Table 5), in which AsB, AsC, DMA, and MMA all exhibited significant transformation to As(V). The transformation seemed to occur rapidly, with a 60% transformation within 30 min in most cases (Table 6). Moreover, the reaction rate in digestion juice was higher than that in glycine buffers, indicating that other components (e.g., enzymes) in the digestion juice may also be responsible for the reaction, and this result was consistent with a previous study (20), in which sodium cholate and pancreatin were suggested as important factors for increasing iAs caused by a demethylation reaction during digestion.

Based on results mentioned above, interconversion among different As species (especially from oAs to iAs) under certain *in vitro* digestion conditions was confirmed, and this was not only consistent with some previous studies of food samples (19–21, 36) but was well supported by recent experiments with mice, in which a significant biotransformation of AsB to As(V) was observed in the gastrointestinal tract following oral administration. On the other hand, the information presented here will increase understanding of the biotransformation among As species in mammals and its influencing factors. However, such an interconversion among As species seems to be a very complex process, and it could be affected by many parameters of the samples and experimental conditions, which indicates that lots of work still needs to be carried out in future to provide more detailed and accurate information. The environmental and culture conditions may influence the accumulation and metabolism of As species, and slight differences in experimental conditions, such as acidity, reaction time, oxygen/light exposure, the digestion juice composition, the As concentration used,

TABLE 8 Transformation of the AsB standard to other As species (calculated for As, ng) during *in vitro* gastric digestion with radical scavengers ($n = 3$).

	AsB	AsC	DMA	MMA	As(III)	As (V)
AsB (421.35 ng)	407.11 ± 3.57	< DL	1.19 ± 0.50	< DL	< DL	< DL

DL, the lowest detection limit.

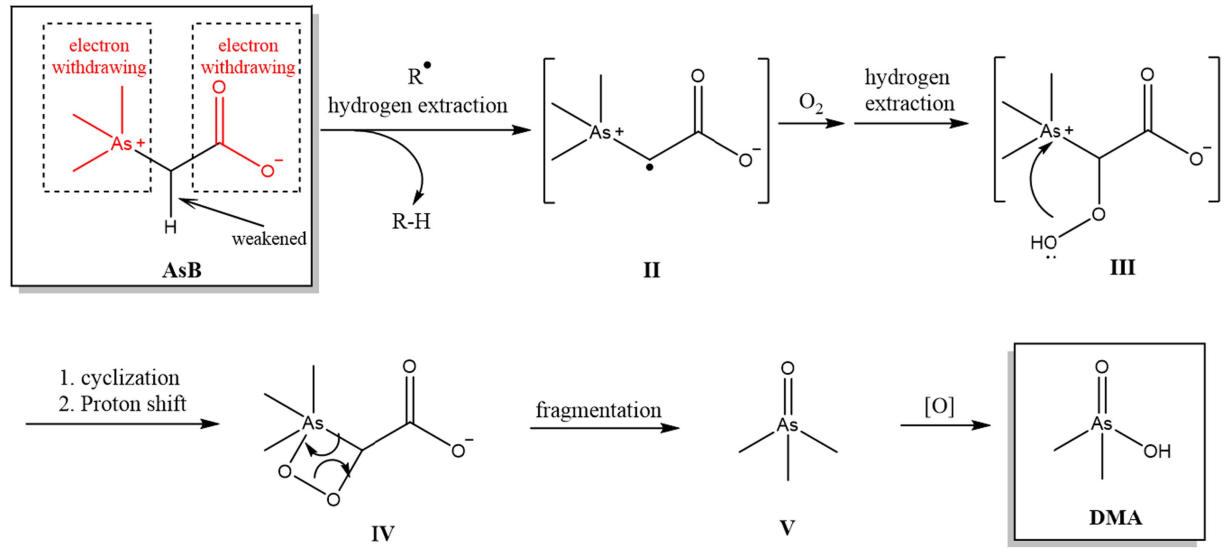

FIGURE 1
Hypothetical oxidation reaction pathway of AsB.

and analytical performance, may result in a failure to observe such interconversion during *in vitro* digestion (15, 40–44) or significant differences in the suggested reaction sites (stomach or intestine), pathways, and transformation rates (19–21, 36). Besides further validation with more samples of different species, areas, culturing, and processing conditions, another possible way to solve the task is the fabrication of more reasonable and standardized *in vitro* digestion models, such as the use of dynamic models in place of traditional static ones to simulate human digestion more accurately. In this case, the influence of gut microbes should be given full emphasis, considering that many studies have confirmed the significant As interconversion by these microbes. The research conducted by Wiele et al. demonstrated the potential of human colonic microbiota to actively metabolize arsenic into methylated arsenic species and thioarsenates. Additionally, Chi et al. found that disruption of the gut microbiota can alter the methylation transformation of arsenic (22, 45).

3.3. Proposed reaction mechanism

Until now, the detailed mechanism of the transformation among different As species remains unclear, especially for oAs such as AsB and AsC. It is widely accepted that the oxidation of As species follows a radical mechanism, although most of these well-controlled transformations utilize photo and/or catalytical activation for the rapid generation of radicals (46–48). However, the absence of these activation factors does not necessarily quench radical oxidation reactions as reactive oxygen radicals exist ubiquitously. Thus, in the present system, we propose that radical oxidation is also the key step governing the degradation of organoarsenic species. To test this hypothesis, AsB was used as a model and a mixture of radical scavengers was added to the oxidation system (keeping the rest of the parameters unchanged). When the concentration of the radical scavengers was in the same order of magnitude as that of AsB, the result (Table 8) showed a significant suppression in DMA formation, which proved the importance of a radical reaction in the process of oxidation.

Another interesting phenomenon was observed during the oxidation of AsB and AsC. The two arsoniums are the major As compounds found in seafood and other foodstuffs, but provide different oxidative products: AsB was oxidized to DMA, whereas AsC yielded MMA. This difference is tentatively explained by different oxidation sequences resulting from structural variation: the oxidation of oAs is a combination of two general types of reaction: the formation of an O-As bond and the breakage of a C-As bond (49). Owing to the different structures of AsB and AsC, the order of these fundamental steps may also be different and result in different key intermediates that yield completely different final products. For example (Figure 1), both the arsonium and carboxylic moieties in AsB are electron withdrawing, which significantly weakens the C-H of the α-H of arsonium through an inducing effect and activates the bond for hydrogen extraction (Figure 1, I- > II) by exiting radicals (R). The resulting radical may be oxygenated (Figure 1, II- > III), then, driven by the high nucleophilicity of peroxide, cyclizes to form a four-membered ring (Figure 1, III- > IV). Owing to the instability of the O-O bond and high ring tension, this 4-membered ring

system is prone to fragment (50–52), yielding intermediate V (Figure 1, IV- > V), and the latter could be further oxidized to DMA. On the other hand, the α-H of AsC is less activated as the adjacent CH_2OH group is generally considered as an electron donating one. Thus, the oxidation might have occurred on As first, and then the C-As bond is cleaved to release MMA. It should be noted that, regarding the difference between the *in vitro* and *in vivo* digestion conditions, the real transformation rate of different As species (and the mechanism involved) in the human body still needs further investigation, in which the influence of food matrix and gut microbes should also be considered.

4. Conclusion

In this study, the bio-accessibility of different As species in different seafood samples was determined, and a significant difference was observed. Unlike fish samples, the content of As(V) in digested crabs and scallops was demonstrated to be approximately 2–3 times greater than those of the pre-digestion samples. These results indicated an underestimated risk of these foodstuffs. In further experiments, arsenocholine, dimethylarsinate, arsenobetaine, and monomethylarsonate were all confirmed to be able to convert to As(V) during *in vitro* digestions with different efficiencies. The transformation could be affected by many factors, such as pH, time, and digestion juice, of which low pH seems particularly important. Free radicals were found to be responsible for the oxidation in the transformation reactions. Unlike arsenobetaine, arsenocholine appears to be able to be transformed directly to monomethylarsonate without the intermediate dimethylarsinate. These results provided much new and detailed information on the dynamic changes of As species during seafood digestion, which allowed us to suggest the great scientific significance of a more accurate risk assessment of As in aquatic products and other foodstuffs.

Data availability statement

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

Ethics statement

The studies involving animals were reviewed and approved by the Animal Experimental Ethics Review Committee of School of Food Science and Engineering, Ocean University of China.

Author contributions

LC, HL, and XS: conceptualization. JS, XH, RF, and BL: experimental design and methodology. BL: formal analysis, investigation, data curation, and writing—original draft preparation. LC and XS: writing—review and editing. LC: funding acquisition. 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/fnut.2023.1207732/full#supplementary-material

References

1. Braman RS, Foreback CC. Methylated forms of arsenic in the environment. *Science*. (1973) 182:1247–9. doi: 10.1126/science.182.4118.1247

2. Craigmile PF, Calder CA, Li H, Rajib P, Noel C. Hierarchical model building, fitting, and checking: a behind-the-scenes look at a Bayesian analysis of arsenic exposure pathways. *Bayesian Anal*. (2009) 4:1–35.

3. Jia Y, Wang L, Ma L, Yang Z. Speciation analysis of six arsenic species in marketed shellfish: extraction optimization and health risk assessment. *Food Chem*. (2018) 244:311–6. doi: 10.1016/j.foodchem.2017.10.064

4. Le XC, Lu X, Li XF. Peer reviewed: arsenic speciation. *Anal Chem*. (2004) 76:26A–33A. doi: 10.1021/ac041492r

5. Zhong Y, Chen J, Wang S, Chen J, Li R, Jia X, et al. Research progress of arsenic speciation and toxicity in seafood. *Food Ferment Indus*. (2022) 48:337–43.

6. IARC Working Group on the Evaluation of Carcinogenic Risks to Humans. Arsenic, metals, fibres, and dusts. *IARC Monogr Eval Carcinog Risks Hum*. (2012) 100:11–465.

7. European, Commission (2006). B commission regulation (EC) no. 1881/2006 of 19 December 2006 setting maximum levels for certain contaminants in foodstuffs.

8. GB2762-2017. China National Food Safety Standard Maximum Limit of Contaminants in Food is a national pollutant limit standard issued by the National Health and Family Planning Commission and the National Medical Products Administration of the PRC on March 17, 2017 and implemented on September 17, 2017. Available at: http://www.nhc.gov.cn/sps/s7891/201704/b83ad058ff544ee39dea811264878981.shtml

9. Signes-Pastor AJ, Carey M, Meharg AA. Inorganic arsenic in rice-based products for infants and young children. *Food Chem*. (2016) 191:128–34. doi: 10.1016/j.foodchem.2014.11.078

10. Zhao QY, Xiong HY, Ye ZJ, Huang LP, Zhang W. Biodegradation of arsenobetaine into inorganic arsenic in mice. *Asian J Ecotoxicol*. (2023) 18:1–11.

11. Rodriguez RR, Basta NT, Casteel SW, Pace LW. An in vitro gastrointestinal method to estimate bioavailable arsenic in contaminated soils and solid media. *Environ Sci Technol Easton Pa*. (1999) 33:642–9. doi: 10.1021/es980631h

12. Ruby MV, Davis A, Link TE, Schoof R, Chaney RL, Freeman GB, et al. Development of an in vitro screening test to evaluate the in vivo bioaccessibility of ingested mine-waste lead. *Environ Forensic*. (1993) 27:2870–7. doi: 10.1021/es00049a030

13. Wragg J, Cave M, Taylor H, Basta N, Brandon E, Casteel S, et al. Inter-laboratory trial of a unified bioaccessibility testing procedure. *Inter-lab Trial Unified Bioaccessibility Test Proced*. (2010) 409:19.

14. Berenguel O, Pessoa GDS, Arruda MAZ. Total content and in vitro bioaccessibility of tellurium in Brazil nuts. *J Trace Elem Med Biol*. (2018) 48:46–51. doi: 10.1016/j.jtemb.2018.02.026

15. Chi H, Zhang Y, Williams PN, Lin S, Hou Y, Cai C. In vitro model to assess arsenic bioaccessibility and speciation in cooked shrimp. *J Agric Food Chem*. (2018a) 66:4710–5. doi: 10.1021/acs.jafc.7b06149

16. Wragg J, Cave M, Basta N, Brandon E, Casteel S, Denys S, et al. An inter-laboratory trial of the unified BARGE bioaccessibility method for arsenic, cadmium and lead in soil. *Sci Total Environ*. (2011) 409:4016–30. doi: 10.1016/j.scitotenv.2011.05.019

17. Mackie A, Mulet-Cabero A-I, Torcello-Gomez A. Simulating human digestion: developing our knowledge to create healthier and more sustainable foods. *Food Funct*. (2020) 11:9397–431. doi: 10.1039/D0FO01981J

18. Wang YA, Liu K, Lu MS, Shi JC, Xu YJ, Liu YF. Comparative evaluation of static and dynamic simulated digestion models. *J Sci Food Agric*. (2023) 103:5893–903. doi: 10.1002/jsfa.12692

19. Chavez-Capilla T, Beshai M, Maher W, Kelly T, Foster S. Bioaccessibility and degradation of naturally occurring arsenic species from food in the human gastrointestinal tract. *Food Chem*. (2016) 212:189–97. doi: 10.1016/j.foodchem.2016.05.163

20. Liao W, Wang G, Li K, Zhao W. Change of arsenic speciation in shellfish after cooking and gastrointestinal digestion. *J Agric Food Chem*. (2018) 66:7805–14. doi: 10.1021/acs.jafc.8b02441

21. Zhao YF, Wu JF, Shang DR, Ning JS, Ding HY, Zhai YX. Arsenic species in edible seaweeds using in vitro biomimetic digestion determined by high-performance liquid chromatography inductively coupled plasma mass spectrometry. *Hindawi Publish Corp*. (2014) 2014:436347

22. Wiele TVD, Gallawa CM, Kubachk KM, Creed JT, Basta N, Dayton EA, et al. Arsenic metabolism by human gut microbiota upon in vitro. *Digest Contamin Soils*. (2010) 118:1004–9.

23. Calatayud M, Bralatei E, Feldmann J, Devesa V, Vélez D. Transformation of arsenic species during in vitro gastrointestinal digestion of vegetables. *J Agric Food Chem*. (2013) 61:12164–70. doi: 10.1021/jf4034738

24. Xu F, Chen P, Yuan Y, Zhang S, Xu Y. Effects of steaming process on the distribution of arsenic in different tissues of the scallops (Chlamys farreri). *Food Control*. (2020) 123:107694

25. Chen P. (2020). Establishment of analysis method of arsenic in scallop skirt. Ocean University of China.

26. Oomen AG, Hack A, Minekus M, Zeijdner E, Wijnen JHV. Comparison of five in vitro digestion models to study the bioaccessibility of soil contaminants. *Environ Sci Technol*. (2002) 36:3326–34. doi: 10.1021/es010204v

27. Maulvault A.L., Machado R., Afonso C., Louren, O, H.M., Nunes M.L., Coelho I., Langerholc T., Marques A. (2011). Bioaccessibility of hg, cd and as in cooked black scabbard fish and edible crab. *Food Chem Toxicol* 49, 2808–2815. doi: 10.1016/j.fct.2011.07.059

28. Aida S, Alma R, Vedrana K, Alma M, Lamija A. The content of arsenic in nectarine fruit (*Prunus persica var. nucipersica* Schnied.) with risk assessment for human health on area of Herzegovina. *Glasilo Future*. (2019) 2:1–2.

29. Ehsan N, Shan A, Riaz S, Zaman QU, Jabeen M. Health risk assessment due to exposure of arsenic contamination in drinking water of district Shiekhupura, Punjab, Pakistan. *Hum Ecol Risk Assess*. (2018) 3:1–15.

30. Uddh-Söderberg TE, Gunnarsson SJ, Hogmalm KJ, Lindegård MIBG, Augustsson ALM. An assessment of health risks associated with arsenic exposure via consumption of homegrown vegetables near contaminated glassworks sites. *Sci Total Environ*. (2015) 536:189–97. doi: 10.1016/j.scitotenv.2015.07.018

31. Kimpe JD, Cornelis R, Vanholder R. In vitro methylation of Arsenite by rabbit liver cytosol: effect of metal ions, metal chelating agents, methyltransferase inhibitors and uremic toxins. *Drug Chem Toxicol*. (1999) 22:613–28. doi: 10.3109/01480549908993171

32. Lu M, Wang H, Li XF, Lu X, Le XC. Enzymatic digestion and chromatographic analysis of arsenic species released from proteins. *J Chromatogr A*. (2009) 1216:3985–91. doi: 10.1016/j.chroma.2009.03.015

33. Shen S, Li XF, Cullen WR, Weinfeld M, Le XC. Arsenic binding to proteins. *Chem Rev*. (2013) 113:7769–92. doi: 10.1021/cr300015c

34. Namsa K, Yoshida T. Nitric acid oxidation. *J Synth Organ Chem Jpn*. (1966) 24:958–69. doi: 10.5059/yukigoseikyokaishi.24.958

35. Al-Abed SR, Jegadeesan G, Purandare J, Allen D. Arsenic release from iron rich mineral processing waste: influence of pH and redox potential. *Chemosphere*. (2007) 66:775–82. doi: 10.1016/j.chemosphere.2006.07.045

36. Leufroy A, Noël L, Beauchemin D, Guérin T. Bioaccessibility of total arsenic and arsenic species in seafood as determined by a continuous online leaching method. *Anal Bioanal Chem*. (2012) 402:2849–59. doi: 10.1007/s00216-012-5774-4

37. Vahter M, Marafante E, Dencker L. Metabolism of arsenobetaine in mice, rats and rabbits. *Sci Total Environ*. (1983) 30:197–211. doi: 10.1016/0048-9697(83)90012-8

38. Atallah RH, Kalman DA. Online PHOTOOXIDATION for the determination of ORGANOARSENIC compounds by atomic-absorption spectrometry with continuous arsine generation. *Talanta*. (1991) 38:167–73. doi: 10.1016/0039-9140(91)80125-J

39. Palacios MA, Gomez M, Camara C, Lopez MA. Stability studies of arsenate, monomethylarsonate, dimethylarsinate, arsenobetaine and arsenocholine in deionized water, urine and clean-up dry residue from urine samples and determination by liquid chromatography with microwave-assisted oxidation-hydride generation atomic absorption spectrometric detection. *Anal Chim Acta*. (1997) 340:209–20. doi: 10.1016/S0003-2670(96)00525-9

40. Calatayud M, Xiong C, Du Laing G, Raber G, Francesconi K, Van De Wiele T. Salivary and gut microbiomes play a significant role in in vitro Oral bioaccessibility, biotransformation, and intestinal absorption of arsenic from food. *Environ Sci Technol*. (2018) 52:14422–35. doi: 10.1021/acs.est.8b04457

41. Fu YQ, Yin NY, Cai XL, Du HL, Wang PF, Sultana MS, et al. Arsenic speciation and bioaccessibility in raw and cooked seafood: influence of seafood species and gut microbiota. *Environ Pollut*. (2021) 280:116958. doi: 10.1016/j.envpol.2021.116958

42. Laparra JM, Velez D, Barbera R, Montoro R, Farre R. Bioaccessibility and transport by Caco-2 cells of organoarsenical species present in seafood. *J Agric Food Chem*. (2007) 55:5892–7. doi: 10.1021/jf070490f

43. Moreda-Pineiro J, Alonso-Rodriguez E, Romaris-Hortas V, Moreda-Pineiro A, Lopez-Mahia P, Muniategui-Lorenzo S, et al. Assessment of the bioavailability of toxic and non-toxic arsenic species in seafood samples. *Food Chem*. (2012) 130:552–60. doi: 10.1016/j.foodchem.2011.07.071

44. Tokalolu E, Clough R, Foulkes M, Worsfold P. Stability of arsenic species during bioaccessibility assessment using the in vitro UBM and HPLC-ICP-MS detection. *Biol Trace Elem Res*. (2020) 198:332–8. doi: 10.1007/s12011-020-02066-2

45. Chi L, Xue J, Tu P, Lai Y, Ru H, Lu K. Gut microbiome disruption altered the biotransformation and liver toxicity of arsenic in mice. *Arch Toxicol*. (2019) 93:25–35. doi: 10.1007/s00204-018-2332-7

46. Guan X, Du J, Meng X, Sun Y, Sun B, Hu Q. Application of titanium dioxide in arsenic removal from water: a review—ScienceDirect. *J Hazard Mater*. (2012) 215-216:1–16. doi: 10.1016/j.jhazmat.2012.02.069

47. Gupta AD, Rene ER, Giri BS, Pandey A, Singh H. Adsorptive and photocatalytic properties of metal oxides towards arsenic remediation from water: a review. *J Environ Chem Eng*. (2021) 9:106376. doi: 10.1016/j.jece.2021.106376

48. Liu YZ, Guo RT, Duan CP, Wu GL, Pan WG. Removal of gaseous pollutants by using 3DOM-based catalysts: a review. *Chemosphere*. (2020) 262:127886

49. Allen DW, Jackson G. Steric and electronic effects of substituents at arsenic on the decomposition of arsonium betaines. *J Organomet Chem*. (1976) 110:315–20. doi: 10.1016/S0022-328X(00)82490-0

50. Akasaka T, Sato R, Miyama Y, Ando W. Reaction of singlet oxygen with indanone phosphazine: formation of ketone and lactone. *Tetrahedron Lett*. (1985) 26:843–6. doi: 10.1016/S0040-4039(00)61944-5

51. Watanabe N, Ijuin HK, Takatsuka H, Matsumoto M. Solvent effect on base-induced chemiluminescent decomposition of bicyclic dioxetanes bearing a 3-hydroxyphenyl group. *Tetrahedron*. (2021) 88:132147. doi: 10.1016/j.tet.2021.132147

52. Watanabe N, Takatsuka H, Ijuin HK, Matsumoto M. Highly effective and rapid emission of light from bicyclic dioxetanes bearing a 3-hydroxyphenyl substituted with a 4-p-oligophenylene moiety in an aqueous system: two different ways for the enhancement of chemiluminescence efficiency. *Tetrahedron*. (2020) 76:131203. doi: 10.1016/j.tet.2020.131203

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EDITED BY
Siddika Yalcin,
Hacettepe University, Türkiye

REVIEWED BY
Carla Ferreri,
National Research Council (CNR), Italy
Anandita Pal,
University of North Carolina at Chapel Hill, United States
Seda Ciftci,
Izmir Democracy University, Türkiye

*CORRESPONDENCE
Roger W. Godschalk
✉ r.godschalk@maastrichtuniversity.nl

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Maternal fatty acid status during pregnancy versus offspring inflammatory markers: a canonical correlation analysis of the MEFAB cohort

Sven H. Rouschop[1], Agnieszka Smolinska[1], Marij Gielen[2], Renate H. M. de Groot[3], Maurice P. Zeegers[2], Antoon Opperhuizen[1,4], Frederik J. van Schooten[1] and Roger W. Godschalk[1]*

[1]Department of Pharmacology and Toxicology, School for Nutrition and Translational Research in Metabolism (NUTRIM), Maastricht University, Maastricht, Netherlands, [2]Department of Epidemiology, CAPHRI Care and Public Health Research Institute, Maastricht University, Maastricht, Netherlands, [3]Department Conditions for Life Long Learning, Faculty of Educational Sciences, Open University of the Netherlands, Heerlen, Netherlands, [4]Nederlandse Voedsel en Warenautoriteit (NVWA), Utrecht, Netherlands

The development of inflammatory lung disorders in children may be related to maternal fatty acid intake during pregnancy. We therefore examined maternal fatty acid (FA) status during pregnancy and its associations with inflammatory markers and lung conditions in the child by analyzing data from the MEFAB cohort using multivariate canonical correlation analysis (CCA). In the MEFAB cohort, 39 different phospholipid FAs were measured in maternal plasma at 16, 22 and 32 weeks of pregnancy, and at day of birth. Child inflammatory markers and self-reported doctor diagnosis of inflammatory lung disorders were assessed at 7 years of age. Using CCA, we found that maternal FA levels during pregnancy were significantly associated with child inflammatory markers at 7 years of age and that Mead acid (20:3n-9) was the most important FA for this correlation. To further verify the importance of Mead acid, we examined the relation between maternal Mead acid levels at the day of birth with the development of inflammatory lung disorders in children at age 7. After stratification for the child's sex, maternal Mead acid levels at day of birth were significantly related with self-reported doctor diagnosis of asthma and lung infections in boys, and bronchitis and total number of lung disorders in girls. Future studies should investigate whether the importance of Mead acid in the relation between maternal FA status and inflammation and lung disorders in the child is due to its role as biomarker for essential fatty acid deficiency or due to its own biological function as pro-inflammatory mediator.

KEYWORDS

childhood asthma, canonical correlation analyses (CCA), fatty acid, pregnancy, inflammation

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Introduction

Inflammatory lung diseases are a major burden to global health. According to the World Health Organization (WHO), respiratory infections are the fourth leading cause of both death and burden of disease in the world (1). Similarly, asthma prevalence has increased over the past few decades (2–4), making asthma the most common chronic disease in children (5). The development of inflammatory lung disorders in children has been related to a maternal fat intake during pregnancy. Additionally, animal studies have demonstrated that maternal high fat diets during gestation predispose weanling offspring to airway inflammation, airway hyperresponsiveness, and increased susceptibility to Respiratory Syncytial Virus (RSV) infection (6, 7). Likewise, both observational and intervention studies in humans have shown that the risk of having asthma and asthma-related symptoms in offspring is negatively correlated with maternal intake of n-3 polyunsaturated fatty acids (PUFAs) (8–13). Furthermore, maternal fatty acid (FA) status during pregnancy has been linked to inflammation in general and the development of the immune system in the child (14); more specifically the n-6/n-3 PUFA ratio in the maternal diet seems to be important, with n-6 FA in the maternal diet as pro-inflammatory mediators, whereas n-3 FA were reported to have anti-inflammatory effects.

When examining maternal FA status during pregnancy and its relation with offspring health, FA levels are often estimated through the use of food frequency questionnaires (FFQs) (9, 15) rather than using objective FA biomarkers, such as plasma phospholipids. Furthermore, studies usually assess maternal FAs at a single prenatal time point (16, 17) instead of measuring the FA status throughout pregnancy. Finally, the relation between maternal FA status and offspring's health is commonly studied by analyzing single associations between an individual exposure variable and an individual outcome parameter (9, 15–17). However, the complex etiology of biological disorders may be better studied by examining a set of exposure variables and a set of outcome parameters simultaneously (18). Therefore, the aim of this study was to examine maternal FA status and the association with inflammation and lung disorders in the child, taking these shortcomings into account.

We used data of the MEFAB cohort that was established to study the associations between maternal FA status during pregnancy and pregnancy outcomes (19). In this cohort, maternal plasma phospholipid FAs were measured at 16, 22, and 32 weeks of pregnancy, and at day of birth, and various child inflammatory markers were measured at 7 years of age. These inflammation endpoints included: total white blood cell, monocyte, granulocyte and lymphocyte count, and blood concentrations of tissue plasminogen activator (tPA), plasminogen activator inhibitor-1 (PAI-1), leptin, fibrinogen and C-reactive protein (CRP). The simultaneous correlation analysis of multiple independent variables (here: FA in maternal blood) and multiple dependent variables (here: inflammatory outcomes) can be performed by canonical correlation analysis (CCA). Although CCA is used in many research areas, such as social and behavioral research (20), bioinformatics (21), and genetics (22), CCA has not yet been used before to study the relation between maternal fatty acid status and child inflammation. Finally, we investigated whether the maternal FA concentrations during pregnancy that contributed most to the CCA analysis correlated with the self-reported doctor diagnosis of bronchitis, asthma and lung infections in the child at the age of 7 years.

Methods

A schematic overview of the study design and analysis workflow are presented in Figure 1.

Study design, setting and participants

Pregnant women attending one of three antenatal clinics in the south the Netherlands between 1989 and 1995 were asked to participate in the cohort. Women were eligible for participation if they were less than 16 weeks pregnant and did not suffer from any cardiovascular, neurological, renal or metabolic condition. Originally, 1,203 women were included in the MEFAB cohort. For the follow-up, 305 singletons born between 1990 and 1994 were included between 1997 and 2000. For the current analysis, only mother–child pairs were included for which there were no missing data in any of the maternal plasma fatty acid concentrations or child plasma inflammatory markers, leading to a total number of 173 mother–child pairs. The study was approved by the medical ethics committee of the University hospital Maastricht and Maastricht University. Written consent was given by pregnant women before enrolment in the initial study and again by both parents (if possible) for the follow-up study.

Exposure variables

To measure maternal FA status during pregnancy, maternal venous blood samples were collected in EDTA tubes around 16, 22 and 32 weeks of pregnancy, and immediately after delivery. Concentrations of 39 different plasma phospholipid FAs were determined by capillary gas–liquid chromatography as described previously (23) and expressed in mg/L. See Supplementary Table S1 for a list of the FAs that were measured. Plasma was stored at −80°C until fatty acid analysis. To check whether the fatty acid profiles changed with different lengths of storage, Pearson correlation coefficients were calculated between the relative amounts of fatty acid in maternal or umbilical plasma phospholipid and storage time. Since no significant correlations were observed, an effect of storage time on the fatty acid profile was excluded. Changes in maternal fatty acid status throughout pregnancy were studied by including all 39 fatty acids in a principal component analysis (PCA) for each of the 4 time points during and directly after pregnancy.

Outcome variables

At 7 years of age, offspring inflammatory markers were measured in plasma. These markers included: total white blood cell, monocyte, granulocyte and lymphocyte count ($\times 10^9$/L) determined by Beckman-Coulter Gen-s (Beckman Coulter, Brea, CA, USA); tissue plasminogen activator (tPA) concentration (ng/ml) and enzyme activity (IU/ml) measured by ELISA and bioimmunoassay, respectively (Biopool International, Ventura, CA, USA); plasminogen activator inhibitor-1 (PAI-1) concentration (ng/ml); leptin concentration (µg/l) determined by RIA (Linco Research, St Charles, MO, USA); fibrinogen concentration (g/l) measured by the Clauss method (24); and C-reactive protein (CRP) concentration (mg/l) assayed with an in-house ELISA using polyclonal antibodies as catching and tagging

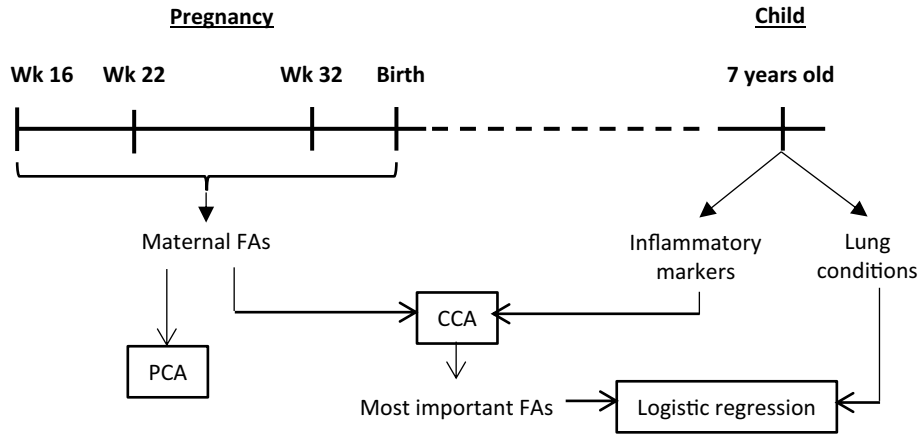

FIGURE 1
Schematic overview of the study design and analysis workflow. Wk, weeks of pregnancy; FAs, fatty acids; PCA, principal component analysis; CCA, canonical correlation analysis.

antibodies labelled with HRP (Dako, Glostrup, Denmark). To assess the children's history of lung disorders, their parents were asked at follow-up whether their child was ever diagnosed by a medical doctor with asthma, bronchitis, or pneumonia which was treated with antibiotics.

Confounding variables

Information on potential confounders was obtained by using medical records and questionnaires for maternal age (years) and BMI (kg/m^2) at study entry, duration of pregnancy (days), child sex, and child birth weight (grams). Information regarding maternal smoking and alcohol consumption during pregnancy (yes or no), breastfeeding (yes or no) with or without a combination of formula feeding, presence of pets (dog, cat, rodent or bird) at home during childhood (yes or no), and daycare attendance during first 4 years (yes or no) was obtained from questionnaires at the age of 7 years of the child. Additionally, the child's BMI (kg/m^2) was assessed by a nurse during a follow-up visit.

Canonical correlation analysis

The relation between maternal FA status and child inflammatory status was studied using CCA. CCA is a method which explores the linear relationship between two multivariate data sets X and Y (Equation 1). CCA does this by making separate linear combinations (called canonical variates) of both data sets by multiplying each separate variable in X or Y with a coefficient (a so-called canonical weight) and taking the sum of these products (Equation 2). Canonical weights are chosen in such a way that the correlation between the canonical variates of X and Y is maximized. CCA develops as many pairs of canonical variates as there are variables in the smallest of the two data sets. Furthermore, all canonical variates are orthogonal (uncorrelated) of each other, to make sure that each pair of canonical variates represents a different relationship between X and Y. To test if the correlation between two canonical variates is significant, an asymptotic test using F-approximation of Wilks' Lambda was applied. Only canonical variate pairs with a significant correlation were used for biological interpretation. Individual variables were considered to have a considerable contribution to a canonical correlation if their canonical loadings, which represent the correlation between the individual variable and the conical variate, were > |0.3|.

$$X = \begin{pmatrix} x_1 \\ x_2 \\ \dots \\ x_p \end{pmatrix}, \mathrm{Y} = \begin{pmatrix} y_1 \\ y_2 \\ \dots \\ y_q \end{pmatrix} \quad (1)$$

$$\begin{aligned} U_1 &= a_{11}x_1 + a_{12}x_2 + \ldots + a_{1p}x_p, V_1 = b_{11}y_1 + b_{12}y_2 + \ldots + b_{1p}y_p \\ U_2 &= a_{21}x_1 + a_{22}x_2 + \ldots + a_{2p}x_p, V_2 = b_{21}y_1 + b_{22}y_2 + \ldots + b_{2p}y_p \\ &\ldots \\ U_p &= a_{p1}x_1 + a_{p2}x_2 + \ldots + a_{ppx_p}, V_{\boldsymbol{p}} = b_{p1}y_1 + b_{p2}y_2 + \ldots + b_{pp}y_p \end{aligned} \quad (2)$$

Canonical correlation analysis explores the linear relationship between two multivariate data sets X and Y (Equation 1). It does so by making separate linear combinations (canonical variates) of both data sets by multiplying each separate variable in X or Y with a canonical weight and taking the sum of these products (Equation 2).

Model optimization

First a full CCA model was obtained using all 39 maternal fatty acids as independent variables and all child inflammatory markers as dependent variables (Figure 2A). To further identify which specific fatty acids were most important for this relation, the number of independent variables in the model was reduced by deleting the fatty acid variable with the smallest contribution to the model (i.e., with the smallest absolute, standardized canonical weight) and the analysis was repeated (Figure 2B). By removing variables, information is withdrawn from the model and the correlation decreases. Therefore, this process of removing the least contributing independent variable was iterated

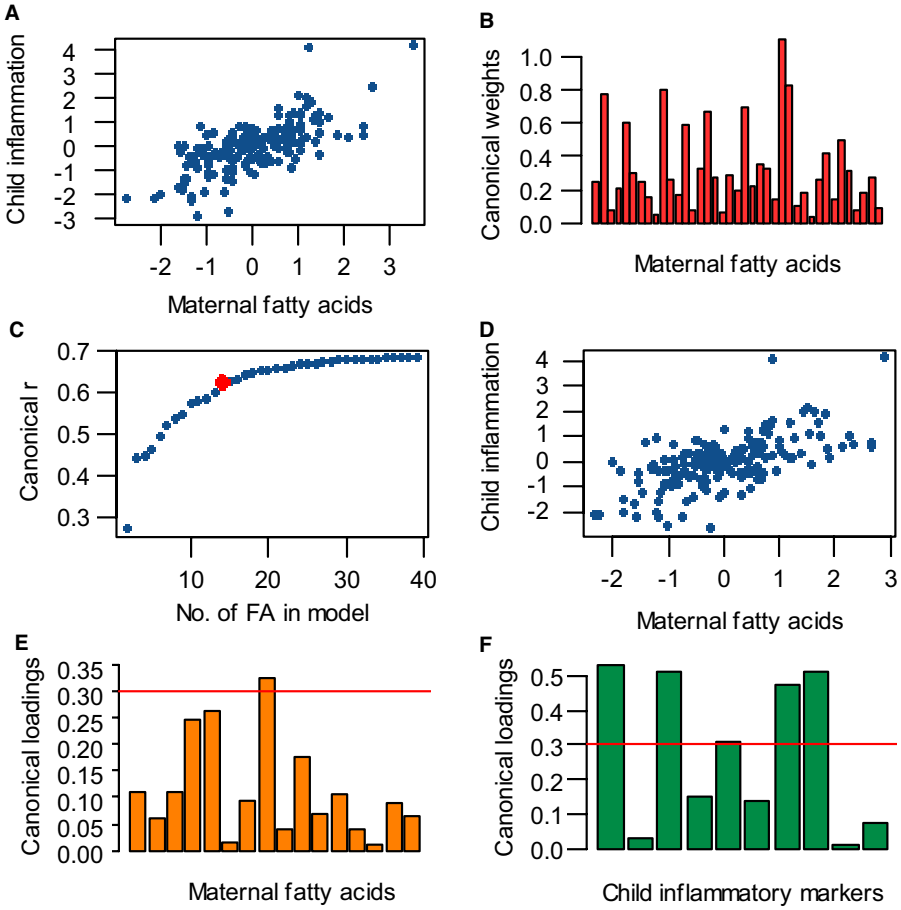

FIGURE 2
Analysis strategy for performing canonical correlation analysis (CCA), reducing the number of independent variables, obtaining an optimal model, and selecting the most important variables. **(A)** A full CCA model was obtained, containing all 39 maternal fatty acids vs. all child inflammatory markers. **(B)** To reduce the number of independent variables in the CCA model, the fatty acid variable with the lowest absolute, standardized weight was eliminated and the analysis was repeated. **(C)** The process of removing the least contributing independent variable and rerunning the analysis was iterated until an optimum was achieved between a low number of variables and a high correlation. This optimum was set at the point where the canonical correlation coefficient (r) starts decreasing with increasing intervals while cutting out independent variables (indicated by the red dot). **(D)** With the optimal number of independent variables, an optimal CCA model was made. **(E,F)** Fatty acids and inflammatory markers with an absolute loading >0.3 (cut-off indicated by the red line) were selected as most important for the canonical correlation.

until an optimal balance was achieved between a low number of independent variables and a high correlation (Figure 2C). This optimum was chosen to be at the point where the correlation coefficient started to visually decrease quicker or at a canonical $r \geq 0.6$. With this optimal number of independent variables, an optimal CCA model was made (Figure 2D). For all optimized CCA models, the most important maternal fatty acids and child inflammatory markers were identified by selecting variables with an absolute canonical loading >|0.3| (Figures 2E,F). Potential confounders were initially not included in the analysis, but were taken into account in follow up logistic regression analyses for the occurrence of lung disorders.

Logistic regression

The relation between maternal FA variables with a canonical loading >|0.3| and offspring lung disorders was assessed using binomial and ordinal logistic regression. The influence of potential confounders on logistic regression was determined by including the potential confounder as a covariate in the analysis. The variable was considered to be a confounder if inclusion of the confounder led to a significant covariate effect, the main effect not being significant anymore, or a change >10% of the main effect odds ratio. For all statistical analyses, $p < 0.05$ was considered significant. All statistical analyses were performed using R version 4.0.2 (R Foundation for Statistical Computing, Vienna, Austria). CCA was done with CCA package version 1.2.

Results

Population characteristics

Practically all mothers included in the analysis were Caucasian (99%; Table 1). On average, mothers were 30 years-old when entering the study and had a BMI of 24 kg/m^2 and a normal pregnancy duration (279 days). Twenty percent of the mothers smoked during pregnancy, with a mean of nine cigarettes per day. Three percent consumed alcohol during pregnancy, but none specified how much. Deliveries were mostly vaginal (95%) and half of the infants were breastfed for

some period of time (47%). About half of the children were female (43%). On average, children had a normal birthweight (3,362 grams) and BMI at 7 years-of-age (15 kg/m^2).

Maternal plasma fatty acid status changed throughout pregnancy

To assess the progression of maternal fatty acid status during pregnancy, PCA was applied to maternal plasma concentrations of 39 different FAs during pregnancy. The result from this is shown in Figure 3, with each dot representing a mother, with different colors representing different prenatal time points and the position of the dot representing the overall FA status. The gradual shift of the cloud of dots from week 16 to week 22, to week 32, to partus indicates that the overall maternal FA profile gradually changed throughout pregnancy, with the largest shift occurring between 16 and 22 weeks of pregnancy.

Maternal fatty acid status during pregnancy correlated with child inflammatory status at 7 years of age

To assess the multivariate relation between maternal FA levels during pregnancy and inflammatory markers in the child at 7 years of age, CCA was used. Since PCA showed that the maternal fatty acid status gradually changed throughout pregnancy, a separate CCA was performed for each prenatal time point. The optimized CCA models for week 16, week 22 and week 32 of pregnancy and partus contained sixteen, fourteen, fifteen, and nineteen independent FA variables, respectively (Table 2) and all models were statistically significant (week 16: $r = 0.60$, $p < 0.001$; week 22: $r = 0.62$, $p < 0.001$; week 32: $r = 0.64$; $p = 0.001$; partus: $r = 0.61$, $p = 0.028$; Table 2). All lower-order canonical variates were not significant and thus not used for further interpretation (Supplementary Table S2). For each significant canonical variate, the most important independent and dependent variables were identified by selecting variables with an absolute canonical loading >0.3. Of these various fatty acids and inflammatory markers that were identified to be important for the different canonical correlations, Mead acid (20:3n9) and granulocyte count, respectively, occurred most often at the different time points (Table 2).

TABLE 1 Population characteristics of the MEFAB participants included in this study ($n = 173$ mother–child pairs).

Variable	Mean (SD)	Median (IQR)
Mother		
Ethnicity (Caucasian *n*, %)	*n* = 172, 99.4%	
Age at entry study (years)	30.12 (4.14)	29.54 (5.33)
BMI at entry study (kg/m^2)	23.56 (3.75)	23.05 (3.66)
Pregnancy		
Duration (days)	279 (11)	281 (12)
Smoking (*n*, %)	*n* = 34, 19.6%	
Cigarettes per day	9.17 (5.62)	9.50 (5.00)
Alcohol consumption (*n*, %)	*n* = 5, 2.9%	
Caesarian section (*n*, %)	*n* = 8, 4.6%	
Breastfeeding (*n*, %)	*n* = 81, 46.8%	
Child		
Sex (Female *n*, %)	*n* = 75, 43.4%	
Birthweight (grams)	3,362 (495)	3,400 (640)
BMI at 7 years*	15.47 (1.88)	15.03 (2.12)
Asthma (*n*, %)	*n* = 12, 6.9%	
Bronchitis (*n*, %)	*n* = 39, 22.5%	
Lung infection (*n*, %)	*n* = 12, 7.0%	

SD, standard deviation; IQR, interquartile range. *Please note that a BMI between 13.5 and 17.6 is considered normal for children at the age of 7 years.

Maternal Mead acid levels at day of birth are positively associated with the development of lung disorders in offspring

Since Mead acid (20:3n-9) showed a canonical loading of >|0.3| at 3 time points (week 22-partus) in the CCA, the relevance of maternal Mead acid levels during pregnancy was further assessed by examining the association between maternal plasma levels of Mead acid at day of birth and the odds of the child developing asthma, bronchitis and/or lung infection during the first 7 years of age. Only Mead acid data from the day of birth were used for this analysis, since Mead acid concentrations consistently increased throughout pregnancy, with the highest concentrations and the largest variation occurring at that specific time point (Figure 4). Using unadjusted binomial regression, a significant, positive relation was found between maternal Mead acid levels (mg/L) and self-reported doctor diagnosis of bronchitis (OR [95% CI] = 1.17 [1.01–1.34]) and lung infection (OR [95% CI] = 1.27 [1.04–1.55]), but not asthma (Table 3). Furthermore, unadjusted ordinal logistic regression showed a significant, positive association between maternal Mead acid concentrations at day of birth (mg/L) and the total number of self-reported doctor-diagnosed lung disorders that the child suffered from during the first 7 years of age (OR [95% CI] = 1.18 [1.03–1.34]; Table 3). Adjusting the logistic regression models for maternal age or BMI at study entry, gestational age, smoking or alcohol use during pregnancy, birthweight, breastfeeding, presence of pets, daycare attendance, or child BMI at 7 years of age left the effect estimate and significance of Mead acid largely unchanged (Supplementary Table S3). Stratification for child sex, however, did lead to sex-specific results. For asthma, bronchitis and number of lung conditions, associations were significant for girls (OR [95% CI] =1.77 [1.09–3.62], 1.32 [1.03–1.72], and 1.40 [1.10–1.80], respectively) but not boys, whereas for lung infection, associations were significant for boys (OR [95% CI] = 1.28 [1.02–1.63]), but not girls (Table 3).

Discussion

The development of inflammatory lung disorders has previously been shown to be related to the total fat content or the fatty acid composition of the maternal diet during pregnancy. However, estimates of prenatal exposures and statistical analysis were often suboptimal in previous studies. Therefore, this study examined the relation between maternal FA status and child inflammation by

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analyzing data from the MEFAB cohort using CCA. This paper showed that maternal plasma FA levels during pregnancy were significantly associated with child inflammatory markers at 7 years of age and that Mead acid was a fatty acid that contributed most to the CCA (loading >|0.3|) at different time points of pregnancy (from 22 weeks until delivery). In addition, we showed that maternal Mead acid levels at day of birth were significantly correlated with self-reported asthma, bronchitis and the total number of lung conditions in girls, and with self-reported lung infection in boys.

First, we confirmed with a PCA that included 39 different FAs that the profile of maternal FAs gradually changed throughout pregnancy. This finding emphasizes the importance of assessing FA levels at consistent prenatal time points when studying the relation between maternal FA status and offspring health, rather than assessing FA exposure at varying prenatal time points, as has been the case for previous studies. For instance, Notenboom et al. measured maternal FA levels in the 34th to 36th week of gestation (16), whereas Rucci et al. measured maternal FA levels around the 20th week of gestation (17), making comparison of both studies suboptimal. Moreover, Miyake et al. assessed maternal FA consumption at study entry, irrespective of the gestational age (9), thereby potentially introducing heterogeneity in the exposure data set. The current study, in contrast, measured maternal FA levels in plasma phospholipids at 16, 22 and 32 weeks of gestation, and at day of birth, thereby covering both the second and third trimester. In addition, maternal FA were assessed in phospholipids, which represent FA intake of the past few days (25). Altogether, the exposure variables as assessed in the current study may thus be considered as a comprehensive representation of prenatal maternal FA status throughout pregnancy.

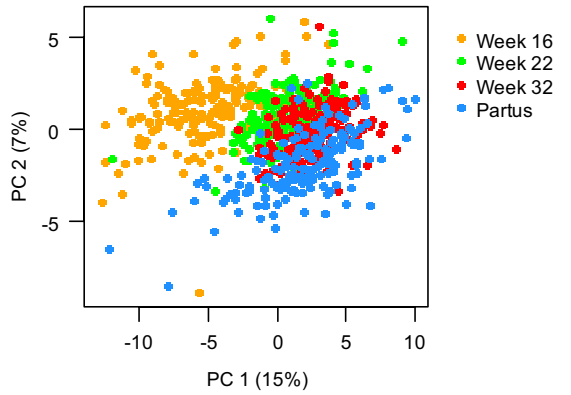

FIGURE 3
Principal component analysis of 39 maternal plasma fatty acid concentrations, measured at 16, 22, and 32 weeks of pregnancy, and at day of birth (partus). Data are presented as scatter plot of the first and second principal component (PC), covering 15 and 5%, respectively, of the total variance, with each dot representing one mother at a specific gestational time point ($n = 173$ mothers).

Using CCA, we examined the multivariate relation between child inflammatory markers at 7 years of age and maternal plasma FA levels at 16, 22 and 32 weeks of gestation, and at day of birth. With this approach, we found that for all prenatal time points, maternal blood concentrations of various FAs were significantly associated with child inflammatory markers at 7 years of age. Of those FA that were related to child inflammatory markers, Mead acid was identified as a significant maternal FA for these multivariate correlations at multiple time points (i.e., all time points after 22 weeks). Mead acid (20:3n-9) is an n-9 PUFA which is synthesized *in vivo* from oleic acid (18,1n-9) through elongation and desaturation by the enzymes Fatty acid desaturase 1 (Fads1), Fatty acid desaturase 2 (Fads2) and Elongation of very long chain fatty acids protein 5 (Elovl5) (26). Fads1, Fads2 and Elovl5 also elongate and desaturate the n-6 and n-3 PUFAs linoleic

TABLE 2 Canonical correlation analysis of maternal plasma fatty acid (FA) concentrations at 16, 22, and 32 weeks of pregnancy, and at day of birth (partus) versus child plasma inflammatory markers at 7 years of age ($n = 173$ mother–child pairs).

	No. of FAs in CCA model	*r*	Value of *p*	FAs |loading| > 0.3	Inflammatory markers |loading| > 0.3
Week 16	16	0.60	<0.001	24:1n9 18:1DMA	Monocytes Granulocytes tPA activity
Week 22	14	0.62	<0.001	20:3n9	White blood cells Granulocytes PAI-1 Leptin
Week 32	15	0.64	0.001	22:2n6 16:0DMA 20:3n9	White blood cells Granulocytes tPA activity tPA concentration PAI-1
Partus	19	0.61	0.028	20:3n9 24:1n9 22:2n6 16:0DMA	White blood cells Granulocytes tPA concentration PAI-1 CRP

r, canonical correlation coefficient.

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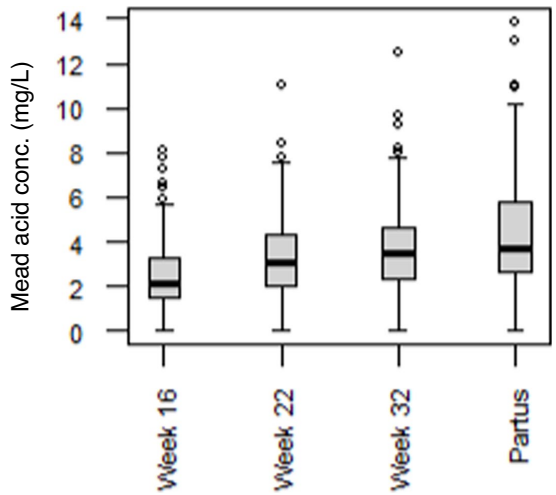

FIGURE 4
Mead acid concentrations in maternal plasma at 16, 22, and 32 weeks of pregnancy, and at day of birth (partus). Data are presented as boxplots, with dots representing values higher than 1.5 times the interquartile range above the third quartile (n = 173 mothers).

TABLE 3 Associations between maternal plasma mead acid concentration at day of birth (mg/L) and self-reported doctor diagnosis of lung disorders in the child during the first 7 years of age (n = 173 mother–child pairs).

	OR	95% CI	Value of p
Asthma			
Unstratified	1.13	0.91–1.38	0.228
Girls	1.77	1.09–3.62	0.038
Boys	1.03	0.77–1.29	0.842
Bronchitis			
Unstratified	1.17	1.01–1.34	0.030
Girls	1.32	1.03–1.72	0.029
Boys	1.11	0.93–1.33	0.227
Lung infection			
Unstratified	1.27	1.04–1.55	0.014
Girls	1.27	0.82–1.91	0.237
Boys	1.28	1.02–1.63	0.029
Number of lung disorders			
Unstratified	1.18	1.03–1.34	0.007
Girls	1.40	1.10–1.80	0.003
Boys	1.12	0.94–1.31	0.093

Statistical analysis by binomial logistic regression (for asthma, bronchitis and lung infection) or ordinal logistic regression (for number of lung conditions). OR (odds ratio), 95% CI (confidence interval) and value of p are shown for both unadjusted results and results stratified for child sex.

acid (LA, 18:2n-6) and α-linolenic acid (ALA, 18:3n-3) into their respective longer-chain derivatives, such as arachidonic acid (AA, 20:4n-6), eicosapentaenoic acid (EPA, 20:5n-3) and docosahexaenoic acid (DHA, 22:6n-3) (27). As a result of these shared metabolic pathways between n-3, n-6 and n-9 PUFAs, Mead acid synthesis is enhanced when n-3 and n-6 PUFA levels are low, making Mead acid to be considered as a biomarker for essential fatty acid deficiency (28). Next to this, Mead acid has its own biological functions. Similar to the conversion of AA into 5-hydroxyeicosatetraenoic acid (5-HETE) by 5-lipoxygenase (5-LOX) and its subsequent oxidation into 5-oxo-eicosatetraenoic acid (5-oxo-ETE) by 5-hydroxyeicosanoid dehydrogenase (5-HEDH), Mead acid is metabolized into 5-hydroxyeicosatrienoic acid (5-HETrE) and 5-oxo-eicosatrienoic acid (5-oxo-ETrE), respectively, by the same enzymes (29, 30). In accordance with these structural analogies, 5-oxo-ETrE is as potent as 5-oxo-ETE in inducing chemotaxis, surface expression of CD11b and calcium mobilization in neutrophils, as well as increasing actin polymerization in eosinophils (29). Mead acid thus may act as a pro-inflammatory mediator, thereby potentially contributing to the course of inflammatory lung disorders, such as lung infections, asthma and bronchitis. From these data, one could speculate that mothers should assure sufficient intake of essential fatty acids during pregnancy to avoid *de novo* synthesis of Mead acid.

In a canonical correlation analysis, multiple variables (here: maternal FA during and directly after pregnancy) are statistically linked to multiple dependent variables (here: inflammatory markers in the child at 7 years of age). Each independent and dependent variable has a certain contribution to the overall relationship. Next to Mead acid being identified as an important independent variable, the outcome variable that contributed most to the multivariate correlation between maternal FA status and child inflammation was granulocyte count. Granulocytes play an important role in various inflammatory lung disorders. For instance, both eosinophils and neutrophils are associated with clinical severity and pulmonary dysfunction in asthma patients (31–34). Similarly, fungal infection of the lungs is associated with pulmonary influx of eosinophils (35), whereas neutrophils contribute to early defense against pulmonary infection with bacteria (36). Mead acid may possibly affect lung conditions through its effect on granulocytes, because both eosinophils and neutrophils are involved in inflammatory lung conditions. Whether this hypothesis is correct and what molecular or cellular mechanisms would be involved in this, remains to be examined by future studies.

To further evaluate the relevance of maternal Mead acid levels during pregnancy, the association between maternal plasma levels of Mead acid at day of birth and the self-reported doctor diagnosis of lung disorders in children was examined. As a result, maternal Mead acid levels at day of birth were positively associated with the diagnosis of several lung conditions in children. After stratification for sex, these associations were significant for asthma, bronchitis and number of lung infections for girls but not boys, whereas for lung infection, associations were significant for boys but not girls. These sex-specific associations may be related to known differences between males and females regarding development of the lungs and lung disorders. For instance, boys are more likely to develop asthma than girls, whereas adult females have a higher prevalence than adult males (37), suggesting the involvement of sex hormones. Furthermore, neonate boys have a higher chance of mortality due to respiratory distress syndrome than neonate girls (38). Next to males and females having different lung development, differences may also occur in the way male and female offspring respond to changes in maternal diet during pregnancy, as we have shown previously (39). Future studies will have

to confirm whether indeed the relation between offspring lung disorders and maternal FA concentrations and specifically Mead acid concentrations differ between male and female offspring, and if so, what the underlying mechanisms are.

When interpreting results from the current study, it should be considered that the MEFAB cohort comprises a relatively healthy population. Although this consistency within the cohort enhances the comparability within participants, it also limits the applicability of the findings to less healthy populations, such as people with obesity. Furthermore, the MEFAB cohort has a relatively homogeneous genetic background, with 99% of the participants in the current analysis being Caucasian. Given the differences that exist in inflammatory markers between ethnicities (40, 41), the associations reported in this study may thus have been different if the ethnic background of the population would have been more diverse. Moreover, solely studying Caucasian participants in biomedical research contributes to health disparity. Future studies should therefore include other ethnicities as well, as did Rucci et al., who's Generation R cohort consisted of 66% Europeans and 34% non-Europeans (17). Finally, health outcome parameters were assessed at the age of 7 years, and there was no control over the child's lifestyle/environment between birth and the age of 7 years. In other words, although we observed a correlation between early life exposures and diseases later in life, it cannot be excluded that these are additionally related to exposures/lifestyle after birth.

In conclusion, our findings support the previously reported relation between inflammatory lung conditions in childhood and maternal fatty acid status during pregnancy. Furthermore, this work demonstrated the application of CCA within this field of research, which may lead to future studies using a similar approach. Lastly, future studies should investigate whether the importance of Mead acid in the relation between maternal FA status and child inflammation is due to its role as biomarker for essential fatty acid deficiency, due to its own biological function as pro-inflammatory mediator, or due to a combination of both.

Data availability statement

The data analyzed in this study is subject to the following licenses/restrictions: data can be made available by sending email to corresponding author. Requests to access these datasets should be directed to marij.gielen@maastrichtuniversity.nl.

Ethics statement

The studies involving humans were approved by Medical Ethics Committee, University Hospital, Maastricht/University of Maastricht, Netherlands. The studies were conducted in accordance with the local legislation and institutional requirements. Written informed consent for participation in this study was provided by the participants' legal guardians/next of kin.

Author contributions

SR: Formal analysis, Investigation, Methodology, Project administration, Visualization, Writing – original draft, Writing – review & editing. AS: Formal analysis, Methodology, Supervision, Writing – review & editing. MG: Conceptualization, Data curation, Project administration, Resources, Writing – review & editing. ReG: Investigation, Writing – review & editing. MZ: Funding acquisition, Investigation, Methodology, Resources, Supervision, Writing – review & editing. AO: Conceptualization, Funding acquisition, Investigation, Methodology, Supervision, Writing – review & editing. FS: Conceptualization, Investigation, Resources, Supervision, Writing – review & editing. RoG: Conceptualization, Investigation, Writing – original draft, Writing – review & editing.

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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

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References

1. World Health Organization. *Global health estimates: life expectancy and leading causes of death and disability*. Available at: https://www.who.int/data/gho/data/themes/mortality-and-global-health-estimates (Accessed August 13, 2021).

2. Centers for Disease Control and Prevention, Asthma. Available at: https://www.cdc.gov/asthma/data-visualizations/default.htm (Accessed June 23, 2021).

3. Asthma UK, Asthma prevalence. Available at: https://public.tableau.com/app/profile/asthmauk/viz/Asthmaprevalence/Asthmaprevalence (Accessed June 23, 2021).

4. Eurostat Data Browser. Persons reporting a chronic disease Available at: http://appsso.eurostat.ec.europa.eu/nui/show.do?dataset=hlth_ehis_cd1e&lang=en (Accessed June 23, 2021).

5. World Health Organization. *Asthma fact sheet*. Available at: https://www.who.int/news-room/fact-sheets/detail/asthma (Accessed August 13, 2021).

6. Griffiths PS, Walton C, Samsell L, Perez MK, Piedimonte G. Maternal high-fat hypercaloric diet during pregnancy results in persistent metabolic and respiratory abnormalities in offspring. *Pediatr Res*. (2016) 79:278–86. doi: 10.1038/pr.2015.226

7. MacDonald KD, Moran AR, Scherman AJ, McEvoy CT, Platteau AS. Maternal high-fat diet in mice leads to innate airway hyperresponsiveness in the adult offspring. *Physiol Rep*. (2017) 5:e13082. doi: 10.14814/phy2.13082

8. Lumia M, Luukkainen P, Tapanainen H, Kaila M, Erkkola M, Uusitalo L, et al. Dietary fatty acid composition during pregnancy and the risk of asthma in the offspring. *Pediatr Allergy Immu*. (2011) 22:827–35. doi: 10.1111/j.1399-3038.2011.01202.x

9. Miyake Y, Sasaki S, Tanaka K, Ohfuji S, Hirota Y. Maternal fat consumption during pregnancy and risk of wheeze and eczema in Japanese infants aged 16–24 months: the Osaka Maternal and Child Health Study. *Thorax*. (2009) 64:815–21. doi: 10.1136/thx.2009.115931

10. Pike KC, Calder PC, Inskip HM, Robinson SM, Roberts GC, Cooper C, et al. Maternal plasma phosphatidylcholine fatty acids and atopy and wheeze in the offspring at age of 6 years. *Clin Dev Immunol*. (2012) 2012:1–13. doi: 10.1155/2012/474613

11. Bisgaard H, Stokholm J, Chawes BL, Vissing NH, Bjarnadóttir E, Schoos A-MM, et al. Fish oil–derived fatty acids in pregnancy and wheeze and asthma in offspring. *N Engl J Med*. (2016) 375:2530–9. doi: 10.1056/NEJMoa1503734

12. Hansen S, Strøm M, Maslova E, Dahl R, Hoffmann HJ, Rytter D, et al. Fish oil supplementation during pregnancy and allergic respiratory disease in the adult offspring. *J Allergy Clin Immun*. (2017) 139:e104:104–111.e4. doi: 10.1016/j.jaci.2016.02.042

13. Olsen SF, Østerdal ML, Salvig JD, Mortensen LM, Rytter D, Secher NJ, et al. Fish oil intake compared with olive oil intake in late pregnancy and asthma in the offspring: 16 y of registry-based follow-up from a randomized controlled trial. *Am J Clin Nutr*. (2008) 88:167–75. doi: 10.1093/ajcn/88.1.167

14. Miles EA, Calder PC. Can early omega-3 fatty acid exposure reduce risk of childhood allergic disease? *Nutrients*. (2017) 9:784. doi: 10.3390/nu9070784

15. Nwaru BI, Erkkola M, Lumia M, Kronberg-Kippilä C, Ahonen S, Kaila M, et al. Maternal intake of fatty acids during pregnancy and allergies in the offspring. *Br J Nutr*. (2012) 108:720–32. doi: 10.1017/S0007114511005940

16. Notenboom M, Mommers M, Jansen E, Penders J, Thijs C. Maternal fatty acid status in pregnancy and childhood atopic manifestations: KOALA birth cohort study. *Clin Exp Allergy*. (2011) 41:407–16. doi: 10.1111/j.1365-2222.2010.03672.x

17. Rucci E, den Dekker H, de Jongste J, Steenweg-de-Graaff J, Gaillard R, Pasmans S, et al. Maternal fatty acid levels during pregnancy, childhood lung function and atopic diseases. The generation R study. *Clin Exp Allergy*. (2016) 46:461–71. doi: 10.1111/cea.12613

18. Thompson B. A primer on the logic and use of canonical correlation analysis. *Meas Eval Couns Dev*. (1991) 24:80–95.

19. van der Wurff IS, de Groot RH, Stratakis N, Gielen M, Hornstra G, Zeegers M. Maastricht essential fatty acid birth cohort. *Lipid Technol*. (2015) 27:59–62. doi: 10.1002/lite.201500007

20. Sherry A, Henson RK. Conducting and interpreting canonical correlation analysis in personality research: a user-friendly primer. *J Pers Assess*. (2005) 84:37–48. doi: 10.1207/s15327752jpa8401_09

21. Tripathi A, Klami A, Kaski S. Simple integrative preprocessing preserves what is shared in data sources. *BMC Bioinformat*. (2008) 9:1–13. doi: 10.1186/1471-2105-9-111

22. Naylor MG, Lin X, Weiss ST, Raby BA, Lange C. Using canonical correlation analysis to discover genetic regulatory variants. *PLoS One*. (2010) 5:e10395. doi: 10.1371/journal.pone.0010395

23. Al MD, Van Houwelingen AC, Kester AD, Hasaart TH, De Jong AE, Hornstra G. Maternal essential fatty acid patterns during normal pregnancy and their relationship to the neonatal essential fatty acid status. *Br J Nutr*. (1995) 74:55–68. doi: 10.1079/BJN19950106

24. Clauss A. Rapid physiological coagulation method in determination of fibrinogen. *Acta Haematol*. (1957) 17:237–46. doi: 10.1159/000205234

25. Arab L. Biomarkers of fat and fatty acid intake. *J Nutr*. (2003) 133:925S–32S. doi: 10.1093/jn/133.3.925S

26. Ichi I, Kono N, Arita Y, Haga S, Arisawa K, Yamano M, et al. Identification of genes and pathways involved in the synthesis of Mead acid (20: 3n− 9), an indicator of essential fatty acid deficiency. *Biochim Biophys Acta*. (2014) 1841:204–13. Available at: doi: 10.1016/j.bbalip.2013.10.013

27. Guillou H, Zadravec D, Martin PG, Jacobsson A. The key roles of elongases and desaturases in mammalian fatty acid metabolism: insights from transgenic mice. *Prog Lipid Res*. (2010) 49:186–99. doi: 10.1016/j.plipres.2009.12.002

28. Holman RT. The ratio of trienoic: tetraenoic acids in tissue lipids as a measure of essential fatty acid requirement. *J Nutr*. (1960) 70:405–10. doi: 10.1093/jn/70.3.405

29. Patel P, Cossette C, Anumolu JR, Gravel S, Lesimple A, Mamer OA, et al. Structural requirements for activation of the 5-oxo-6E, 8Z, 11Z, 14Z-eicosatetraenoic acid (5-oxo-ETE) receptor: identification of a mead acid metabolite with potent agonist activity. *J Pharmacol Exp Ther*. (2008) 325:698–707. doi: 10.1124/jpet.107.134908

30. Powell WS, Rokach J. Biochemistry, biology and chemistry of the 5-lipoxygenase product 5-oxo-ETE. *Prog Lipid Res*. (2005) 44:154–83. doi: 10.1016/j.plipres.2005.04.002

31. Bousquet J, Chanez P, Lacoste JY, Barnéon G, Ghavanian N, Enander I, et al. Eosinophilic inflammation in asthma. *N Engl J Med*. (1990) 323:1033–9. doi: 10.1056/NEJM199010113231505

32. Louis R, Lau LC, Bron AO, Roldaan AC, Radermecker M, Djukanovic R. The relationship between airways inflammation and asthma severity. *Am J Respir Crit Care Med*. (2000) 161:9–16. doi: 10.1164/ajrccm.161.1.9802048

33. Shaw DE, Berry MA, Hargadon B, McKenna S, Shelley MJ, Green RH, et al. Association between neutrophilic airway inflammation and airflow limitation in adults with asthma. *Chest*. (2007) 132:1871–5. doi: 10.1378/chest.07-1047

34. Woodruff PG, Khashayar R, Lazarus SC, Janson S, Avila P, Boushey HA, et al. Relationship between airway inflammation, hyperresponsiveness, and obstruction in asthma. *J Allergy Clin Immun*. (2001) 108:753–8. doi: 10.1067/mai.2001.119411

35. Wiesner DL, Smith KD, Kashem SW, Bohjanen PR, Nielsen K. Different lymphocyte populations direct dichotomous eosinophil or neutrophil responses to pulmonary Cryptococcus infection. *J Immunol*. (2017) 198:1627–37. doi: 10.4049/jimmunol.1600821

36. Lowe DM, Redford PS, Wilkinson RJ, O'Garra A, Martineau AR. Neutrophils in tuberculosis: friend or foe? *Trends Immunol*. (2012) 33:14–25. doi: 10.1016/j.it.2011.10.003

37. Crawford W, Beedham C. The changing demographic pattern in asthma related to sex and age: a study of 13,651 patients on sodium cromoglycate (Intal). *Med J Aust*. (1976) 1:430–4. doi: 10.5694/j.1326-5377.1976.tb140741.x

38. Perelman RH, Palta M, Kirby R, Farrell PM. Discordance between male and female deaths due to the respiratory distress syndrome. *Pediatrics*. (1986) 78:238–42. doi: 10.1542/peds.78.2.238

39. Rouschop SH, Snow SJ, Kodavanti UP, Drittij M-J, Maas LM, Opperhuizen A, et al. Perinatal high-fat diet influences ozone-induced responses on pulmonary oxidant status and the molecular control of mitophagy in female rat offspring. *Int J Mol Sci*. (2021) 22:7551. doi: 10.3390/ijms22147551

40. Lim EM, Cembrowski G, Cembrowski M, Clarke G. Race-specific WBC and neutrophil count reference intervals. *Int J Lab Hematol*. (2010) 32:590–7. doi: 10.1111/j.1751-553X.2010.01223.x

41. Paalani M, Lee JW, Haddad E, Tonstad S. Determinants of inflammatory markers in a bi-ethnic population. *Ethn Dis*. (2011) 21:142–9.

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EDITED BY
Michael Rychlik,
Technical University of Munich, Germany

REVIEWED BY
Mustafa Soylak,
Erciyes University, Türkiye
Levent Bat,
Sinop University, Türkiye
Michael Rychlik,
Technical University of Munich, Germany

*CORRESPONDENCE
Tuba Esatbeyoglu
✉ esatbeyoglu@lw.uni-hannover.de
Ali Riza Kosker
✉ alirizakosker@gmail.com;
✉ akosker@cu.edu.tr

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Metal levels of canned fish sold in Türkiye: health risk assessment

Ali Riza Kosker [1]*, Sedat Gundogdu[2], Tuba Esatbeyoglu [3]*, Deniz Ayas[4] and Fatih Ozogul[1,5]

[1]Department of Seafood Processing Technology, Faculty of Fisheries, Cukurova University, Adana, Türkiye, [2]Department of Basic Science, Faculty of Fisheries, Cukurova University, Adana, Türkiye, [3]Department of Food Development and Food Quality, Institute of Food Science and Human Nutrition, Gottfried Wilhelm Leibniz University Hannover, Hannover, Germany, [4]Fisheries Faculty, Mersin University, Mersin, Türkiye, [5]Biotechnology Research and Application Center, Cukurova University, Adana, Türkiye

This study analyzed 34 canned fish products, including 28 tuna specimens, 3 salmon specimens, 1 mackerel specimen, and 1 anchovy specimen, from 13 different brands purchased in Türkiye. The study aimed to determine metal/metalloid levels in canned fish and potential health risks for both children and adult consumers. The metal/metalloid levels in the samples were determined using an Inductively Coupled Plasma–Mass Spectrometer (ICP–MS), with the range of levels found as follows (mg/kg, ww): Fe (12.12–101.4), Cu (2.19–11.68), Zn (4.06–33.56), Se (0.24–10.74), Al (1.41–14.45), Cr (0.06–4.08), Pb (0.10–0.43), Cd (0.001–0.110), and As (0.01–0.13). Estimated weekly intake (EWI) levels were found that the consumption of canned fish products did not pose any risk based on the EWI levels and provisional tolerable weekly intake (PTWI) limits. However, three tuna samples had target hazard quotient (THQ) levels above the threshold (>1). Arsenic levels were found to increase the carcinogenic risk for child consumers if they heavily consumed 18 canned fish products, including 15 tuna, 2 salmon, and 1 mackerel. The maximum allowable consumption rates (CRmm) for each canned fish product were calculated monthly. Consequently, the consumption of canned fish by children can pose health risks.

KEYWORDS

tuna fish, salmon, mackerel, toxic metals, Inductively Coupled Plasma–Mass Spectrometer

1. Introduction

Canning is a healthy alternative food to reduce the occurrence of non-communicable diseases caused by nourishment (1). This process was established after close observation of heat treatment and high-quality preservation of food stored in sealed glass bottles. It became even more common right after the invention of metal cans. Canned fish was first introduced to the United States in 1815. Fish such as tuna, shad, and alewives were first canned in the early twentieth century (2). With the COVID-19 pandemic, consumers have tended to pay more attention to their diet, and the place of fish in the diet and canned products has increased due to consumer demand. In this period, processed and packaged products were preferred more than fresh or chilled products all over the world (3–9). Processed product consumption in Europe increased from 424 thousand tons to 511 thousand tons from 2019 to 2020 with the pandemic (10). The increase in consumers' preference for processed and packaged products instead of fresh or chilled products has increased the purchase of canned products. For instance, canned food consumption increased 21% in Portugal, 14% in Italy, and 13% in Luxembourg during the pandemic period (11). The increase in consumers' orientation toward canned products caused an increase of 7% in all tuna fish imports in Europe and 11% in filet tuna imports in 2020 (10). However,

as with all food products, there are risks in canned products that may adversely affect consumer health. For example, in canned fish, which is one of the most common canned products, contaminations can be observed in the transportation and processing processes, as well as the contaminations that may occur in the habitat of the fish (12–15). For this reason, it is essential to monitor regularly the canned products offered to the consumer for contaminants. In recent years, studies on microplastic and Bisphenol-A contamination in canned products have also been carried out (15–19). There is also a lot of research on metal pollution, one of the most common risks in canned fish (13, 14, 20–32). Studies on metal contamination in food products have largely concentrated on the quantification aspect. Authorities such as the European Union (EC, 1881/2006), World Health Organization (WHO), the US Environmental Protection Agency (US EPA), and the Turkish Food Codex (TGK) have already established limit values with regard to the potential risks of metal contamination of food products for human consumption. However, estimating the health risks that the metal levels in the product will create for the consumers has become even more essential regarding food safety. As a result, the evaluation of metal contamination in food products has expanded to include both consumer health and ecological pollution (38). In addition to establishing limit values, several consumer risk assessment criteria, such as estimated weekly intake (EWI), target hazard quotient (THQ), and lifetime cancer risk (CR), have become increasingly relevant to ensure consumer health and food safety. Moreover, contamination of processed seafood can also occur during transportation, processing and packaging (12, 14, 24). For this reason, it is important for food safety to calculate the metal levels and related health risk estimation calculations of the canned seafood products offered to consumers, which have an increasing market share worldwide.

Therefore, this study investigated health risk calculations based on metal/metalloid levels in 34 canned fish samples of 13 different brands purchased from grocery stores in Turkey during the summer of 2021. Initially, the levels of elements such as Fe, Cu, Zn, and Se were investigated due to their effects on the nutritional quality of canned fish products and potentially toxic metals/metalloids such as Al, Cr, Pb, Cd, and As, which have a risk of adversely affecting consumer health. Then, based on the metal/metaloids concentrations, health risk calculations were carried out. In this context, the calculations of health risk analysis (EWI, THQ, and CR) were made in adults and children in case of consumption once, three, and 5 days a week to predict the possible risks in terms of consumer health consuming of 34 different canned fish products. In addition, the maximum allowable consumption for canned fish was measured daily and monthly for the samples examined.

2. Materials and methods

2.1. Canned fish

This study involved the procurement of 34 canned fish samples from seven companies that were obtained from local markets in Türkiye in 2021. These canned fish samples were derived from various fish species such as Black Sea anchovy, Norwegian salmon, longtail tuna, yellowfin tuna, skipjack, and mackerel (Table 1).

2.2. Elemental analyses

Metal analysis of canned fish was performed using the method of Canli and Atli (33). Canned fish samples with a wet weight (ww) of 0.1 g were treated using a solution of 2 ml perchloric acid and 4 ml concentrated nitric acid (Merck, Darmstadt, Germany). The canned fish samples were subjected to digestion by placing them on a hot plate set at 150°C until complete dissolution of the tissue. The levels of various trace elements including Iron (Fe), zinc (Zn), copper (Cu), selenium (Se), aluminum (Al), cadmium (Cd), chrome (Cr), lead (Pb), and arsenic (As) present in the canned samples (mg/kg) were determined using Inductively Coupled Plasma-Mass Spectrometer (ICP-MS) (Agilent, 7500ce, Japan). The operating conditions of ICP-MS were as below: radio frequency (RF) 1,500 W; plasma gas flow rate, 15 L min^{-1}; auxiliary gas flow rate, 1 L min^{-1}; carrying gas flow rate, 1.1 L min^{-1}; spray chamber T, 2°C; sample depth, 8.6 mm; sample entry rate, 1 mL min^{-1}; nebuliser pump, 0.1 rps. The ICP-MS was calibrated with a high-purity multi-standard (Charleston, SC 29423) mixture for the elemental analysis. Standard solutions for calibration curves were prepared by the dilution of a stock solution of selected elements. Standard solutions prepared for toxic metals were in the 1–50 ppb (0.001–0.050 mg/L) range, while for macro and trace elements, they were in the 1–50 ppm (1–50 mg/L) range. The accuracy of the metal analysis was ensured through the use of the International Atomic Energy Agency's (IAEA) Certified Reference Material (CRM) IAEA-436. The IAEA reference material prepared by the International Atomic Energy Agency's Marine Environmental Studies Laboratory (MESL) was used for tuna meat homogenate. The same methodology as that used for analyzing the samples under study was employed for the reference material utilized in this research. The certified value of the IAEA436 reference material was compared to the observed value. Repeated analysis of the reference material demonstrated good accuracy (Table 2). The limit of detection (LOD) for Fe, Cu, Zn, Se, Al, Cr, Cd, and Pb and As, were 0.018, 0.056, 0.108, 0.027, 0.001, 0.007, 0.0004, and 0.048 and 0.003, mg/kg, respectively (Table 2).

2.3. Health risk estimation

In order to assess the risks associated with consuming the canned fish samples, EWI, THQ, and CR values were calculated for consumption frequencies of once, three, and five times per week. In health risk estimation calculations, using seafood consumption data (16.82 g/person/day) provided by Turkish Statistical Institute (T.S.I., 2020) since specific canned fish consumption data for Turkey from the were not available. The calculations were conducted separately for both adults and children. According to data from the United States Environmental Protection Agency (34), a body weight of 70 kg and a lifespan of 70 years for adult consumers, and a body weight of 32 kg for children (35) and a lifespan of seven years were considered.

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TABLE 1 Characteristics of sampled canned fish. In the code column, the letters indicate brands, and the numbers indicate products.

Code	Package type	Fish species	Additives	Product weight	Party-no
P-1	Can	Tuna	Water, salt	160 g	9283
P-2	Can	Tuna	Sunflower oil (%27), water, salt	80 g	20842E
D1	Can (BPA free)	Yellowfin Tuna	Olive oil, salt	75 g	19/10/2020
D2	Can	Skipjack	Sunflower oil, canola oil, salt	80 g	07-09-2020
D3	Can (BPA free)	Yellowfin Tuna	Water	75 g	05-10-2020
D4	Aluminum	Norwegian Salmon	Olive oil, salt	100 g	16.11.2024
D5	Aluminum	Skipjack	Olive oil, salt	125 g	10-06-2023
D6	Aluminum	Blacksea Anchovy	Sunflower oil, salt	110 g	23.09.2024
D7	Aluminum	Mackerel	Olive oil, salt	110 g	19-10-2024
D8	C/PP (90)	Norwegian Salmon	Olive oil, lemon water, salt	85 g	27.01.2022
D9	C/PP (90)	Skipjack	Sunflower oil, salt	120 g	20.10.2022
D10	C/PP (90)	Yellowfin Tuna	Water	120 g	18-03-2023
D11	C/PP (90)	Yellowfin Tuna	Olive oil, salt	185 g	02-06-2023
D12	Glass	Skipjack	Olive oil, salt	185 g	25.09.2023
C1	Can	Tuna	Sunflower oil, salt	80 g	20848E
W1	Can	Tuna	Sunflower oil, salt	160 g	20861E CO
DE1	Can	Tuna	Sunflower oil, salt	160 g	20861E
MI1	Can	Tuna	Sunflower oil, canola oil, salt	160 g	20860E CO
M1	Can	Tuna	Water, salt	80 g	25
M2	Can	Skipjack	Sunflower oil (%25), water, salt	80 g	26
M3	Can	Yellowfin Tuna	Olive oil (%25), water, salt	160 g	46
Y1	Can	Tuna	Sunflower oil (%25), water, salt	104 g	20
Y2	Aluminum	Mackerel	Sunflower oil, water, salt	160 g	14
SF1	Can	Tuna	Olive oil (%25), water, salt	75 g	0265
SF2	Can	Tuna	Sunflower oil (%27), water, salt	80 g	0051
SF3	Can	Tuna	Sunflower oil, water, salt	80 g	0197
SF4	Can	Tuna	Water, salt	80 g	7339
V1	Can	Tuna	Sunflower oil, water, salt	160 g	0346
F1	Can	Tuna	Sunflower oil, salt	160 g	L1720
SA1	Can	Salmon	Sunflower oil, water, salt	160 g	302507280
SA2	Can	Tuna	Water, salt	160 g	435375812
SA3	Can	Tuna	Olive oil, salt	160 gr	3021078T3
SA4	Can	Tuna	Sunflower oil, salt, water	80 gr	406009812
T1	Can	Tuna	Sunflower oil, salt	80 gr	17848E

TABLE 2 The confirmed and observed values of reference material (IAEA-436) and the quantitative limits for the elements.

Analyte	Certificated value (mg/kg)	Observed value (mg/kg)	95% Confidence interval (mg/kg)	Recovery %	LOD (mg/kg)	LOQ (mg/kg)
Fe	88	86 ± 4.30	80.0–92.0	97.73	0.0175	0.0541
Cu	1.74	1.73 ± 0.04	1.68–1.79	99.31	0.0565	0.1985
Zn	18.00	17.2 ± 1.30	16.00–19.00	95.56	0.1075	0.3165
Se	4.43	4.25 ± 0.25	3.97–4.51	95.98	0.0275	0.0846
Al	3.92	3.83 ± 0.07	3.76–3.92	97.76	0.001	0.0031
Cr	0.13	0.13 ± 0.01	0.11–0.14	98.46	0.0068	0.0229
Cd	0.05	0.05 ± 0.00	0.04–0.05	96.87	0.0004	0.0012
Pb	-	0.10 ± 0.00	-	-	0.0379	0.0965
As	1.98	1.96 ± 0.04	1.91–2.02	98.89	0.0026	0.0086

LOD, Limit of detection; LOD, Limit of quantification.

All metals except for As were directly analyzed using instrumental analysis values. Total As has a higher proportion of organic forms than inorganic forms, with organic As being less toxic than the inorganic form (36). Consequently, this makes it difficult to assess the potential health risks associated with its concentration in fish samples (37, 38). To evaluate the risk factors (EWI, THQ, and CR) associated with As concentration, the toxic form was assumed to be 3% of the total As concentration, as suggested in previous studies (38–41).

EWI was calculated using the formula determined by USEPA (34):

$$EWI = (C_M.CR)/BW \quad (1)$$

The EWI equation used in this study includes the metal concentration (CM), consumption rate (CR), and consumer body weight (BW). The calculated EWI values were compared with the provisional tolerable weekly intake (PTWI) levels established by the FAO/WHO Joint Expert Committee on Food Additives (JECFA) and the European Food Safety Authority (EFSA). PTWI represents the lifetime weekly intake of a substance in food or drinking water that is unlikely to cause significant health risks, based on body weight (mg/kg body weight).

The THQ calculation represents the ratio of exposure to metals, metalloids and reference doses (RfD), which is used to assess the non-carcinogenic risks of metals. THQ values were determined using methods established by the USEPA (42).

$$THQ = [(EF.ED.CR.C_M)/(RfD.BW.AT)].10^{-3} \quad (2)$$

In this equation, EF represents the frequency of exposure to the metal or metalloid of interest at 52, 156, and 260 days per year for weekly, 3 and 5-day exposures, respectively. ED stands for lifetime exposure time. This is 70 years for adults while it is 7 years for children. CR represents the consumption rate, and CM represents the metal concentration in the tissues of the samples investigated. RfD represents the oral reference dose. Based on US EPA (42) data, RfD values used for As, Cd, Pb, Cr, Fe, Cu, Zn, Se, and Al are 3.10^{-4}, 1.10^{-3}, 4.10^{-3}, 3.10^{-3},0.7, 0.04, 0.3, 5.10^{-3}, and 1.00, respectively. BW indicates body weight. As reported by USEPA, 70 kg was used for adults and 32 kg for children. AT indicates the average non-carcinogenic time; the AT value was calculated as 365 days/year × ED. The THQ value of >1 indicates that consuming the examined canned fish samples may cause different non-carcinogenic health problems for consumers (42, 43).

∑THQ is the sum of the THQ values of all elements studied.

$$\sum THQ(TTHQ) = THQ_{As} + THQ_{Al} + \ldots + THQn) \quad (3)$$

CR calculations were applied according to US EPA, 2019. CR calculates cancer risk in people exposed to metal pollution through consumption. CR values above 10^{-5} include a high risk of developing cancer.

$$CR = [(EF.ED.CR.C_M.CsF)/(BW.AT)].10^{-3} \quad (4)$$

The CR equation includes a modification where the cancer slope factor (CsF) value is used. For the metals Pb, As, Cr, and Cd, the *CsF* values used were $8.5.10^{-3}$, 1.5, 0.5, and 6.3, respectively, according to the US EPA (42).

2.4. Maximum allowable consumption rate

The US EPA suggests that the daily limits on fish consumption should be expressed as the number of meals that can be safely consumed in a given period for a specific meal size. Therefore, in this study, the daily fish consumption limit (CRlim) and the number of meals per month (CRmm) were calculated. For non-carcinogenic heavy metals, the CRlim was determined using Equation (5). For carcinogenic metals and

TABLE 3 Metal levels in 34 canned fish products (mg/kg ww).

Products	Fe Mean ± SD	Cu Mean ± SD	Zn Mean ± SD	Se Mean ± SD	Al Mean ± SD	Cr Mean ± SD	Pb Mean ± SD	Cd Mean ± SD	As Mean ± SD	Fish species
P-1	70.78 ± 10.1	10.3 ± 5.16	15.6 ± 0.87	5.89 ± 0.34	4.67 ± 0.57	1.81 ± 0.48	0.43 ± 0.01	0.001 ± 0.00	0.05 ± 0.00	Tuna
P-2	29.54 ± 2.06	11.7 ± 0.65	9.07 ± 0.72	5.88 ± 0.65	3.52 ± 0.45 0.45	0.36 ± 0.08	0.26 ± 0.03	0.001 ± 0.00	0.07 ± 0.00	Tuna
D1	101.4 ± 3.28	5.47 ± 0.25	17.0 ± 0.51	8.34 ± 0.29	NA	0.99 ± 0.06	0.34 ± 0.03	0.002 ± 0.00	0.08 ± 0.00	Yellowfin Tuna
D2	22.77 ± 0.57	4.88 ± 0.41	6.86 ± 1.08	4.57 ± 0.83	3.88 ± 0.29	4.08 ± 0.67	0.27 ± 0.03	0.001 ± 0.00	0.04 ± 0.00	Skipjack
D3	49.51 ± 2.30	3.23 ± 0.15	9.28 ± 0.43	1.29 ± 0.06	14.45 ± 0.67	0.61 ± 0.03	0.31 ± 0.01	0.001 ± 0.00	0.04 ± 0.00	Yellowfin Tuna
D4	15.90 ± 0.89	2.19 ± 0.21	8.70 ± 0.44	2.76 ± 0.10	3.13 ± 0.49	0.12 ± 0.05	0.27 ± 0.04	0.013 ± 0.00	0.07 ± 0.01	Norwegian Salmon
D5	40.61 ± 4.42	2.47 ± 0.26	7.21 ± 0.57	3.38 ± 0.33	8.56 ± 0.54	0.10 ± 0.10	0.27 ± 0.03	0.014 ± 0.00	0.07 ± 0.00	Skipjack
D6	44.25 ± 3.12	4.60 ± 0.81	33.6 ± 3.69	0.23 ± 0.03	7.89 ± 0.60	0.23 ± 0.23	0.34 ± 0.02	0.012 ± 0.00	0.02 ± 0.00	Anchovy
D7	13.16 ± 1.24	4.80 ± 0.88	5.85 ± 0.53	3.38 ± 0.33	6.44 ± 0.48	0.50 ± 0.14	0.26 ± 0.03	0.003 ± 0.00	0.13 ± 0.00	Mackerel
D8	13.31 ± 0.31	3.69 ± 0.22	11.0 ± 0.83	1.43 ± 0.21	6.08 ± 0.49	0.25 ± 0.05	0.10 ± 0.02	0.011 ± 0.00	0.03 ± 0.00	Norwegian Salmon
D9	26.76 ± 1.82	3.88 ± 0.28	13.7 ± 0.80	2.35 ± 0.36	3.71 ± 0.50	0.20 ± 0.06	0.23 ± 0.03	NA	0.06 ± 0.00	Skipjack
D10	20.79 ± 1.29	3.73 ± 0.71	14.6 ± 0.65	3.79 ± 0.59	5.17 ± 0.86	0.15 ± 0.03	0.17 ± 0.03	0.003 ± 0.00	NA	Yellowfin Tuna
D11	17.56 ± 1.42	2.63 ± 0.26	9.33 ± 0.95	5.94 ± 0.65	8.35 ± 0.44	0.27 ± 0.03	0.18 ± 0.02	0.027 ± 0.00	NA	Yellowfin Tuna
D12	25.38 ± 2.66	3.38 ± 0.28	10.6 ± 0.31	1.13 ± 0.78	13.8 ± 1.18	0.16 ± 0.07	0.17 ± 0.00	0.110 ± 0.01	0.04 ± 0.01	Skipjack
C1	41.39 ± 2.40	3.40 ± 0.65	12.6 ± 0.98	4.69 ± 0.35	3.85 ± 0.74	0.13 ± 0.01	0.22 ± 0.05	0.009 ± 0.00	0.06 ± 0.01	Tuna
W1	86.88 ± 10.0	3.62 ± 0.40	13.3 ± 0.66	6.69 ± 1.62	13.8 ± 1.42	0.18 ± 0.06	0.27 ± 0.03	0.109 ± 0.03	0.10 ± 0.00	Tuna
DE1	32.97 ± 2.20	3.13 ± 0.27	6.29 ± 0.64	5.13 ± 0.57	5.24 ± 1.17	0.06 ± 0.01	0.25 ± 0.08	0.108 ± 0.02	0.12 ± 0.01	Tuna
MI1	27.13 ± 1.66	2.92 ± 0.21	11. 6 ± 1.85	3.53 ± 0.09	4.46 ± 0.36	0.11 ± 0.02	0.15 ± 0.05	0.099 ± 0.00	0.02 ± 0.00	Tuna
M1	59.84 ± 3.67	6.73 ± 0.38	15.0 ± 1.63	2.67 ± 0.98	8.04 ± 0.51	0.24 ± 0.09	0.23 ± 0.01	0.001 ± 0.00	0.05 ± 0.00	Tuna
M2	16.70 ± 0.57	2.35 ± 0.21	8.60 ± 0.91	1.93 ± 0.18	3.63 ± 0.65	0.17 ± 0.02	0.33 ± 0.07	0.047 ± 0.01	0.05 ± 0.00	Skipjack
M3	12.81 ± 1.18	2.86 ± 0.26	8.16 ± 0.75	1.91 ± 0.16	2.68 ± 0.25	0.12 ± 0.02	0.18 ± 0.02	0.018 ± 0.00	0.04 ± 0.00	Yellowfin Tuna
Y1	42.70 ± 1.82	2.57 ± 0.10	7.60 ± 0.49	4.84 ± 0.60	6.38 ± 0.31	0.09 ± 0.06	0.16 ± 0.00	NA	0.03 ± 0.00	Tuna
Y2	97.36 ± 2.67	2.90 ± 0.22	11.4 ± 2.37	2.98 ± 0.72	13.5 ± 1.76	0.31 ± 0.02	0.27 ± 0.06	0.040 ± 0.00	0.09 ± 0.01	Mackerel

(Continued)

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TABLE 3 (Continued)

Products	Fe Mean ± SD	Cu Mean ± SD	Zn Mean ± SD	Se Mean ± SD	Al Mean ± SD	Cr Mean ± SD	Pb Mean ± SD	Cd Mean ± SD	As Mean ± SD	Fish species
SF1	35.47 ± 2.34	8.60 ± 0.57	4.06 ± 0.27	4.29 ± 0.28	1.98 ± 0.13	0.31 ± 0.02	NA	0.001 ± 0.00	0.05 ± 0.00	Tuna
SF2	12.12 ± 1.09	6.05 ± 0.50	5.31 ± 0.71	10.7 ± 1.26	NA	NA	0.23 ± 0.00	0.001 ± 0.00	0.02 ± 0.00	Tuna
SF3	30.67 ± 2.50	4.08 ± 0.72	15.7 ± 1.97	2.78 ± 0.37	5.63 ± 0.91	0.18 ± 0.04	0.41 ± 0.05	0.010 ± 0.00	0.02 ± 0.00	Tuna
SF4	35.17 ± 20.5	2.53 ± 0.47	10.9 ± 0.92	4.62 ± 0.78	4.13 ± 0.47	0.23 ± 0.11	0.25 ± 0.05	0.010 ± 0.00	0.02 ± 0.00	Tuna
V1	22.17 ± 1.71	4.15 ± 0.81	24.6 ± 1.66	7.37 ± 0.61	7.82 ± 0.97	0.48 ± 0.36	0.26 ± 0.02	0.002 ± 0.00	0.01 ± 0.00	Tuna
F1	37.69 ± 4.61	2.58 ± 0.23	8.52 ± 0.94	4.61 ± 0.14	11.5 ± 3.97	0.12 ± 0.01	0.29 ± 0.06	0.032 ± 0.00	NA	Tuna
SAS1	14.04 ± 2.13	5.09 ± 0.59	8.35 ± 7.13	4.43 ± 2.23	1.41 ± 0.15	0.17 ± 0.04	0.21 ± 0.06	0.001 ± 0.00	0.04 ± 0.00	Salmon
SAS2	30.14 ± 3.37	7.63 ± 0.31	15.2 ± 1.30	5.09 ± 0.63	2.03 ± 0.26	0.32 ± 0.18	0.20 ± 0.02	0.002 ± 0.00	0.03 ± 0.00	Tuna
SAS3	32.91 ± 1.50	6.54 ± 0.35	12.3 ± 0.59	1.66 ± 0.59	10.7 ± 1.70	0.19 ± 0.10	0.27 ± 0.01	0.003 ± 0.00	0.07 ± 0.01	Tuna
SAS4	31.50 ± 3.03	4.53 ± 0.48	14.3 ± 1.37	8.09 ± 0.76	6.01 ± 0.48	0.12 ± 0.01	0.21 ± 0.03	0.056 ± 0.00	0.07 ± 0.01	Tuna
T1	40.93 ± 2.08	2.21 ± 0.26	5.74 ± 0.53	2.93 ± 0.16	14.1 ± 0.31	0.28 ± 0.07	0.16 ± 0.04	0.003 ± 0.00	0.06 ± 0.01	Tuna

NA, Not analyzed.

metalloids (Cd, Cr, Pb, and As), CRlim was calculated using Equation (6).

$$CRlim : (RfD.BW)/C_M \quad (5)$$

$$CRlim^* : (ARL.BW)/(C_M.CSF) \quad (6)$$

Information on the number of meals that a consumer can safely consume is more practical than daily limits. The maximum allowable consumption rate, CRmm, is expressed in terms of the number of meals per month. If the consumption rate of a contaminated fish species is more than 16 meals per month, it suggests that consuming this species does not pose a significant risk to human health (34). Therefore, the number of meals allowed per month for a consumer was calculated by considering multiple pollutants for both carcinogenic and non-carcinogenic effects using the following Equation (7) proposed by the US EPA (34):

$$CRmm : (.CRlim.Tap)/MS \quad (7)$$

In these Equations (5–7), ARL is the maximum acceptable individual lifetime risk level (unitless; it was used the risk level of 10–5). The TAP refer to the average time interval (365.25 days/12 months = 30.44 days/month) and MS refer to the amount of food per meal that is 0.227 kg fish/meal for adults, 0.114 kg fish/meal for children.

2.5. Statistical analyses

Results are reported as the mean and standard deviation of the measurements. SPSS version 17.0 (SPSS Inc., Chicago, IL. USA) was used for statistical evaluations of the changes of values among 34 different brands for each metals. To determine significant differences between the levels detected for each metal/metalloid in canned fish samples, a one-way analysis of variance (ANOVA) combined with Duncan's multiple range test comparisons at $p < 0.05$ were performed.

3. Results and discussions

Fe, Cu, Zn, Se, Al, Cr, Cd, Pb, and As levels (mg/kg, ww) were determined for 34 canned fish samples from 13 brands with three replicates purchased from Turkish markets during the summer of 2021 (Table 3). In addition, consumer health risk assessment was performed by calculating EWI and THQ values (Table 3), as well as CR and CRmm values (Tables 5, 6, respectively).

3.1. Metal/metalloid levels of canned fish samples

The levels of Fe, Cu, Zn, Se, Al, Cr, Cd, Pb, and As in different canned fish samples are presented in Table 3. Iron, Cu, Zn, and

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Cr values were observed in all samples (Figure 1). However, in a few samples, Al, Cd, and As concentrations were <LOD values. The highest metal levels were found for Fe, Zn, and Al in canned fish samples while the lowest amounts were found for Cr, Cd, Pb, and As. The mean of metal/metalloid concentrations found in all canned fish samples was as follows (mg/kg, ww): Fe (36.25), Cu (4.45), Zn (11.52), Se (4.16), Al (6.77), Cr (0.41), Cd (0.02), Pb (0.25), and As (0.05). Mean metal/metalloid concentrations were compared with maximum limit values set by the Turkish Food Codex (TFC), Food and Agriculture Organization (FAO)/World Health Organization (WHO) and European Commission (EC). FAO/WHO (44) maximum limits for Cu, Zn, Cd, and As were reported as 30, 40, 0.5, and 0.3 mg/kg, respectively. The EC (45) and TFC (46) reported maximum limits for Cd and Pb as 0.05 and 0.2 and 0.05 and 0.3 mg/kg, respectively. The mean Cu concentration is above the maximum limits reported by FAO, while the Zn concentration is below the maximum limit. Cd value were below the maximum limits for all codexes. While the mean Pb concentration was below the maximum limit value according to FAO and TFC, it was above the EC limits. In terms of statistical evaluation, significant differences were observed between the samples for Al. There are statistical differences between D3 and all other samples ($p < 0.05$). For Cr, there are statistical differences between P1, D1, and D2, and there are statistical differences between these three and all others ($p < 0.05$). For Fe, there was no statistical difference between Y2 and D1 ($p > 0.05$), while a statistical difference was found between these two and all other samples ($p < 0.05$). For Cu, while there were statistical differences between SF1, SAS2, and P2, there were also statistical differences between these three and all other samples ($p < 0.05$). For Zn, there were statistical differences between V1 and D6, while these two were statistically different from all other samples ($p < 0.05$). For Se, statistical differences were found between SF2 and all samples ($p < 0.05$). For As, there was a statistical difference between W1 and D7, while these two samples were statistically different from all other samples ($p < 0.05$). For Pb, there was no statistical difference between SF3 and P1 ($p > 0.05$), while statistical differences were found between these two and all other groups ($p < 0.05$). For Cd, while there was no statistical difference between D12 and MI1 ($p > 0.05$), these two were statistically different from all other groups ($p < 0.05$).

Iron, Cu, Zn, and Se are essential for fish nutrition (26). In this study, Fe, Cu, Zn, and Se values in canned fish are similar to the values reported by the several authors (13, 14, 22, 26, 29). It was determined that the highest Fe, Zn, and Se values were found in tuna fish compared to the canned fish species tested. Similarly, Alcala-Orozco et al. (14) found that these elements were higher in tuna whereas it was determined that Cu levels were high in tuna and anchovy. Tuzen and Soylak (21) also reported similar results. Another remarkable finding in the canned anchovy samples examined is the high levels of Zn, which is in agreement with work of Tuzen and Soylak (21) since higher zinc levels was reported by these researcher in canned anchovy. However, the Se values were lower in canned anchovy compared to other investigated canned fish samples. Selenium is a nutritionally essential trace element for the activity of over 30 enzymes with vital functions. Nevertheless, the canning process can decrease the Se level (47), which has a negative effect on the nutritional value of canned anchovies.

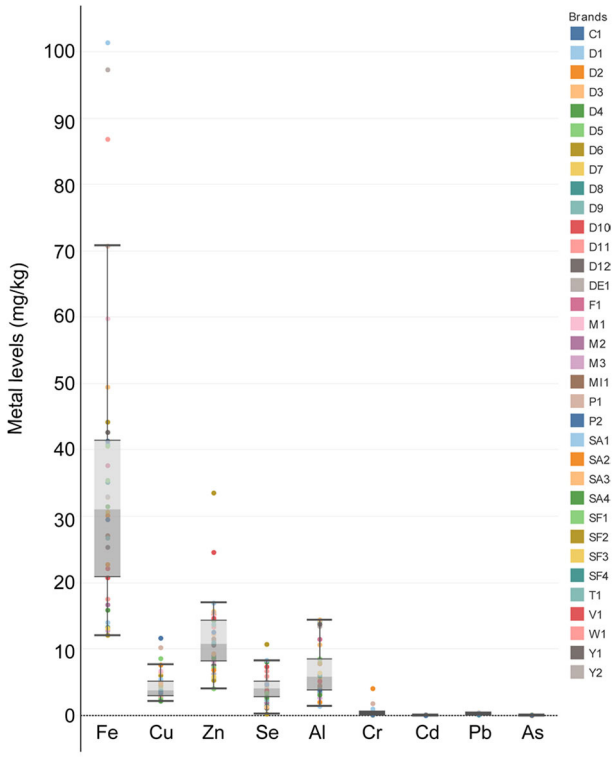

FIGURE 1
Metal levels in 34 canned fish products.

One of the important elements in terms of consumer health is Al. Aluminum is closely associated with many neurological diseases, such as Alzheimer's, Parkinson's, and MS (48). Although Al has been studied relatively less in canned fish, in the current study Al values observed in all four canned fish species were higher than in several studies (21, 49, 50). Al Ghoul et al. (22) reported similar levels of Al values in canned tuna. High Al values may be due to contamination in the fish's transportation, processing, and packaging processes. It is known that in canned fish products, transportation, processing, and packaging processes can cause contamination as well as the habitat of the fish used for canning fish products (14).

In this study, metals and metalloids such as Cr, Cd, Pb, and As, which may pose health risks for consumers, were also investigated. ATSDR (51) reported that Cd, Pb, and As elements are among 10 most dangerous toxic substances in the Priority List of Hazardous Substances, while Cr metal is among the 100 most dangerous substances (38). The canned fish product with the highest Cr and Cd levels was tuna. Mackerel was the species of canned fish with the highest value for As (0.11 mg/kg), a critical toxic metalloid. Chromium, Cd, Pb, and As values in tuna samples were determined as 0.43, 0.03, 0.25, and 0.05 mg/kg, respectively. The level of Cr, Pb, and Cd values found in this study are similar to the values by Alcala-Orozco et al. (14), Miedico et al. (20), Kowalska et al. (23), Ulusoy (24), Ashraf et al. (25), Novakov et al. (26), Popovic et al. (27), and Rahman et al. (29). Arsenic values in canned fish, in contrast, are similar to the values by Ulusoy (24); however, it is lower than reported by several studies (14, 26, 29, 49). This difference is due to

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the direct use of As values determined as a result of instrumental analyses in most studies. However, a significant portion of the As value in fish is organic, and organoarsenic are not as toxic as inorganic forms (36). Therefore, the toxic As value is assumed to be 3% of the total As concentration (37–41).

Girolametti et al. (52) reported Cd, Pb, and Fe levels in wild and farmed tuna fish as 0.01 and 0.02, 0.11 and 0.03, 13 and 7, respectively. Although the reported Cd levels were similar to the present study, Pb and Fe levels were lower than the present study. This difference may be due to the additives such as oil and water used during the canning process and may also be related to the size of the fish used in packaging. For example, in the study by Milatou et al. (53) investigating the metal levels of Atlantic bluefin tuna fish according to different size groups; Fe levels in tuna fish of 250–289 cm length are similar to the data in the present study, while there are differences in other lengths. This emphasizes how crucial it is to provide comprehensive information on packaging regarding the methods of processing and the fish utilized. Because there is a possibility that product quality may be affected during transportation and canning processes.

3.2. Health risk analysis

EWI, THQ (Table 4), and CR (Table 5) values were calculated to assess consumer health risks associated with the consumption of canned fish samples from the different brands. The present calculations were based on the assumption that individuals in two age groups (children and adults) consumed canned fish at different frequencies, including once, three times, or five times a week.

3.2.1. EWI

The results of the study indicated that the estimated weekly intake (EWI) values of all the metals detected in canned fish were lower than the provisional tolerable weekly intake (PTWI) limits set by the relevant authorities (Table 4). The PTWI levels for iron (Fe), copper (Cu), and zinc (Zn) were determined by the Food and Agriculture Organization/World Health Organization (FAO/WHO) in 1983, while the levels for aluminum (Al) were established by the Joint Expert Committee on Food Additives (JECFA) in 2011. The European Food Safety Authority (EFSA) set the PTWI level for arsenic (As) in 2009. The PTWI levels for Fe, Cu, Zn, Al, and As were established at 5,600, 125 μg/kg/day, a range of 300–1,000, 2,000, and 15 μg/kg/day, respectively. Since there is no PTWI value for Se determined by the authorities, a PTWI calculation was not carried out. The tolerable monthly intake of cadmium (Cd) was updated to 25 μg/kg body weight (bw) by the Joint FAO/WHO Expert Committee on Food Additives (JECFA) in 2013. However, in this study, the provisional tolerable weekly intake (PTMI) was converted to PTWI, and a weekly value of 6.25 μg/kg bw was used instead. The ratios of EWI to PTWI for Fe, Cu, Zn, Al, Cd, and As ranged from 0.05 to 4.88%, 0.43 to 25.19%, 0.01 to 9.05%, 0.02 to 1.95%, and 0.01 to 2.33%, respectively. The calculations showed that the EWI levels were higher in children than in adults, as expected. Additionally, an increase in the frequency of canned fish consumption led to

TABLE 4 Estimated weekly intake (EWI; μg/kg BW) and target hazard quotient (THQ) values for each metal analyzed according to different consumption frequencies.

	Consumer groups	Day	Fe	Cu	Zn	Se	Al	Cr	Cd	Pb	As
EWI	A	5	44.86	5.49	14.36	5.14	8.44	0.51	0.03	3.04	0.06
		3	26.91	3.30	8.61	3.08	5.06	0.30	0.02	1.82	0.04
		1	8.97	1.10	2.87	1.03	1.69	0.10	0.01	0.61	0.01
	C	5	98.13	12.02	31.41	11.24	18.46	1.11	0.07	6.65	0.13
		3	58.88	7.21	18.85	6.74	11.08	0.66	0.04	3.99	0.08
		1	19.63	2.40	6.28	2.25	3.69	0.22	0.01	1.33	0.03
THQ	A	5	0.01	0.02	0.01	0.15	0.00	0.24	0.00	0.11	0.03
		3	0.01	0.01	0.00	0.09	0.00	0.14	0.00	0.06	0.02
		1	0.00	0.00	0.00	0.03	0.00	0.05	0.00	0.02	0.01
	C	5	0.02	0.04	0.01	0.32	0.00	0.53	0.01	0.24	0.06
		3	0.01	0.03	0.01	0.19	0.00	0.32	0.01	0.14	0.04
		1	0.00	0.01	0.00	0.06	0.00	0.11	0.00	0.05	0.37

A, adult; C, children.
Exposure time of 5, 3 and 1 days/week. Arsenic calculation was made by assuming the inorganic.

TABLE 5 Target carcinogenic risk (CR) values for Cr, As, Cd, and Pb according to different consumption frequencies.

	Consumer groups	Day	Cr	Cd	Pb	As
CR	A	5	**3.5E-05**	**2.89E-05**	3.57E-06	**1.31E-05**
		3	**2.1E-05**	**1.74E-05**	2.14E-06	7.84E-06
		1	7.01E-06	5.78E-06	7.15E-07	2.61E-06
	C	5	**7.66E-05**	**6.33E-05**	7.82E-06	**1.31E-04**
		3	**4.6E-05**	**3.79E-05**	4.69E-06	**1.72E-05**
		1	**1.53E-05**	**1.27E-05**	1.56E-06	5.72E-06

A, adult; C, children.
Exposure time of 5. 3 and 1 days/week. CR values $>10^{-5}$ are indicated in bold.

TABLE 6 The maximum allowable consumption rates for each investigated metal.

		Fe	Cu	Zn	Se	Al	Cr	Cd	Pb	As
A	CR(lim)kg/day	1.86	0.76	2.16	0.16	14.70	0.12	0.04	0.04	0.01
	CR(mm)	>16	>16	>16	>16	>16	>16	4.74	4.95	1.87
C	CR(lim)kg/day	4.49	2.27	5.17	0.48	32.54	0.25	0.10	0.07	0.02
	CR(mm)	>16	>16	>16	>16	>16	13.89	3.98	4.29	1.63

>16: More than 16 meals in a month.

higher EWI levels. Based on the EWI levels and PTWI limits, the consumption of the canned fish samples was deemed safe with regards to the studied metals. Besides, some studies report that the EDI/EWI values in canned fish are acceptable (29, 31, 32). Rahman et al. (29) reported acceptable EDI values for As, Cr, Cd, Cu, and Zn. Herrera-Herrera et al. (32) reported that Cd EDI/EWI values in canned tuna samples were similar to results of the current study, but the Zn value was higher. Ulusoy (24) reported slightly higher EDI/EWI values in research with 222 different canned fish than the present study. There are few studies on EDI/EWI values in canned fish. Additionally, most of these studies reported that EDI/EWI values for Fe, Cu, Zn, Se, Al, Cr, Cd, and As are under PTDI/PTWI levels determined by regulatory authorities (24, 32). EWI was calculated for Pb although no PTWI comparison was made. Even though there was established PTWI for Pb (25 μg/kg bw), the FAO/WHO, based on analysis of epidemiological data, noted that the Pb of PTWI provided was associated with an increase in systolic blood pressure in adults and at least 3 points IQ loss and adverse neurodevelopmental effects in children. For this reason, it was reported that the PTWI value for Pb could not be considered (Table 5) protective for health and was therefore withdrawn, and a new PTWI that could be regarded as protective for health could not be formulated (54).

3.2.2. Target hazard quotient

Based on the THQ calculations, it was found that the values for Fe, Cu, Zn, Se, Al, Cr, Cd, and As were below the threshold value of 1, as shown in Table 4. The THQ value is not a direct measure of the health risks associated with exposure to metal or metalloid pollutants, but rather serves as an indicator of potential risk (38). A THQ value >1 indicates that the amount of metal intake exceeds the RfD, as defined by the US EPA (42) and Yi et al. (43), which suggests that the metal poses a risk to the consumer. The order of metals based on THQ in both adult and child age groups is Cr > As > Se > Pb > Cu > Cd > Fe > Zn. The mean THQ values of all 34 canned fish samples for adult and child consumers were <1. In singular samples, THQ levels above the threshold value were calculated for Cr only in three samples (P1, D2, and D2). The concentrations of all other metals and metalloids were found to be below the threshold value of 1, as presented in Table 4. Similarly, Ulusoy (24) for Cd and As; Mansouri et al. (31) for Cd; Rahmani et al. (50) for As, Se, Cu, Al, Zn, and Fe reported THQ values below the threshold value (=1). Among all THQ values calculated according to the metal levels found in all canned fish samples, the highest values were observed for chromium (Cr).

Due to the Cr levels in canned fish, one of the examined samples (P1) (tuna) had non-carcinogenic risks when consumed 5 days a week for adults and 3 and 5 days a week for children. While consumption of canned yellowfin Indian ocean tuna fish (D1) did not pose any risk for adults, it is risky for children to consume it 3 days a week. In the canned Pacific skipjack tuna sample (D2), however, according to the THQ values calculated for Cr, consumption by children was risky under all conditions and for adults more than once a week. The fact that this situation was observed in a small number of tuna samples suggested that there might be contamination during processing. Salmon, mackerel, and anchovy samples examined were not THQ risky for adults and children.

TTHQ, which indicates the cumulative non-carcinogenic risk associated with exposure to all studied elements, was also evaluated in this study. A TTHQ value > 10 suggests that there may be non-carcinogenic risks that could cause health problems for consumers over an extended period. However, the results of this study indicate that not all tested canned fish samples pose a risk for TTHQ to both adults and children.

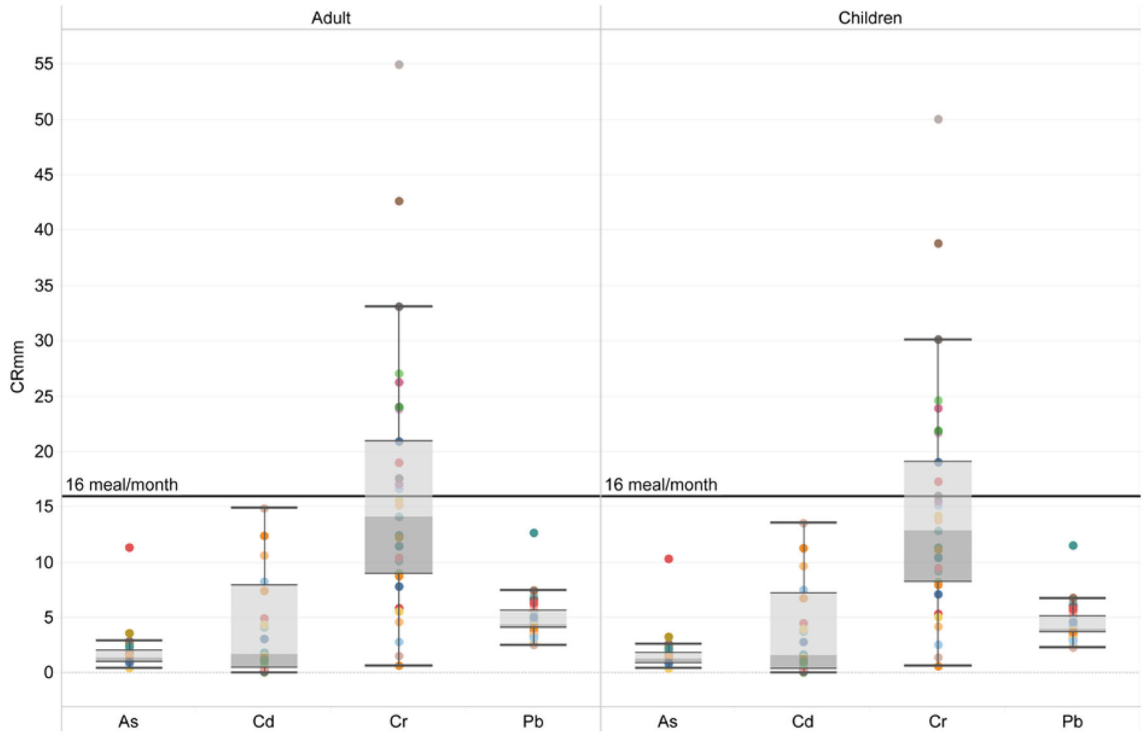

FIGURE 2
The maximum allowable consumption rate (CRmm) for Cr, As, Cd, and Pb.

3.2.3. Lifetime cancer risk

The mean CR values for Cd and Cr of all 34 canned fish samples were found to be risky in children under all conditions and adults when consumed 3 and 5 days a week (Table 5). According to the US EPA (34), the probability of a healthy individual developing cancer is 10^{-5}. Therefore, the CR value is expected to be below this threshold. Values for CR $> 10^{-5}$ include a high risk of developing cancer. For arsenic, intensive consumption was found to be risky. Mansouri et al. (31) reported that CR values in relation to Cd levels were similar to the current study when four different cans of tuna were consumed once a week. While four other products pose carcinogenic risks in relation to chromium levels when consumed heavily, the risk was determined in only one sample (P1) for adults if consumed 5 days a week. In this study, no risk was determined for adult consumers regarding carcinogenic risk owing to arsenic levels. Nevertheless, it was determined that carcinogenic risk increased for child consumers if 18 different products, including 15 tuna, two salmon, and a can of mackerel were consumed 5 days a week. Ulusoy (24) found 222 samples of canned tuna from 36 countries to have high CR values depending on the amount of As consumed 3 days a week or more. Rahmani et al. (50) also stated that attention should be paid to As of CR values originating from canned fish consumption. Arsenic, a naturally occurring metalloid, is widely distributed and considered to be the most significant toxic substance in terms of potential harm to human health due to its known or suspected toxicity. It is known to be a potent poison, a co-carcinogen, and even at low concentrations, has been shown to cause damage to almost all major organs, including the lungs, liver, brain, and bladder (55). Therefore, regular monitoring of toxic metals in processed seafood, especially As levels is important for consumer health. The CR values for Pb in 34 canned fish samples did not show any carcinogenic risk in children and adults.

3.3. Maximum allowable limits

The US EPA recommends the maximum allowable consumption rate (CRmm) for daily fish consumption limits to express the permissible number of fish meals consumed in a given meal size and a given period. If the number of meals of a contaminated fish species is <16 per month, it is thought that consuming this fish species may pose a risk to human health (34). In this study, CRmm values for Fe, Cu, and Zn were <16 (meals/month) for both children and adults (Table 6). While the risk for chromium was not detected in adults, it was found to be 14.68 (meals/month) in children. However, monthly food consumption levels were low for both consumer groups for Cd, Pb, and As. Particularly As levels are quite limiting. Health risks can be observed if it is consumed more than 1.70 meals per month for children and 1.87 meals per month for adults (Figure 2).

According to all these findings, it was determined that 34 different canned fish products tested did not contain significant

risks for consumers in terms of EWI and THQ. It was determined that Fe, Cu, Zn, Se, and Al levels in canned fish were not risky regarding health risk assessment in terms of metals and metalloids tested. However, it was reported that toxic metals such as Cr, Cd, Pb, and As carried carcinogenic risks and risks in terms of CRmm. It was determined that Cr, Cd, and As levels in canned fish were risky for adult and child consumers if consumed 3 days or more a week. In addition, it was observed that toxic metals had a restrictive effect on canned fish consumption in terms of maximum permissible consumption rate (CRmm). Metals and metalloids have long been recognized as critical toxic agents causing acute and chronic poisoning cases in environmental exposure situations (56). These health risks from canned fish consumption were thought to arise from the processing process. During the process of food packaging and preservation, metals can act as a source of contamination and can contaminate food through various pathways (12). Contamination can occur during food processing owing to the direct interaction of equipment, tanks, tubes, as well as other parts of processing equipment prepared from toxic metal. Moreover, contamination can also occur throughout the entire container, especially during storage stages such as canning and packaging. Although the 34 different canned products tested contain different fish caught from different regions, similar risk values were observed, particularly concerning toxic metals.

4. Conclusion

Canned fish has been a popular food globally for many years because of its long shelf life and microbiological protection from the canning process. However, the fish may be contaminated by metals, pesticides, microplastics, etc., from its habitat or during processing. A study of the potential health impacts of metal content in 34 canned fish products found that higher attention should be paid to contamination from processing. Although EWI levels in tuna, salmon, mackerel, and anchovy were not found to pose a risk, increased THQ and CR values were observed with intensive consumption. Although fish consumption in Türkiye is lower than in other countries, it is still important to test regularly the canned fish for toxic metals and metalloids to protect consumer health. To ensure the safety of canned fish, there are regulations and standards in place to control its production. Regular monitoring and careful regulation of production facilities can minimize contamination and provide consumers with the confidence that the canned fish they purchase is safe for consumption.

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

TE: Funding acquisition, Writing—review and editing. AK: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Writing—original draft. SG: Conceptualization, Data curation, Investigation, Writing—original draft. DA: Data curation, Formal analysis, Writing—review and editing. FO: Data curation, Writing—review and editing.

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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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References

1. Bell JD, Sharp MK, Havice E, Batty M, Charlton KE, Russell J, et al. Realising the food security benefits of canned fish for Pacific Island countries. *Mar Policy.* (2019) 100:183–91. doi: 10.1016/j.marpol.2018.10.034

2. Joardder MUH, Masud MH. *Food Preservation in Developing Countries: Challenges and Solutions*. (2019). p. 1–245.

3. White ER, Froehlich HE, Gephart JA, Cottrell RS, Branch TA, Agrawal Bejarano R, et al. Early effects of COVID-19 on US fisheries and seafood consumption. *Fish Fish.* (2021) 22:232–9. doi: 10.1111/faf.12525

4. Kitz R, Walker T, Charlebois S, Music J. Food packaging during the COVID-19 pandemic: consumer perceptions. *Int J Consum Stud.* (2022) 46:434–48. doi: 10.1111/ijcs.12691

5. Knorr D, Khoo CSH. COVID-19 and food: challenges and research needs. *Front Nutr.* (2020) 7:1–5. doi: 10.3389/fnut.2020.598913

6. Chenarides L, Grebitus C, Lusk JL, Printezis I. Food consumption behavior during the COVID-19 pandemic. *Agribusiness.* (2021) 37:44–81. doi: 10.1002/agr.21679

7. de Oliveira WQ, de Azeredo HMC, Neri-Numa IA, Pastore GM. Food packaging wastes amid the COVID-19 pandemic: trends and challenges. *Trends Food Sci Technol.* (2021) 116:1195–9. doi: 10.1016/j.tifs.2021.05.027

8. Li Z, Zhao A, Li J, Ke Y, Huo S, Ma Y. Food and nutrition related concerns post lockdown during covid-19 pandemic and their association with dietary behaviors. *Foods.* (2021) 10:2858. doi: 10.3390/foods10112858

9. Fernández-González R, Pérez-Pérez MI, Pérez-Vas R. Impact of the COVID-19 crisis: analysis of the fishing and shellfishing sectors performance in Galicia (Spain). *Mar Pollut Bull.* (2021) 169:112463. doi: 10.1016/j.marpolbul.2021.112463

10. EUMOFA. *The EU Fish Market 2021 Edition*. (2021). Brussels: EUMOFA. p. 101.

11. European Commission. *EU Consumer Habits Regarding Fishery and Aquaculture Products.* Brussels: European Commission (2021).

12. Ysart G, Miller P, Croasdale M, Crews H, Robb P, Baxter M, et al. 1997 UK Total Diet Study dietary exposures to aluminium, arsenic, cadmium, chromium, copper, lead, mercury, nickel, selenium, tin and zinc. *Food Addit Contam.* (2000) 17:775–86. doi: 10.1080/026520300415327

13. Mol S. Levels of heavy metals in canned bonito, sardines, and mackerel produced in Turkey. *Biol Trace Elem Res.* (2011) 143:974–82. doi: 10.1007/s12011-010-8909-5

14. Alcala-Orozco M, Balcom PH, Sunderland EM, Olivero-Verbel J, Caballero-Gallardo K. Essential and toxic elements in sardines and tuna on the Colombian market. *Food Addit Contam Part B Surveill.* (2021) 14:206–18. doi: 10.1080/19393210.2021.1926547

15. Gündogdu S, Köşker AR. Microplastic contamination in canned fish sold in Türkiye. *PeerJ.* (2023) 11:1–20. doi: 10.7717/peerj.14627

16. Karami A, Golieskardi A, Choo CK, Larat V, Karbalaei S, Salamatinia B. Microplastic and mesoplastic contamination in canned sardines and sprats. *Sci Total Environ.* (2018) 612:1380–6. doi: 10.1016/j.scitotenv.2017.09.005

17. Akhbarizadeh R, Dobaradaran S, Nabipour I, Tajbakhsh S, Darabi AH, Spitz J. Abundance, composition, and potential intake of microplastics in canned fish. *Mar Pollut Bull.* (2020) 160:111633. doi: 10.1016/j.marpolbul.2020.111633

18. Diaz-Basantes MF, Nacimba-Aguirre D, Conesa JA, Fullana A. Presence of microplastics in commercial canned tuna. *Food Chem.* (2022) 385:132721. doi: 10.1016/j.foodchem.2022.132721

19. Tokat İE, Cumbul D, Yavaş H, Birlik PM. Yağlı Ton Balığı Konserveleri ve Kullanılan Metal Ambalajlarda Bisfenol A (BFA) Miktarının Araştırılması. *Gıda ve Yem Bilimi Teknolojisi Dergisi.* (2018) 1–8.

20. Miedico O, Pompa C, Moscatelli S, Chiappinelli A, Carosielli L, Chiaravalle AE. Lead, cadmium and mercury in canned and unprocessed tuna: six-years monitoring survey, comparison with previous studies and recommended tolerable limits. *J Food Compos Anal.* (2020) 94:103638. doi: 10.1016/j.jfca.2020.103638

21. Tuzen M, Soylak M. Determination of trace metals in canned fish marketed in Turkey. *Food Chem.* (2007) 101:1378–82. doi: 10.1016/j.foodchem.2006.03.044

22. Al Ghoul L, Abiad MG, Jammoul A, Matta J, El Darra N. Zinc, aluminium, tin and Bis-phenol a in canned tuna fish commercialized in Lebanon and its human health risk assessment. *Heliyon.* (2020) 6:e04995. doi: 10.1016/j.heliyon.2020.e04995

23. Kowalska G, Pankiewicz U, Kowalski R. Determination of the level of selected elements in canned meat and fish and risk assessment for consumer health. *J Anal Methods Chem.* (2020) 2020:2148794. doi: 10.1155/2020/2148794

24. Ulusoy S. Determination of toxic metals in canned tuna sold in developed and developing countries: health risk assessment associated with human consumption. *Mar Pollut Bull.* (2023) 187:114518. doi: 10.1016/j.marpolbul.2022.114518

25. Ashraf W, Seddigi Z, Abulkibash A, Khalid M. Levels of selected metals in canned fish consumed in kingdom of Saudi Arabia. *Environ Monit Assess.* (2006) 117:271–9. doi: 10.1007/s10661-006-0989-5

26. Novakov NJ, Mihaljev ŽA, Kartalović BD, Blagojević BJ, Petrović JM, Cirković MA, et al. Heavy metals and PAHs in canned fish supplies on the Serbian market. *Food Addit Contam Part B Surveill.* (2017) 10:208–15. doi: 10.1080/19393210.2017.1322150

27. Popovic AR, Djinovic-Stojanovic JM, Djordjevic DS, Relic DJ, Vranic D V, Milijasevic MP, et al. Levels of toxic elements in canned fish from the Serbian markets and their health risks assessment. *J Food Compos Anal.* (2018) 67:70–6. doi: 10.1016/j.jfca.2018.01.003

28. Boadi N, Twumasi S. Heavy metal contamination in canned fish marketed in Ghana. *Am J Sci Ind Res.* (2011) 2:877–82. doi: 10.5251/ajsir.2011.2.6.877.882

29. Rahman MM, Shehzad MT, Nayak AK, Sharma S, Yeasmin M, Samanta S, et al. Health risks from trace elements in muscles of some commonly available fish in Australia and India. *Environ Sci Pollut Res.* (2020) 27:21000–12. doi: 10.1007/s11356-020-08600-y

30. Sobhanardakani S. Tuna fish and common kilka: health risk assessment of metal pollution through consumption of canned fish in Iran. *J fur Verbraucherschutz und Leb.* (2017) 12:157–63. doi: 10.1007/s00003-017-1107-z

31. Mansouri B, Azadi NA, Albrycht M, Binkowski LJ, Błaszczyk M, Hamesadeghi U, et al. Metal risk assessment study of canned fish available on the Iranian market. *Biol Trace Elem Res.* (2021) 199:3470–7. doi: 10.1007/s12011-020-02446-8

32. Herrera-Herrera C, Fuentes-Gandara F, Zambrano-Arévalo A, Higuita FB, Hernández JP, Marrugo-Negrete J. Health risks associated with heavy metals in imported fish in a coastal city in Colombia. *Biol Trace Elem Res.* (2019) 190:526–34. doi: 10.1007/s12011-018-1561-1

33. Canli M, Atli G. The relationships between heavy metal (Cd, Cr, Cu, Fe, Pb, Zn) levels and the size of six Mediterranean fish species. *Environ Pollut.* (2003) 121:129–36. doi: 10.1016/S0269-7491(02)00194-X

34. US EPA. *Guidance for Assessing Chemical Contaminant Data for Use in Fish Advisories, Vol. I: Fish Sampling and Analysis*. Washington, DC: Office of Water. U.S. Environmental Protection Agency (2000)

35. US EPA. *Child-Specific Exposure Factors Handbook (Final Report) 2008. EPA/600/R-06/096F*. Washington, DC: National Center for Environmental Assessment Office of Research and Development (2008).

36. Castro-González MI, Méndez-Armenta M. Heavy metals: implications associated to fish consumption. *Environ Toxicol Pharmacol.* (2008) 26:263–71. doi: 10.1016/j.etap.2008.06.001

37. Kosker AR. Metal and fatty acid levels of some commercially important marine species from the northeastern Mediterranean: benefits and health risk estimation. *Environ Monit Assess.* (2020) 192:1–16. doi: 10.1007/s10661-020-08287-1

38. Traina A, Bono G, Bonsignore M, Falco F, Giuga M, Quinci EM, et al. Heavy metals concentrations in some commercially key species from Sicilian coasts (Mediterranean Sea): potential human health risk estimation. *Ecotoxicol Environ Saf.* (2019) 168:466–78. doi: 10.1016/j.ecoenv.2018.10.056

39. Andaloro F, Romeo T, Renzi M, Guerranti C, Perra G, Consoli P, et al. Alteration of potential harmful elements levels in sediments and biota from the central Mediterranean Sea (Aeolian Archipelago) following an episode of intense volcanic activity. *Environ Monit Assess.* (2012) 184:4035–47. doi: 10.1007/s10661-011-2242-0

40. Copat C, Arena G, Fiore M, Ledda C, Fallico R, Sciacca S, et al. Heavy metals concentrations in fish and shellfish from eastern Mediterranean Sea: consumption advisories. *Food Chem Toxicol.* (2013) 53:33–7. doi: 10.1016/j.fct.2012.11.038

41. EFSA. EFSA Panel on Contaminants in the Food Chain (CONTAM). *EFSA J.* (2009) 7:1351. doi: 10.2903/j.efsa.2009.1351

42. US EPA. *USEPA (United States Environmental Protection Agency* (2019). Available online at: https://www.epa.gov/risk/regional-screening-levels-rsls-equations (accessed October 01, 2023).

43. Yi Y, Yang Z, Zhang S. Ecological risk assessment of heavy metals in sediment and human health risk assessment of heavy metals in fishes in the middle and lower reaches of the Yangtze River basin. *Environ Pollut.* (2011) 159:2575–85. doi: 10.1016/j.envpol.2011.06.011

44. FAO/WHO. *General Standard for Contaminants and Toxins in Food and Feed*. CODEX Aliment (2019). Available online at: https://www.fao.org/fao-who-codexalimentarius/sh-proxy/en/?lnk=1&url=https%253A%252F%252Fworkspace.fao.org%252Fsites%252Fcodex%252FStandards%252FCXS%2B193-1995%252FCXS_193e.pdf (accessed October 01, 2023).

45. The Commission of the European Communities. *EC 2001 Commission Regulation (EC) No 466/2001. Document 32001R0466. Section 3: Heavy Metals*. (2001). Available online at: https://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=CONSLEG:2001R0466:20060701:EN:PDF (accessed October 01, 2023).

46. Turkish Food Codex. *Bulaşanlar Yönetmeligi*. (2011). Available online at: https://www.resmigazete.gov.tr/eskiler/2008/05/20080517-7.htm (accessed October 01, 2023).

47. Afonso C, Costa S, Cardoso C, Oliveira R, Lourenço HM, Viula A, et al. Nunes ML. Benefits and risks associated with consumption of raw, cooked, and canned tuna (*Thunnus* spp.) based on the bioaccessibility of selenium and methylmercury. *Environ Res.* (2015) 143:130–7. doi: 10.1016/j.envres.2015.04.019

48. Inan-Eroglu E, Ayaz A. Is aluminum exposure a risk factor for neurological disorders? *J Res Med Sci.* (2018) 23:51. doi: 10.4103/jrms.JRMS_921_17

49. Akalın S, Eroğlu Eİ, Güleç A, Ayaz A. Yetişkinlerde Konserve Balık Tüketimine Bağlı Ağır Metal Maruziyet Riskinin Değerlendirilmesi. *Beslenme ve Diyet Dergisi.* (2020) 48:15–23. doi: 10.33076/2020.BDD.1298

50. Rahmani J, Fakhri Y, Shahsavani A, Bahmani Z, Urbina MA, Chirumbolo S, et al. systematic review and meta-analysis of metal concentrations in canned tuna fish in Iran and human health risk assessment. *Food Chem Toxicol.* (2018) 118:753–65. doi: 10.1016/j.fct.2018.06.023

51. ATSDR. *ATSDR (Agency for Toxic Substance and Disease Rigestry) 2017*. Atlanta, GA: Priority List of Hazardous Substances (2017).

52. Girolametti F, Annibaldi A, Carnevali O, Pignalosa P, Illuminati S, Truzzi C. Potential toxic elements (PTEs) in wild and farmed Atlantic bluefin tuna (Thunnus thynnus) from Mediterranean Sea: risks and benefits for human consumption. *Food Control.* (2021) 125:108012. doi: 10.1016/j.foodcont.2021.108012

53. Milatou N, Miliou H, Dassenakis M, Megalofonou P. Trace metal accumulation in Atlantic bluefin tuna and correlations with protein-lipid composition. *Food Chem.* (2023) 404:134691. doi: 10.1016/j.foodchem.2022.134691

54. JECFA. *Evaluation of Certain Food Additives and Contaminants: Seventyfourth Report of the Joint FAO/WHO Expert Committee on Food Additives*. WHO Tech Rep Ser. Geneva: JECFA (2011)

55. Flora SJS. *Handbook of Arsenic Toxicology*. Eds, Flora, S. J. S. Amsterdam: Elsevier (2015).

56. Nordberg GF, Costa M. *Handbook on the Toxicology of Metals*, 5th ed. London: Academic Press (2021). p. 1–1011.

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EDITED BY
Misha Vrolijk,
Maastricht University, Netherlands

REVIEWED BY
Yue Zhang,
Fudan University, China
Pei Xiao,
Capital Medical University, China

*CORRESPONDENCE
Fang Xia
✉ 1621387342@qq.com
Xin Luo
✉ 1836735412@qq.com

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The association of blood metals with latent tuberculosis infection among adults and adolescents

Jinyi Wu, Kai Wang, Fengxi Tao, Qingwen Li, Xin Luo* and Fang Xia*

Department of Public Health, Wuhan Fourth Hospital, Wuhan, China

Objective: We aimed to investigate the relationship of metal exposure and latent tuberculosis infection (LTBI) among US adults and adolescents.

Methods: Participants from the National Health and Nutrition Examination Surveys (NHANES 2011 ~ 2012) were included. Multiple logistic regression models were used to explore the associations between metal exposure and LTBI. A total of 5,248 adults and 1,860 adolescents were included in the present analysis.

Results: For adults, we only found a positive association between total mercury and LTBI (OR: 1.411; 95% CI: 1.164 ~ 1.710) when used as a continuous variable. Compared with Q1, Q4 increased the prevalence of LTBI (2.303; 1.455 ~ 3.644) when used as a quartile. The OR of total mercury and LTBI was higher among females (1.517; 1.009 ~ 2.279), individuals aged 45 ~ 64 (1.457; 1.060 ~ 2.002), and non-Hispanic White individuals (1.773; 1.316 ~ 2.388). A relationship was observed among only participants with obesity (1.553; 1.040 ~ 2.319) or underweight (1.380; 1.076 ~ 1.771), with college or above (1.645; 1.184 ~ 2.286), with PIR > 3.0 (1.701; 1.217 ~ 2.376), reported smoking (1.535; 1.235 ~ 1.907) and drinking (1.464; 1.232 ~ 1.739). For adolescents, blood manganese was positively associated with LTBI. The OR and 95% CIs for each one-unit increase in the log-transformed level of blood manganese with LTBI were 9.954 (1.389 ~ 71.344).

Conclusion: Significant associations were observed in girls, aged ≥12years and in the non-Hispanic white population. In conclusion, total mercury is associated with an increased prevalence of LTBI among adults and positive association between blood manganese and LTBI was observed among adolescents. Further studies should be conducted to verify the results and explore potential biological mechanisms.

KEYWORDS

blood metals, latent tuberculosis infection, NHANES, LTBI, TB

Introduction

In 2020, approximately 1.5 million people died from tuberculosis (TB), and it was estimated that 10 million people fell ill with tuberculosis (TB) worldwide. TB was the 13th leading cause of death and the second leading infectious killer after COVID-19. By 2022, a total of 13 billion dollars are needed annually for TB prevention, diagnosis, treatment and care in the US (1). Fortunately, TB is curable and preventable. According to the United Nations High Level Meeting on TB in 2018, effective management and treatment of latent

tuberculosis infection (LTBI) is one of the TB preventive care approaches to control and reduce the incidence of newly diagnosed active TB (2). Individuals with LTBI are clinically asymptomatic; however, to diagnose and to treat them for LTBI could be beneficial in preventing TB (3). A previous study revealed that major reductions in US tuberculosis incidence could be achieved if LTBI treatment was substantially increased (4), and LTBI was considered as the final frontier of tuberculosis elimination.

LTBI is an asymptomatic status in which individuals demonstrate an immunological response to mycobacterium tuberculosis that confers a heightened risk of subsequently developing TB (5), and approximately one-third of the world's population may harbor LTBI. LTBI is caused by mycobacterium tuberculosis; however, many other factors (6), such as socioeconomic status (7) and diabetes (8), also increase the risk of LTBI. Previous studies showed that heavy metals were associated with adverse impacts on immune function and lung host defense (9, 10), which may increase the risk of LTBI. It was reported that an exposure-response relationship between cumulative respirable metal dust exposures with deterioration of lung function among steel workers. The economic cost to society from COPD is tied to dysfunctional innate immune responses to infection, leading to repetitive and severe exacerbations of this existing lung disease (9, 10). In addition, some studies also indicated that chronic low-grade inflammation may contribute to susceptibility to TB infection (11), while metals such as zinc, lead and cadmium have been demonstrated to be associated with systemic inflammation (12, 13). Therefore, we speculate that metals may be associated with LTBI.

To address the gap in knowledge related to metal exposure and LTBI, we conducted the study to determine the association between blood metals including lead, cadmium, total mercury, selenium and manganese and LTBI using the National Health and Nutrition Examination Survey (NHANES).

Methods

Study population

The study population was from NHANES 2011 ~ 2012 and data were obtained by questionnaire and interview, mobile physical examination and laboratory tests with a complex, multistage, probability sampling method. Details of NHANES have been described online[1] (14–17). In the present analysis, participants with basic characteristics (N = 9,756), blood metals (N = 8,956), and LTBI (N = 7,821) were first enrolled. After excluding data without incomplete information on blood metals (N = 1,810) and LTBI (N = 669), a total of 7,108 individuals (N = 5,248 for adults and N = 1,860 for adolescents aged 12–17 years old) were included. The flowchart of the study is presented in Supplementary Figure S1. The NHANES protocol was approved by the National Center for Health Statistics Institutional Review Board, and written informed consent is obtained.

Definition of LTBI

QuantiFERON-TB gold In-Tube (QFT-GIT) was analyzed according to manufacturer instructions. The results were interpreted according to guidelines from the Centers for Disease Control and Prevention (CDC) for using interferon-gamma release assays (IGRAs) (18). Participants with positive QFT-GIT results were classified as LTBI positive, and participants with negative QFT-GIT results were classified as LTBI negative. Participants with indeterminate QFTGIT results or missing were classified as LTBI positive if induration ≥10 mm in response to PPD regardless of participants' LTBI risk factors (19, 20).

Measurement of blood metals

We choose the blood metals according to data from NHANES (15–17). 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. The metals measured in whole blood were lead, cadmium, total mercury, selenium and manganese. The detection limit for all analytes was constant in the data set. The lower detection limits for lead were 0.25 μg/dL, and 0.16 μg/L for cadmium, 30 μg/dL for selenium, 1.06 μg/dL for manganese, and 0.16 μg/L for total mercury. Among the samples where values were below the limit of detection, they were imputed as the lowest LOD value for each metal.[2]

Ascertainment of covariates

We included covariates based on previous reports (15–17). Gender, age, race/ethnicity (Mexican American, non-Hispanic white, non-Hispanic black, non-Hispanic Asian and others), educational level (less than high school, high school or equivalent, and college or above), family income-poverty ratio (PIR), smoking status (only for adults), drinking status (only for adults), and body mass index (BMI) were obtained by interviews and physical examinations. BMI was calculated as weight (kg) divided by the square of height (m^2), and was categorized into three groups: <25, 25 ~ 30 and ≥ 30 kg/ m^2. PIR was grouped into three categories: 0–1.0, 1.1–3.0, and > 3.0. Current smokers were defined as those who smoked at least 100 cigarettes and smoked at the time of the survey (only for adults). Current drinkers were defined as those who had at least 12 alcohol drinks per year (only for adults).

1 https://wwwn.cdc.gov/nchs/nhanes/analyticguidelines.aspx

2 https://wwwn.cdc.gov/Nchs/Nhanes/2011-2012/PBCD_G.htm

Abbreviations: LTBI, latent tuberculosis infection; NHANES, national health and nutrition examination surveys; TB, tuberculosis; QFT-GIT, QuantiFERON-TB gold in-tube; CDC, centers for disease control and prevention; ORs, odds ratios; 95%CIs, 95%confidence intervals; PIR, family income-poverty ratio; BMI, body mass index.

Statistical analysis

Complex survey design factors including sample weights, clustering, and stratification were considered for all analyses with instructions for using NHANES data. We compared baseline characteristics by LTBI in the two intervals by using the Rao-Scott χ^2 test for categorical variables and analysis of variance and Kruskal-Wallis test adjusted for sampling weights for continuous variables. Logistic regression models were used to estimate the odds ratios (ORs) with 95% confidence intervals (CIs) for each metal and LTBI. The baseline age (years, continuous), gender, race/ethnicity (non-Hispanic white, non-Hispanic black, Mexican American, and others) and education level (less than high school, high school or equivalent, and college or above) were adjusted in model 2. Furthermore, family income-poverty ratio level (0–1.0, 1.1–3.0, >3.0), BMI (<25, 25 ~ 30 and ≥ 30 kg/ m^2), smoking status (no, yes) and drinking status (yes, no) were adjusted in model 3. In subgroup analyses, we examined the bivariate association between all participant characteristics and lifestyles among adults and that between gender, age and PIR among adolescents adjusted for gender, age, race, education, income, BMI, smoking status and drinking status.

All statistical analyses were performed using SAS version 9.4 (SAS Institute Inc., Cary, NC, United States) with a 2-sided $p < 0.05$ considered statistically significant.

Results

Basic characteristics

Tables 1 and 2 show the basic characteristics of the 5,248 adults and 1,860 adolescents. For adults, 2,599 (49.52%) were males, and more than 50% were individuals with college and above educational levels. The weighted genome means of metals (95%CI) including lead, cadmium, total mercury, selenium, and manganese were 1.067 (1.003 ~ 1.136), 0.332 (0.317 ~ 0.347), 0.849 (0.742 ~ 0.972), 192.908 (190.047 ~ 195.812), and 9.101 (8.954 ~ 9.250) μg/dL, respectively. For adolescents, 951 (51.13%) were boys, 1,019 (54.78%) were adolescents aged 6 ~ 12 years old. The weighted genome means of metals (95%CI) including lead, cadmium, total mercury, selenium, and manganese were 0.604 (0.564 ~ 0.647), 0.146 (0.141 ~ 0.151), 0.355 (0.304 ~ 0.414), 182.502 (177.846 ~ 187.281), and 10.226 (10.002 ~ 10.456) μg/dL, respectively. The distribution of blood metals in US adults and adolescents are presented in Supplementary Tables S1, S2, and significant differences were observed for all metals between most groups.

Associations between blood metals and LTBI among adults and adolescents

The associations between blood metals and LTBI among adults are presented in Table 3. A significant positive association of total mercury with LTBI was found, and OR (95% CI) for each 1-unit increase in log-transformed levels of total mercury with LTBI was 1.411 (1.164 ~ 1.710) after adjusting for all variables. Compared with Q1, Q4 was significantly associated with an increased prevalence of LTBI (2.303; 1.455 ~ 3.644). Furthermore, subgroup analyses of the association of total mercury with LTBI were conducted (Supplementary Table S3). The OR of total mercury and LTBI was higher among females (1.517; 1.009 ~ 2.279), individuals aged 45 ~ 64 (1.457; 1.060 ~ 2.002), and non-Hispanic White individuals (1.773; 1.316 ~ 2.388). In addition, a positive relationship was observed among participants with obesity (1.553; 1.040 ~ 2.319) and underweight (1.380; 1.076 ~ 1.771), with college or above (1.645; 1.184 ~ 2.286), with PIR > 3.0 (1.701; 1.217 ~ 2.376), reported smoking (1.535; 1.235 ~ 1.907), and drinking (1.464; 1.232 ~ 1.739).

Associations between blood metals and LTBI among adolescents are presented in Table 4, only blood manganese was positively associated with LTBI. The OR and 95% CIs for each one-unit increase in the log-transformed level of blood manganese with LTBI were 9.954 (1.389 ~ 71.344). Subgroup analyses of the association of blood manganese with LTBI showed significant associations in girls, aged ≥12 years and with a PIR > 3.0 (Supplementary Table S4).

Discussion

In the present study, we found positive relationships between total mercury and LTBI in US adults. Meanwhile, a positive association of blood manganese with LTBI among US adolescents was observed. The findings in the present study have important implications for public health, as both metal pollution and LTBI are major health concerns. The results indicated that controlling for environmental metals may be an effective measure to control LTBI. Besides, significant differences in age, educational levels, income and smoking status in participants with LTBI vs. non-LTBI in the present study were also observed.

The underlying biological mechanism between metals and LTBI remains unclear. The inflammation process is reported to be associated not only with heavy metals (21, 22), but also with LTBI (23). However, information on biomarkers of inflammation in NHANES data (2011 ~ 2012) is missing, and further study needs to be conducted in the future to explore the mechanism. In addition, heavy metals including mercury could increase lipid peroxidation and related oxidative stress (24, 25), and cardiometabolic risks (26, 27), which are also risk factors for LTBI (8, 28–30). However, these factors need to be confirmed in future studies involving animals and humans.

Subgroup analyses showed that the relationship between total mercury and LTBI was observed in US obese adults. A previous study showed that cumulative exposure to heavy metals was associated with obesity (31); meanwhile, obese individuals may be more susceptible to chronic systemic inflammation (32) and metabolic disorders (33), which may reinforce the risk of LTBI. On the other hand, their association was also observed in underweight adults, and a previous study showed that undernutrition was associated with gut microbiota composition and inflammation (34), which may provide some clue to understand the results; however, more studies are needed to explore the related mechanism. In addition, an association of

TABLE 1 Basic characteristics of the adults in NHANES 2011–2012 according to LTBI ($N = 5,248$).

Characteristics	All participants	Non-LTBI (4742)	LTBI (506)	Rao-Scott χ^2	*p* value
Gender				11.0154	0.0009*
Male	2,599 (49.52)	2,306 (48.63)	293 (57.91)		
Female	2,649 (50.48)	2,436 (51.37)	213 (42.09)		
Age				23.2327	<0.0001*
18 ~ 44	2,461 (46.89)	2,338 (49.30)	123 (24.31)		
45 ~ 64	1713 (32.64)	1,490 (31.42)	223 (44.07)		
≥65	1,074 (20.46)	914 (19.27)	160 (31.62)		
Race				225.424	<0.0001*
Mexican American	538 (10.25)	462 (9.74)	76 (15.02)		
Non-Hispanic White	1926 (36.70)	1854 (39.10)	72 (14.23)		
Non-Hispanic Black	1,372 (26.14)	1,250 (26.36)	122 (24.11)		
Non-Hispanic Asian	712 (13.57)	574 (12.10)	138 (27.27)		
Others	700 (13.34)	602 (12.70)	98 (19.37)		
Educational levels				41.1142	<0.0001*
Less than high school	1,251 (23.84)	1,059 (22.33)	192 (37.94)		
High school or equivalent	1,122 (21.38)	1,017 (21.45)	105 (20.75)		
College or above	2,875 (54.78)	2,666 (56.22)	209 (41.30)		
[a]**PIR**				14.9649	0.0006*
0–1.0	1,272 (26.44)	1,139 (26.09)	133 (29.89)		
1.1–3.0	1862 (38.71)	1,680 (38.49)	182 (40.90)		
>3.0	1,676 (34.84)	1,546 (35.42)	130 (29.21)		
[b]**BMI, kg/m^2**				0.5966	0.7421
<25	1,699 (32.86)	1,533 (32.83)	166 (33.07)		
25–30	1,639 (31.70)	1,468 (31.44)	171 (34.06)		
≥30	1833 (35.45)	1,668 (35.72)	165 (32.87)		
[c]**Smoking status**				3.067	0.0799
No	2,835 (57.09)	2,573 (57.60)	262 (52.51)		
Yes	2,131 (42.91)	1894 (42.40)	237 (47.49)		
[d]**Drinking status**				11.9029	0.0006*
No	3,419 (72.59)	1,142 (26.71)	149 (34.33)		
Yes	1,291 (27.41)	3,134 (73.29)	285 (65.67)		
Blood metals (ug/dL) (GM, 95%CI)					
Blood lead	1.067 (1.003 ~ 1.136)	1.051 (0.989 ~ 1.118)	1.374 (1.210 ~ 1.560)	−1.9	0.075
Blood cadmium	0.332 (0.317 ~ 0.347)	0.329 (0.314 ~ 0.345)	0.382 (0.337 ~ 0.432)	−0.44	0.6681
Total mercury	0.849 (0.742 ~ 0.972)	0.832 (0.727 ~ 0.951)	1.205 (0.988 ~ 1.469)	−3.94	0.0011*
Blood Selenium	192.908 (190.047 ~ 195.812)	192.962 (190.038 ~ 195.932)	192.009 (187.698 ~ 196.420)	0.47	0.642
Blood manganese	9.101 (8.954 ~ 9.250)	9.076 (8.935 ~ 9.219)	9.529 (9.027 ~ 10.058)	−1.93	0.0708

LTBI: latent tuberculosis infection; PIR: family income-poverty ratio; BMI: body mass index. [a]438 individuals missing; [b]77 individuals missing; [c] 282 individuals missing; [d]538 individuals missing. * $p < 0.05$.

total mercury was found among smokers, but not among nonsmokers. A previous study showed that the impact of smoking on heavy metal contamination (35, 36), and our study also revealed that the level of total mercury was higher in smokers than in nonsmokers. Similar findings were observed among drinkers, which may be due to the higher concentration of metals according to our results. Besides, the ORs of total mercury and LTBI in adults and blood manganese with LTBI in

TABLE 2 Basic characteristics of the adolescents in NHANES 2011–2012 according to LTBI ($N = 1860$).

Characteristics	All participants	Non-LTBI (4742)	LTBI (506)	*Rao-Scott* χ^2	*p* value
Gender				0.0271	0.8692
Boy	951 (51.13)	939 (51.09)	12 (54.55)		
Girl	909 (48.87)	899 (48.91)	10 (45.45)		
Age				0.8378	0.36
6 ~ 12	1,019 (54.78)	1,010 (54.95)	9 (40.91)		
≥12	841 (45.22)	828 (45.05)	13 (59.09)		
Race				3.2549	0.5161
Mexican American	369 (19.84)	364 (19.80)	5 (22.73)		
Non-Hispanic White	422 (22.69)	418 (22.74)	4 (18.18)		
Non-Hispanic Black	557 (29.95)	554 (30.14)	3 (13.64)		
Non-Hispanic Asian	212 (11.40)	207 (11.26)	5 (22.73)		
Others	300 (16.13)	295 (16.05)	5 (22.73)		
[a]**PIR**				3.4157	0.1813
0–1.0	611 (35.07)	605 (35.17)	6 (27.27)		
1.1–3.0	689 (39.55)	678 (39.42)	11 (50.00)		
>3.0	442 (25.37)	437 (25.41)	5 (22.73)		
Blood metals (ug/dL) (GM, 95%CI)					
Blood lead	0.604 (0.564 ~ 0.647)	0.605 (0.565 ~ 0.647)	0.546 (0.344 ~ 0.868)	0.37	0.7194
Blood cadmium	0.146 (0.141 ~ 0.151)	0.146 (0.141 ~ 0.151)	0.146 (0.115 ~ 0.186)	0.05	0.959
Total mercury	0.355 (0.304 ~ 0.414)	0.354 (0.302 ~ 0.415)	0.448 (0.343 ~ 0.585)	−0.76	0.4557
Blood Selenium	182.502 (177.846 ~ 187.281)	182.435 (177.767 ~ 187.226)	188.484 (177.564 ~ 200.076)	−1.07	0.2986
Blood manganese	10.226 (10.002 ~ 10.456)	10.203 (9.964 ~ 10.447)	12.529 (10.910 ~ 14.388)	−2.57	0.0198*

LTBI: latent tuberculosis infection; PIR: family income-poverty ratio; BMI: body mass index. [a]118 individuals missing. * $p < 0.05$.

adolescents were different in sociodemographic characteristics, which implied more concerns should be given to certain population (29, 37, 38).

Our study was the first investigation to examine the relationship between metal exposure and LTBI among adults as well as in adolescents based on a large multiethnic, nationally representative sample of U.S. population. Our results suggested that more attention should be given to environmental pollution problems, especially for total mercury and blood manganese. However, some limitations should also be addressed. First, the data set is cross-sectional and causal inference could not be made. Second, our findings may be only representative of the US population, and further study should be conducted to validate the generalizability to other populations.

Conclusion

The present study reveals that the positive associations between total mercury exposure and LTBI among adults and blood manganese are related to LTBI among adolescents in the United States. The findings indicate that exposure to specific metals is associated with increased prevalence of LTBI among adults and adolescents, which may have profound implications in light of the importance of controlling LTBI.

Data availability statement

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

Author contributions

JW: Software, Writing – original draft. KW: Formal analysis, Methodology, Software, Writing – original draft. FT: Investigation, Methodology, Writing – original draft. QL: Formal analysis, Writing – original draft. XL: Supervision, Writing – review & editing. FX: Conceptualization, Writing – review & editing.

TABLE 3 Associations between blood metals and LTBI among adults.

	Model 1	Model 2	Model 3
Blood lead (ug/dl)			
Continuous log-transformed	**1.657 (1.398 ~ 1.962)***	1.213 (0.973 ~ 1.513)	1.201 (0.881 ~ 1.639)
Q1 (0.18 ~ 0.68)	Ref.		
Q2 (0.69 ~ 1.08)	2.114 (1.438 ~ 3.109)	1.537 (0.998 ~ 2.367)	1.661 (1.013 ~ 2.723)
Q3 (1.09 ~ 1.73)	2.625 (1.663 ~ 4.143)	1.512 (0.958 ~ 2.388)	1.449 (0.932 ~ 2.251)
Q4 (1.74 ~ 61.29)	3.121 (2.045 ~ 4.763)	1.580 (0.952 ~ 2.623)	1.529 (0.844 ~ 2.770)
Blood cadmium (ug/dl)			
Continuous log-transformed	**1.218 (1.023 ~ 1.450)** *	1.058 (0.890 ~ 1.259)	0.914 (0.738 ~ 1.132)
Q1 (0.11 ~ 0.20)	Ref.		
Q2 (0.21 ~ 0.33)	1.038 (0.695 ~ 1.551)	0.824 (0.567 ~ 1.198)	0.799 (0.503 ~ 1.270)
Q3 (0.34 ~ 0.61)	1.527 (0.941 ~ 2.476)	1.009 (0.607 ~ 1.676)	0.718 (0.385 ~ 1.336)
Q4 (0.62 ~ 9.30)	1.751 (1.188 ~ 2.581)	1.174 (0.806 ~ 1.709)	0.876 (0.544 ~ 1.413)
Total mercury (ug/dl)			
Continuous log-transformed	**1.403 (1.232 ~ 1.598)** *	**1.282 (1.126 ~ 1.459)** *	**1.411 (1.164 ~ 1.710)** *
Q1 (0.11 ~ 0.42)	Ref.		
Q2 (0.43 ~ 0.83)	1.196 (0.763 ~ 1.875)	1.142 (0.762 ~ 1.710)	1.270 (0.756 ~ 2.135)
Q3 (0.84 ~ 1.86)	1.214 (0.788 ~ 1.870)	1.176 (0.843 ~ 1.642)	1.414 (0.972 ~ 2.059)
Q4 (1.87 ~ 50.81)	**2.263 (1.662 ~ 3.080)** *	**1.861 (1.328 ~ 2.609)** *	**2.303 (1.455 ~ 3.644)** *
Blood selenium (ug/dl)			
Continuous log-transformed	0.760 (0.222 ~ 2.595)	0.961 (0.350 ~ 2.639)	1.083 (0.354 ~ 3.312)
Q1 (105.39 ~ 176.46)	Ref.		
Q2 (176.48 ~ 190.97)	1.020 (0.604 ~ 1.723)	1.090 (0.620 ~ 1.915)	1.010 (0.524 ~ 1.950)
Q3 (191.00 ~ 207.01)	0.986 (0.639 ~ 1.521)	1.104 (0.739 ~ 1.650)	0.998 (0.600 ~ 1.659)
Q4 (207.02 ~ 692.52)	0.905 (0.566 ~ 1.447)	0.978 (0.650 ~ 1.472)	1.016 (0.623 ~ 1.659)
Blood manganese (ug/dl)			
Continuous log-transformed	**1.498 (1.001 ~ 2.240)** *	1.287 (0.850 ~ 1.949)	1.317 (0.759 ~ 2.286)
Q1 (1.61 ~ 7.26)	Ref.		
Q2 (7.27 ~ 9.11)	1.093 (0.809 ~ 1.478)	1.124 (0.824 ~ 1.533)	1.106 (0.728 ~ 1.682)
Q3 (9.12 ~ 11.46)	1.233 (0.859 ~ 1.771)	1.234 (0.882 ~ 1.728)	1.092 (0.696 ~ 1.713)
Q4 (11.47 ~ 62.51)	1.500 (1.070 ~ 2.103)	1.262 (0.831 ~ 0.838)	1.268 (0.800 ~ 2.010)

Model 1: unadjusted; Model 2: adjusted for gender, age, race, education; Model 3: adjusted for gender, age, race, education, income, BMI, smoking, drinking. Including 4,054 participants in the analysis. *$p < 0.05$.
Bold value means statistically significant.

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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.

The reviewer PX declared a past co-authorship with the author JW to the handling editor.

Publisher's note

Supplementary material

The Supplementary material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fnut.2023.1259902/full#supplementary-material

TABLE 4 Associations between blood metals and LTBI among adolescents.

	Model 1	Model 2	Model 3
Blood lead (ug/dl)			
Continuous log-transformed	0.719 (0.158 ~ 3.278)	0.853 (0.245 ~ 2.963)	0.853 (0.221 ~ 3.297)
Q1 (0.18 ~ 0.45)	Ref.		
Q2 (0.46 ~ 0.62)	0.536 (0.148 ~ 1.937)	0.557 (0.172 ~ 1.808)	0.593 (0.192 ~ 1.830)
Q3 (0.63 ~ 0.90)	1.076 (0.186 ~ 6.216)	1.327 (0.320 ~ 5.503)	1.334 (0.304 ~ 5.860)
Q4 (0.91 ~ 15.37)	0.733 (0.114 ~ 4.709)	0.973 (0.211 ~ 4.492)	1.001 (0.192 ~ 5.227)
Blood cadmium (ug/dl)			
Continuous log-transformed	1.001 (0.274 ~ 3.663)	0.693 (0.131 ~ 3.675)	0.662 (0.129 ~ 3.410)
Q1 (0.11 ~ 0.11)	Ref.		
Q2 (0.16 ~ 0.19)	0.424 (0.102 ~ 1.762)	0.364 (0.076 ~ 1.751)	0.378 (0.079 ~ 1.810)
Q3 (0.20 ~ 0.21)	1.092 (0.267 ~ 4.461)	0.758 (0.140 ~ 4.098)	0.725 (0.137 ~ 3.839)
Total mercury (ug/dl)			
Continuous log-transformed	1.358 (0.854 ~ 2.160)	1.188 (0.664 ~ 2.125)	1.299 (0.656 ~ 2.570)
Q1 (0.11 ~ 0.22)	Ref.		
Q2 (0.23 ~ 0.37)	0.530 (0.046 ~ 6.075)	0.504 (0.041 ~ 6.161)	0.478 (0.038 ~ 6.018)
Q3 (0.38 ~ 0.68)	0.983 (0.222 ~ 4.342)	0.899 (0.187 ~ 4.316)	0.972 (0.205 ~ 4.602)
Q4 (0.69 ~ 9.39)	2.201 (0.423 ~ 11.456)	1.757 (0.247 ~ 12.475)	2.138 (0.261 ~ 17.497)
Blood selenium (ug/dl)			
Continuous log-transformed	8.005 (0.296 ~ 216.828)	2.631 (0.177 ~ 39.165)	2.747 (0.256 ~ 29.448)
Q1 (109.26 ~ 166.09)	Ref.		
Q2 (166.13 ~ 179.68)	1.216 (0.217 ~ 6.814)	1.124 (0.200 ~ 6.304)	1.068 (0.174 ~ 6.541)
Q3 (179.69 ~ 194.21)	2.036 (0.606 ~ 6.840)	1.730 (0.444 ~ 6.742)	1.712 (0.432 ~ 6.787)
Q4 (194.22 ~ 310.81)	1.767 (0.244 ~ 12.811)	1.223 (0.182 ~ 8.234)	1.240 (0.192 ~ 7.986)
Blood manganese (ug/dl)			
Continuous log-transformed	**8.523 (1.893 ~ 38.368)** *	**8.298 (1.154 ~ 59.653)** *	**9.954 (1.389 ~ 71.344)** *
Q1 (3.21 ~ 8.25)	Ref.		
Q2 (8.27 ~ 10.04)	12.730 (1.221 ~ 132.718)	12.781 (1.264 ~ 129.223)	11.905 (1.232 ~ 115.037)
Q3 (10.05 ~ 12.48)	12.578 (1.302 ~ 121.461)	13.229 (1.373 ~ 127.454)	13.977 (1.463 ~ 130.880)
Q4 (12.49 ~ 58.86)	31.576 (2.856 ~ 349.101)	31.666 (2.870 ~ 249.319)	32.292 (3.141 ~ 331.992)

Model 1: unadjusted; Model 2: adjusted for gender, age, race, education; Model 3: adjusted for gender, age, race, education and income. Including 1742 participants in the analysis. ***p* < 0.05.** Bold value means statistically significant.

References

1. WHO (2021): *Tuberculosis key facts*. Available at: https://www.who.int/news-room/factsheets/detail/tuberculosis

2. Wong YJ, Noordin NM, Keshavjee S, Lee SWH. Impact of latent tuberculosis infection on health and wellbeing: a systematic review and meta-analysis. *Eur Respir Rev*. (2021) 30:200260. doi: 10.1183/16000617.0260-2020

3. LoBue PA, Mermin JH. Latent tuberculosis infection: the final frontier of tuberculosis elimination in the USA. *Lancet Infect Dis*. (2017) 17:e327–33. doi: 10.1016/S1473-3099(17)30248-7

4. HILL AN, BECERRA JE, CASTRO KG. Modelling tuberculosis trends in the USA. *Epidemiol Infect*. (2012) 140:1862–72. doi: 10.1017/S095026881100286X

5. Fox GJ, Dobler CC, Marais BJ, Denholm JT. Preventive therapy for latent tuberculosis infection-the promise and the challenges. *Int J Infect Dis*. (2017) 56:68–76. doi: 10.1016/j.ijid.2016.11.006

6. Lönnroth K, Jaramillo E, Williams BG, Dye C, Raviglione M. Drivers of tuberculosis epidemics: the role of risk factors and social determinants. *Soc Sci Med*. (2009) 68:2240–6. doi: 10.1016/j.socscimed.2009.03.041

7. Bennett DE, Courval JM, Onorato I, Agerton T, Gibson JD, Lambert L, et al. Prevalence of tuberculosis infection in the United States population. *Am J Respir Crit Care Med*. (2008) 177:348–55. doi: 10.1164/rccm.200701-057OC

8. Barron MM, Shaw KM, Bullard KM, Ali MK, Magee MJ. Diabetes is associated with increased prevalence of latent tuberculosis infection: findings from the National Health and nutrition examination survey, 2011-2012. *Diabetes Res Clin Pract*. (2018) 139:366–79. doi: 10.1016/j.diabres.2018.03.022

9. Hamzah NA, Mohd Tamrin SB, Ismail NH. Metal dust exposure and lung function deterioration among steel workers: an exposure-response relationship. *Int J Occup Environ Health*. (2016) 22:224–32. doi: 10.1080/10773525.2016.1207040

10. Knoell DL, Wyatt TA. The adverse impact of cadmium on immune function and lung host defense. *Semin Cell Dev Biol*. (2021) 115:70–6. doi: 10.1016/j.semcdb.2020.10.007

11. Martinez N, Kornfeld H. Diabetes and immunity to tuberculosis. *Eur J Immunol*. (2014) 44:617–26. doi: 10.1002/eji.201344301

12. Bonaventura P, Benedetti G, Albarède F, Miossec P. Zinc and its role in immunity and inflammation. *Autoimmun Rev*. (2015) 14:277–85. doi: 10.1016/j.autrev.2014.11.008

13. Werder EJ, Beier JI, Sandler DP, Falkner KC, Gripshover T, Wahlang B, et al. Blood BTEXS and heavy metal levels are associated with liver injury and systemic inflammation in gulf states residents. *Food Chem Toxicol*. (2020) 139:111242. doi: 10.1016/j.fct.2020.111242

14. Wang K, Xia F, Li Q, Luo X, Wu J. The associations of weekend warrior activity patterns with the visceral adiposity index in US adults: repeated cross-sectional study. *JMIR Public Health Surveill*. (2023) 9:e41973. doi: 10.2196/41973

15. Xia F, Li Q, Luo X, Wu J. Identification for heavy metals exposure on osteoarthritis among aging people and machine learning for prediction: a study based on NHANES 2011-2020. *Front Public Health*. (2022) 10:906774. doi: 10.3389/fpubh.2022.906774

16. Xia F, Li Q, Luo X, Wu J. Machine learning model for depression based on heavy metals among aging people: a study with National Health and nutrition examination survey 2017-2018. *Front Public Health*. (2022) 10:939758. doi: 10.3389/fpubh.2022.939758

17. Xia F, Li Q, Luo X, Wu J. Association between urinary metals and leukocyte telomere length involving an artificial neural network prediction: findings based on NHANES 1999-2002. *Front Public Health*. (2022) 10:963138. doi: 10.3389/fpubh.2022.963138

18. Mazurek GH, Jereb J, Vernon A, LoBue P, Goldberg S, Castro K, et al. Updated guidelines for using interferon gamma release assays to detect *Mycobacterium tuberculosis* infection – United States, 2010. *MMWR Recomm Rep*. (2010) 59:1–25.

19. Khan K, Wang J, Hu W, Bierman A, Li Y, Gardam M. Tuberculosis infection in the United States. *Am J Respir Crit Care Med*. (2008) 177:455–60. doi: 10.1164/rccm.200706-950OC

20. Zheng X, Shi J, Wu J. Analysis of factors and corresponding interactions influencing clinical management assistant ability using competency model in China. *Medicine (Baltimore)*. (2020) 99:e23516. doi: 10.1097/MD.0000000000023516

21. Pollard KM, Cauvi DM, Toomey CB, Hultman P, Kono DH. Mercury-induced inflammation and autoimmunity. *Biochim Biophys Acta Gen Subj*. (2019) 1863:129299. doi: 10.1016/j.bbagen.2019.02.001

22. Renu K, Chakraborty R, Myakala H, Koti R, Famurewa AC, Madhyastha H, et al. Molecular mechanism of heavy metals (Lead, chromium, arsenic, mercury, nickel and cadmium) – induced hepatotoxicity – a review. *Chemosphere*. (2021) 271:129735. doi: 10.1016/j.chemosphere.2021.129735

23. Pavan Kumar N, Nair D, Banurekha VV, Dolla C, Kumaran P, Sridhar R, et al. Type 2 diabetes mellitus coincident with pulmonary or latent tuberculosis results in modulation of adipocytokines. *Cytokine*. (2016) 79:74–81. doi: 10.1016/j.cyto.2015.12.026

24. Kobal AB, Horvat M, Prezelj M, Briski AS, Krsnik M, Dizdarevic T, et al. The impact of long-term past exposure to elemental mercury on antioxidative capacity and lipid peroxidation in mercury miners. *J Trace Elem Med Biol*. (2004) 17:261–74. doi: 10.1016/S0946-672X(04)80028-2

25. Şener G, Şehirli AÖ, Ayanoğlu-Dülger G. Melatonin protects against mercury(II)-induced oxidative tissue damage in rats. *Pharmacol Toxicol*. (2003) 93:290–6. doi: 10.1111/j.1600-0773.2003.pto930607.x

26. Roy C, Tremblay PY, Ayotte P. Is mercury exposure causing diabetes, metabolic syndrome and insulin resistance? A systematic review of the literature. *Environ Res*. (2017) 156:747–60. doi: 10.1016/j.envres.2017.04.038

27. Stratakis N, Conti DV, Borras E, Sabido E, Roumeliotaki T, Papadopoulou E, et al. Association of Fish Consumption and Mercury Exposure during Pregnancy with Metabolic Health and inflammatory biomarkers in children. *JAMA Netw Open*. (2020) 3:e201007. doi: 10.1001/jamanetworkopen.2020.1007

28. Lee MR, Huang YP, Kuo YT, Luo CH, Shih YJ, Shu CC, et al. Diabetes mellitus and latent tuberculosis infection: a systematic review and Metaanalysis. *Clin Infect Dis*. (2017) 64:719–27. doi: 10.1093/cid/ciw836

29. Li W, Chen D, Yueqing Tao ZL, Wang D. Association between triglyceride-glucose index and carotid atherosclerosis detected by ultrasonography. *Cardiovasc Diabetol*. (2022) 21:137. doi: 10.1186/s12933-022-01570-0

30. Ugarte-Gil C, Carrillo-Larco RM, Kirwan DE. Latent tuberculosis infection and non-infectious co-morbidities: diabetes mellitus type 2, chronic kidney disease and rheumatoid arthritis. (2019): *Int J Infect Dis*, 80, S29–S31, doi: 10.1016/j.ijid.2019.02.018

1201-9712

31. Wang X, Mukherjee B, Park SK. Associations of cumulative exposure to heavy metal mixtures with obesity and its comorbidities among U.S. adults in NHANES 2003-2014. *Environ Int*. (2018) 121:683–94. doi: 10.1016/j.envint.2018.09.035

32. Cox AJ, West NP, Cripps AW. Obesity, inflammation, and the gut microbiota. *Lancet Diabetes Endocrinol*. (2015) 3:207–15. doi: 10.1016/S2213-8587(14)70134-2

33. Monteiro R, Azevedo I. Chronic inflammation in obesity and the metabolic syndrome. *Mediat Inflamm*. (2010) 2010:1–10. doi: 10.1155/2010/289645

34. Huey SL, Yu EA, Finkelstein JL, Glesby MJ, Bonam W, Russell DG, et al. Nutrition, inflammation, and the gut microbiota among outpatients with active tuberculosis disease in India. *Am J Trop Med Hyg*. (2021) 105:1645–56. doi: 10.4269/ajtmh.21-0310

35. Ahmed AS, Aldubayan MA, Ahmed HA, Refaat AM, Alsalloumi AS, Almasuood RA, et al. Impact of smoking on heavy metal contamination and DNA fragmentation. *Environ Sci Pollut Res Int*. (2021) 28:13931–41. doi: 10.1007/s11356-020-11633-y

36. Bao X, Asgari A, Najafi ML, Mokammel A, Ahmadi M, Akbari S, et al. Exposure to waterpipe smoke and blood heavy metal concentrations. *Environ Res*. (2021) 200:111460. doi: 10.1016/j.envres.2021.111460

37. Li W, Chen D, Peng Y, Lu Z, Wang D. Association of polycyclic aromatic hydrocarbons with systemic inflammation and metabolic syndrome and its components. *Obesity (Silver Spring)*. (2023) 31:1392–401. doi: 10.1002/oby.23691

38. Li W, Zhang H, Xiao Y, Tao Y, Chen W, Wang D. Association between occupational noise exposure duration and heart rate variability among Chinese adults: the role of plasma miRNAs. *Environ Pollut*. (2023b) 323:121324. doi: 10.1016/j.envpol.2023.121324

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EDITED BY
Misha Vrolijk,
Maastricht University, Netherlands

REVIEWED BY
Azalia Avila-Nava,
Hospital Regional de Alta Especialidad de la Península de Yucatán, Mexico
Dorota Formanowicz,
Poznan University of Medical Sciences, Poland

*CORRESPONDENCE
Xin Liu
✉ liuxinhook@126.com
Xiwu Jia
✉ jiaxiwu212@126.com

†These authors have contributed equally to this work

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Assessment of the temporal trend and daily profiles of the dietary purine intake among Chinese residents during 2014 to 2021

Shiwen Li, Xin Liu*†, Xiwu Jia*†, Min Fang, Qing Yang and Zhiyong Gong

College of Food Science and Engineering, Key Laboratory for Deep Processing of Major Grain and Oil (The Chinese Ministry of Education), Hubei Key Laboratory for Processing and Transformation of Agricultural Products, Wuhan Polytechnic University, Wuhan, China

The incidence of hyperuricemia is on the rise in China, primarily due to dietary habits. However, limited data exists regarding dietary purine intake in the country. This study aimed to estimate the daily dietary purine intake among Chinese residents from 2014 to 2021 and evaluate the temporal trend using joinpoint regression analysis. The analysis revealed an annual percentage change (APC) of 0.8% (95% CI: 0.1–1.5%) in dietary purine intake prior to the joinpoint (2014–2019). Following the joinpoint (2019–2021), the APC significantly increased to 6.5% (95% CI: 3.3–9.8%), indicating a noteworthy upward trend ($p = 0.045$). Furthermore, the average daily purine intake varied significantly among different regions of China, with the southern region showing the highest dietary intake of purines. Considering the diverse contributions of various food sources to dietary purine intake, it was observed that meat consumption had the greatest impact, accounting for 36.2% of purine intake, followed by cereals consumption (25.3%) and vegetables and edible fungi (24.2%). These findings hold significance for dietary intervention and management strategies aimed at reducing purine intake among the population.

KEYWORDS

purine, dietary intake, Chinese residents, temporal trend, joinpoint analyzes

1. Introduction

Purine, an essential base, plays a crucial role as a building block of deoxyribonucleic acid (DNA) and ribonucleic acid (RNA) in living organisms (1). It consists primarily of guanine, adenine, hypoxanthine, and xanthine, which are commonly found in various food sources. However, excessive dietary intake of purines can lead to elevated serum uric acid levels and pose a significant risk of developing hyperuricemia. In the human body, purines are eventually oxidized into uric acid (2, 3). Roughly 20% of the uric acid in the body is derived from purine-rich foods, while the remaining 80% originates from the synthesis of amino acids, nucleotides, other small-molecule compounds, and the catabolism of nucleic acids (4). Lack of timely excretion of uric acid can lead to its deposition in human joints and other tissues, causing hyperuricemia, gout, and other associated diseases (5–7).

Epidemiological studies have particularly focused on dietary interventions as important tools to prevent or slow down the adverse prognosis of gout (8). With economic development and changes in dietary habits, the prevalence of hyperuricemia and gout is increasing yearly and

affecting the younger population (9). Hyperuricemia has become the "fourth highest" after diabetes, hypertension, and hyperlipidemia (10). In 2021, a white paper on high uric acid and gout trends in China revealed an overall prevalence of hyperuricemia of 13.3%, affecting approximately 177 million people, and an overall incidence of gout of 1.1%, affecting approximately 14.66 million people. Other data show that nearly 60% of patients with hyperuricemia and gout in China are aged 18–35.[1] Thus, gout is no longer a disease affecting the middle-aged and old. The young population is suggested to incorporate the prevention and treatment of gout into their knowledge, especially those who prefer eating high-purine foods such as seafood, drinking excessively, working and resting irregularly, being overweight, and smoking (11).

For a long time, dietary purine content has been debated as the leading cause of hyperuricemia and gout in the academic community (12). However, most studies suggest that a high-purine diet increases the serum uric acid content, which is implicated in hyperuricemia and gout (13–15). In addition to drug therapy, a low-purine diet is key to treating patients with gout. Further research on hyperuricemia and gout, as well as the purine content in foods, is imperative. It has been reported that purine intake in patients with acute and chronic gout should be <150 mg/day (16); thus, it is important to know the daily intake of dietary purine. However, few reports evaluate purine intake in Chinese residents' diet.

Socioeconomic development has led to significant improvements in living standards and changes in the dietary structure of Chinese residents. Urbanization and agricultural policies have played a role in shaping changes in the dietary structure of Chinese residents, which may greatly influence the dietary intake of purine and the management of purine-related chronic diseases (17). Therefore, it is essential to analyze the temporal trends and differences in purine intake in Chinese residents.

This study evaluated the national level of total purine intake from commonly consumed foods of Chinese residents. Based on the statistics of different kinds of food consumption from 2014 to 2021 and the purine content database, a temporal trend was also assessed for the purine intake from different foods.

2. Materials and methods

2.1. Data source for food consumption of the Chinese population

National-level dietary information of Chinese residents from 2014 to 2021 was obtained from the China Statistical Yearbooks, published by the National Bureau of Statistics. Specific variables were meticulously chosen to represent various dietary components and trends pertinent to the study objectives. The national statistical data included 10 categories of staple foods: grain, oil, vegetables and edible fungi, livestock meat, poultry, aquatic products, eggs, dairy products, fruits, and sugar. This data has been updated to the year 2021. A detailed breakdown of this dataset, including the presentation format, variables, and categories, can be found in Supplementary Tables S1–S3.

1 www.cbndata.com

We utilized a comprehensive dataset from this database, which offers detailed information on dietary patterns from 2014 to 2021. The staple food consumption data in the China Statistical Yearbooks covered 31 provincial regions stratified by area (urban/rural) and gender. Various sampling methods were comprehensively applied to the sample size of 66,000 urban and 74,000 rural households to investigate their main food consumption (18). All the data reported in this study were analyzed in adherence to relevant guidelines and regulations of National Bureau of Statistics of China.

2.2. Purine content in different food

Food purine contents were obtained from the Chinese food composition tables (19). Purines, including adenine, hypoxanthine, guanine, and xanthine in different foods, have been measured using high-performance liquid chromatography (HPLC) (20). Total purine content was the sum of adenine, guanine, hypoxanthine, and xanthine levels. The approximate values of specific purine content in each food are shown in Supplementary Table S1. For each type of food, several foods frequently consumed by residents in various categories were selected. The average purine content of each type of food was calculated (Supplementary Table S2).

2.3. Estimated dietary intake of purine

Foods frequently consumed by Chinese residents in each food category were selected, and the average purine content was calculated. Based on the amount of food consumed and the purine content of the food in each category, the estimated dietary intake (EDI) of purine in food is calculated as follows (21):

$$EDI = C \times IR$$

EDI, estimated daily intake (mg/d); C, purine concentration in foods (mg/100 g); IR, intake rate (g/d).

2.4. Statistical analysis

Temporal trends of dietary purine intake were analyzed using joinpoint regression analysis, a statistical method that fits a series of joined straight lines between statistically significant changes in trend (joinpoints) and estimates the change between joinpoints (22). The analysis was performed using National Cancer Institute (NCI) Joinpoint Regression Program software (Version 4.1.1). Areas covered by population-based purine intake were classified into urban/rural areas and 31 administrative regions of the Chinese mainland, according to the National Bureau of Statistics of China. Urban areas were defined as administrative divisions encompassing larger territorial and population scales, including municipalities, prefecture-level cities. In contrast, rural areas referred to lower-tier administrative units such as counties, county-level cities, and districts. Data are expressed as mean ± standard deviation (SD). Statistical analysis was performed using Origin Lab software (version 2020). Geographic spatial data were visualized using ArcGIS software (version 10.4).

3. Results

3.1. Trends in daily dietary intake of purine for Chinese residents from 2014 to 2021

We conducted a joinpoint regression analysis to examine the dietary purine intake trend from 2014 to 2021 (as shown in Figure 1). The analysis identified one significant joinpoint in the year 2019. The residents in urban areas have shown higher *per capita* daily purine intake than that in rural areas. It was observed that the difference in *per capita* daily purine intake between urban and rural areas is gradually narrowing from 2014 to 2021. The joinpoint regression parameters are displayed in Table 1. At the national level, the annual percentage change (APC) in dietary purine intake was 0.8% (95% CI: 0.1–1.5%) before the joinpoint (2014–2019), and the APC dramatically increased to 6.5% (95% CI: 3.3–9.8%) after the joinpoint (2019–2021), indicating a significant increasing trend ($p = 0.045$). The annual average percent change (AAPC) for the entire study period (2014–2021) was 2.4% (95% CI: 1.7–3.0%). As for the trend at urban level, the APC in dietary purine intake was 0.4% (95% CI: −0.0–0.8%). After the joinpoint (2019–2021), the APC increased to 5.0% (95% CI: 3.0–7.1%), indicating a significant increasing trend ($p = 0.038$). A non-significant increasing trend was observed for dietary purine intake in rural area. While the AAPC of the rural level is greater than urban level (3.1% vs. 1.7%).

3.2. Trends of dietary intake of purine by different foods from 2014 to 2021

Figure 2 shows trends in dietary purine intake in different food groups from 2014 to 2021. Dietary purine intake from cereals had a low level of 101.6 mg in 2018 and a high level of 114.8 mg in 2014. Purine intake from vegetables and edible fungi increased from 2014 to 2021, reaching a peak of 119.6 mg in 2021. Purine intake from Meat gradually increased, reaching a peak of 197.2 mg in 2021. Purine intake from aquatic products showed an overall increasing trend, peaking at 65.8 mg in 2021. Purine intake from beans increased, reaching a peak of 56.6 mg in 2021.

3.3. The averaged geographical distribution of dietary purine intake from 2014 to 2021

Figure 3 shows the geographical distribution of dietary purine intake from 2014 to 2021. It was observed that the average daily purine intake for different regions of China significantly varied, as displayed in a geographical heat map. The highest dietary intake of purine was observed for the population in Southern China, followed by the population in Eastern and Southwestern China. The population in Northwestern China has the lowest dietary intake of purine.

3.4. Contribution of different foods consumption to the daily purine intake

Figure 4 depicts averaged purine intake profiles (2014–2021) in seven food categories. In all the survey years (2014–2021), meat has the largest contribution to the dietary intake of purine (36.2%). Cereals (25.3%) and vegetables and edible fungi (24.2%) consumption have a comparable contribution rate to the dietary intake of purine. Though aquatic products have a high purine content, it has a modest contribution rate (11.0%) to the daily dietary intake of purine for Chinese residents. The above trends were observed in most of the survey year.

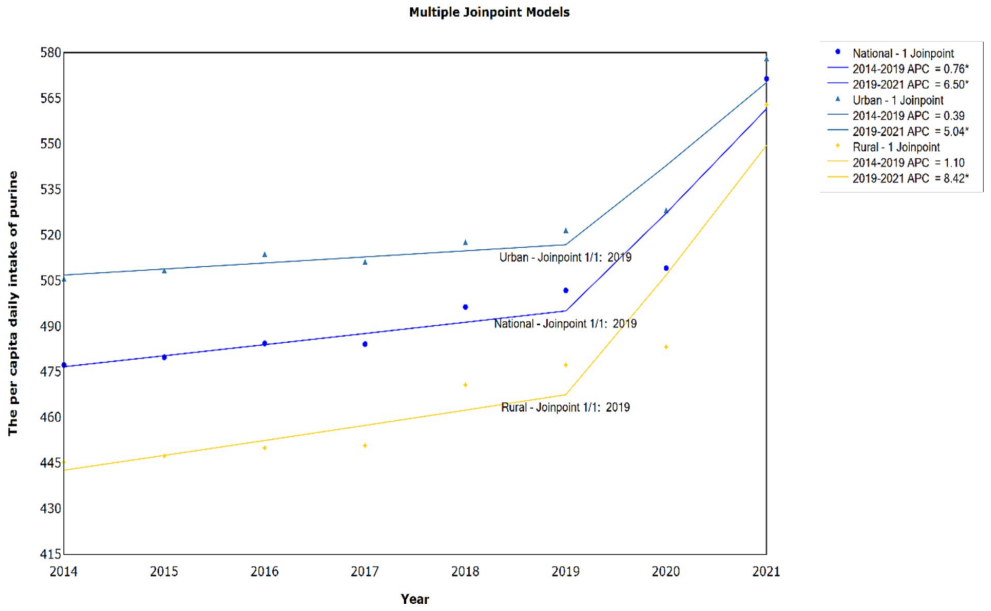

FIGURE 1
Joinpoint trends for *per capita* daily purine intake in Chinese residents, 2014–2021.

TABLE 1 Joinpoint analysis of *per capita* daily purine intake in urban, rural, and national areas.

	Total study period		Period 1			Period 2			*p*-value
	AAPC (%)	95% CI	Years	APC (%)	95% CI	Years	APC (%)	95% CI	
National	2.4	1.7–3.0	2014–2019	0.8	0.1–1.5	2019–2021	6.5	3.3–9.8	0.045
Urban	1.7	1.3–2.1	2014–2019	0.4	−0.0-0.8	2019–2021	5.0	3.0–7.1	0.038
Rural	3.1	1.8–4.4	2014–2019	1.1	−0.3-1.8	2019–2021	8.4	2.5–15.4	0.075

AAPC, annual average percent change; APC, annual percent change; CI, annual percent change.

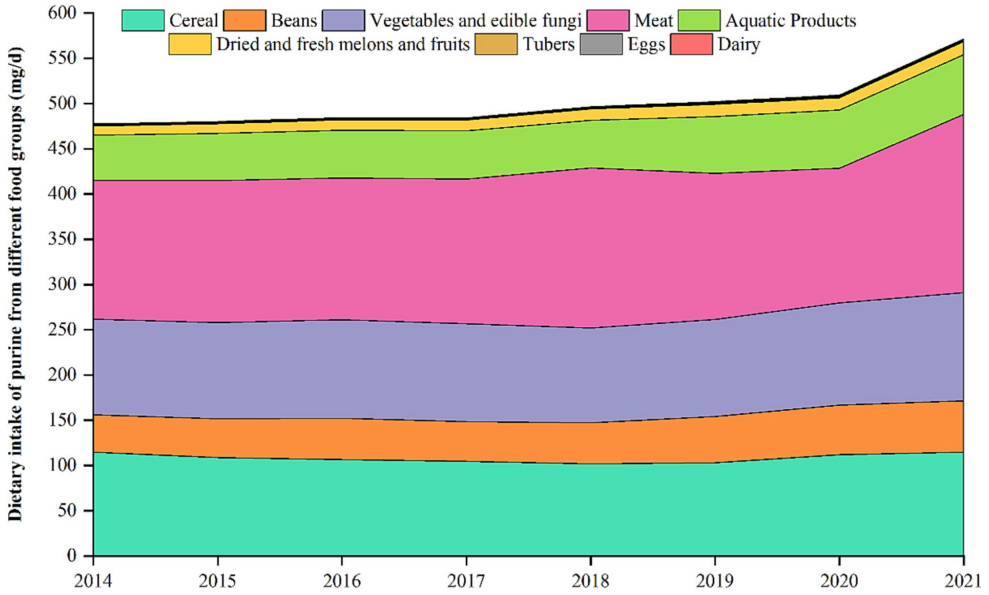

FIGURE 2
Trends in dietary purine intake from different foods, 2014–2021.

4. Discussion

Our research found that from 2014 to 2021, the *per capita* total purine intake of Chinese residents showed an overall upward trend. The average daily intake increased from 477.3 mg to 509.2 mg. Dietary meat products showed the highest average contribution rate (32.45%) of dietary purine, followed by vegetables and edible fungi (21.97%) and grains (21.90%).

Between 2014 to 2021, China's economy developed from high-speed growth to high-quality development, and the *per capita* disposable income of the population showed a yearly growth (23). The eating and consumption habits of Chinese residents have experienced great changes (24). It is manifested that refined grain consumption significantly decreased, whereas coarse grain consumption increased (25). In addition, staple food varieties showed diversification; animal food, fresh fruits, and vegetables consumption has also increased significantly. Compared to 2016, the Pagoda of Balanced Diet for Chinese Residents (2022) subdivided staple foods and separated tubers and cereals, highlighting the importance of tubers and roots. Due to environmental or economic constraints in different regions, animal foods are consolidated so that their inhabitants can more easily implement them to meet nutritional requirements. Simultaneously, the recommended intake of dairy products has also increased. Compared with the data in 2021, the intake of milk, vegetables and edible fungi, dried and fresh melons and fruits, and tubers and roots of Chinese residents did not meet the requirements, while the intake of cereals exceeded recommended values.

At the same time, different storage conditions and processing methods also affect the purine content of foods. Some researchers have studied the effects of photodynamic treatment on the storage life and purine content of *Litopenaeus vannamei* under low-temperature conditions (26). HPLC was performed to determine the shelf-life indices of foods. The results showed that the storage life of shrimp could be extended from 3 to 8 days by microcrystalline refrigeration. Photodynamic treatment combined with microcrystalline storage prolonged the storage life of shrimp to 12 days, and hypoxanthine content decreased significantly after 24 h of storage (26).

Moreover, different processing methods have different effects on the purine content of foods (27). Previous studies have shown the changes in purine content in food by different cooking methods, such as high-temperature heating, boiling, steaming, roasting, ultrasound, and microwaving. Reverse-phase HPLC (RP-HPLC) and ultraviolet detection (UV) were used to analyzes the effects of cooking (including various combinations of boiling, roasting, blanching, baking, and oven drying) on the purine content (including adenine, guanine, xanthine, and hypoxanthine) and their metabolites (uric acid) in three insects (*Tenebrio molitor*, *Gryllus assimilis*, and *Acheta domesticus*) suitable for human consumption (28, 29). According to the data obtained, boiling for 15 min significantly reduced the purine content of *T. molitor* but did not affect the purine content of *A. domesticus* and *G. assimilis*. In

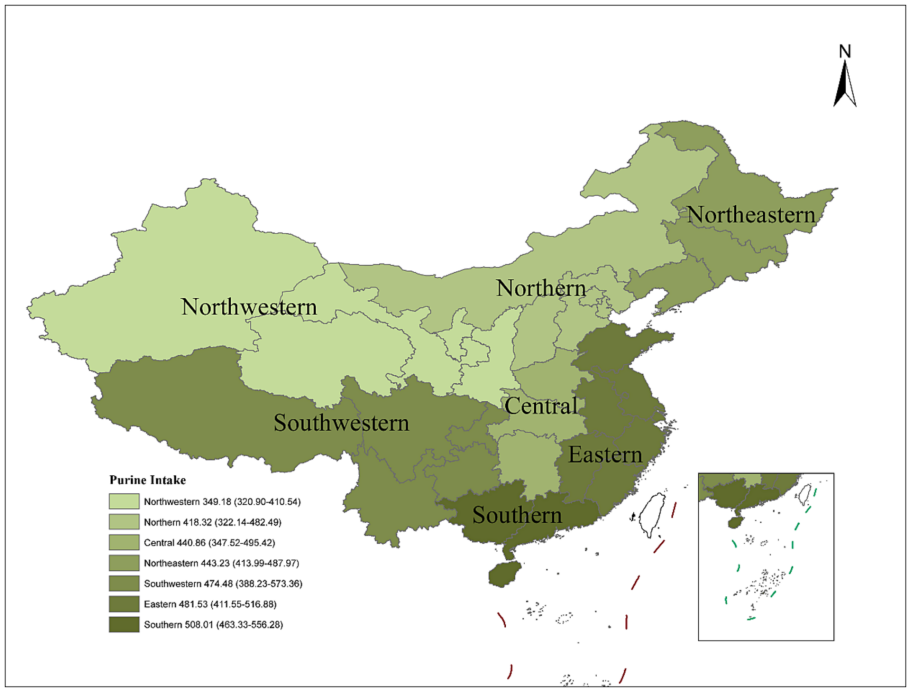

FIGURE 3
The averaged geographical distribution of dietary purine intake, 2014–2021.

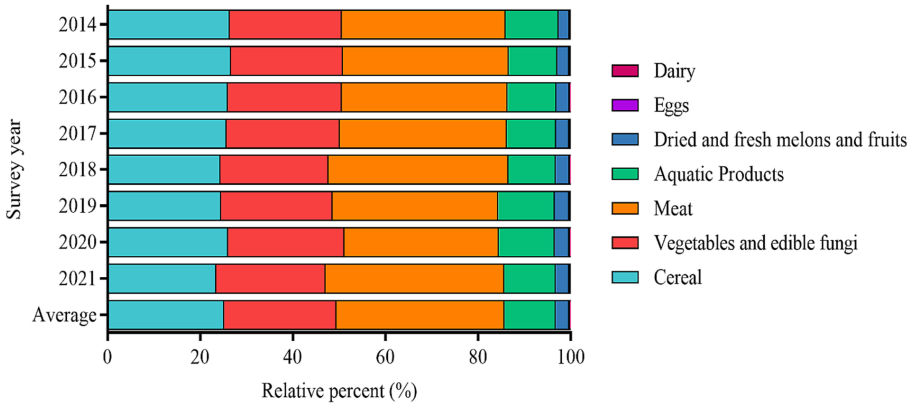

FIGURE 4
Contribution of different foods consumption to the daily purine intake.

contrast, after baking (especially at 220°C), the purine content increased in all insects (30).

Shiitake mushrooms are popular worldwide, and this food is also considered purine-rich. However, the types of purines and changes they undergo during food processing have received little attention. The effects of baking drying, freeze-drying, and sun drying on purine content in Lentinula edodes were compared using acid hydrolysis and HPLC (28). The results showed that adenine content decreased after drying at 120°C, possibly due to thermal damage to the DNA. The total purine content decreased significantly after lyophilisation but remained unchanged after drying and sun exposure. The effects of moisture and heat on the purines of Lentinula edodes were studied. An increase in xanthine content led to an increase in total purine content. The purine content of the cooking liquid was higher than that of the solid. Compared with drying methods, lyophilisation significantly affected purine release and reduced purine content in Lentinula edodes. Therefore, lyophilisation is more suitable for patients with hyperuricemia and gout (28). By understanding the purine content of various foods, consumers can choose foods with low-purine content and reduce their intake in their diets. Food's low, medium, and high-purine contents are generally less than 50 mg/100 g, 50–150 mg/100 g, and 150–1,000 mg/100 g, respectively.

In interpreting the results of this study, several limitations warrant consideration. Firstly, the dietary evaluation was derived from a predefined set of 10 items within the survey, potentially omitting other relevant dietary contributors to purine intake. Furthermore, this analysis did not account for variations in purine content resulting from different food preparation and cooking methods, both of which

can modulate the nutritional profile of food items. Given these constraints, the extrapolation of these findings to a broader dietary context should be approached with caution. Comprehensive assessments encompassing a wider array of food items and considering food preparation nuances are recommended for more detailed elucidation of dietary patterns and their consequent impact on serum uric acid concentrations.

In conclusion, Chinese residents' total daily purine intake gradually increased from 2014 to 2021. Meat products accounted for the largest proportion of the total dietary intake of purine. Our research underscores the nuanced relationship between China's socioeconomic progression and its shifting dietary patterns concerning purine intake. Recognizing the potential health implications of increased purine consumption, such as heightened gout risk, we recommend targeted public health campaigns to educate various population segments. This study also illuminates the need for deeper explorations into micro-level factors influencing these dietary shifts, including cultural and regional determinants. While our approach is primarily epidemiological, it's crucial to note its foundational role in paving the way for multidisciplinary inquiries. By highlighting these trends, we aim to catalyze a broader academic dialog on the implications of China's evolving dietary landscape.

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

SL: Formal analysis, Visualization, Writing - original draft. XL: Conceptualization, Methodology, Funding acquisition, Writing - review & editing. XJ: Methodology, Resources, Writing - review & editing. MF: Methodology, Writing - review & editing. QY: Methodology, Supervision, Writing - original draft. ZG: Supervision, Resources, Writing - review & editing.

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Conflict of interest

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

Publisher's note

Supplementary material

The Supplementary material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fnut.2023.1259053/full#supplementary-material

References

1. Pang B, McFaline JL, Burgis NE, Dong M, Taghizadeh K, Sullivan MR, et al. Defects in purine nucleotide metabolism lead to substantial incorporation of xanthine and hypoxanthine into DNA and RNA. *Proc Natl Acad Sci*. (2012) 109:2319–24. doi: 10.1073/pnas.1118455109

2. Wu B, Roseland JM, Haytowitz DB, Pehrsson PR, Ershow AG. Availability and quality of published data on the purine content of foods, alcoholic beverages, and dietary supplements. *J Food Compos Anal*. (2019) 84:103281. doi: 10.1016/j.jfca.2019.103281

3. Kaneko K, Aoyagi Y, Fukuuchi T, Inazawa K, Yamaoka N. Total purine and purine base content of common foodstuffs for facilitating nutritional therapy for gout and hyperuricemia. *Biol Pharm Bull*. (2014) 37:709–21. doi: 10.1248/bpb.b13-00967

4. Pedley AM, Benkovic SJ. A new view into the regulation of purine metabolism: the purinosome. *Trends Biochem Sci*. (2017) 42:141–54. doi: 10.1016/j.tibs.2016.09.009

5. Aihemaitijiang S, Zhang Y, Zhang L, Yang J, Ye C, Halimulati M, et al. The association between purine-rich food intake and hyperuricemia: a cross-sectional study in Chinese adult residents. *Nutrients*. (2020) 12:3835. doi: 10.3390/nu12123835

6. Huanhuan W, Lingzhu X, Xuhong S, Jing W, Xinyan L, Zhike L, et al. Purine-induced IFN-γ promotes uric acid production by upregulating xanthine oxidoreductase expression. *Front Immunol*. (2022) 13:13. doi: 10.3389/fimmu.2022.773001

7. Ponticelli C, Podestà MA, Moroni G. Hyperuricemia as a trigger of immune response in hypertension and chronic kidney disease. *Kidney Int*. (2020) 98:1149–59. doi: 10.1016/j.kint.2020.05.056

8. Zhang Y, Chen S, Yuan M, Xu Y, Xu H. Gout and diet: a comprehensive review of mechanisms and management. *Nutrients*. (2022) 14:3525. doi: 10.3390/nu14173525

9. Chen Y, Tang Z, Huang Z, Zhou W, Li Z, Li X, et al. The prevalence of gout in mainland China from 2000 to 2016: a systematic review and meta-analysis. *J Public Health*. (2017) 25:521–9. doi: 10.1007/s10389-017-0812-5

10. Zhu Q, Yu L, Li Y, Man Q, Jia S, Zhou Y, et al. Association between dietary Fiber intake and hyperuricemia among Chinese adults: analysis of the China adult chronic disease and nutrition surveillance (2015). *Nutrients*. (2022) 14:1433. doi: 10.3390/nu14071433

11. Goldberg EL, Asher JL, Molony RD, Shaw AC, Zeiss CJ, Wang C, et al. β-Hydroxybutyrate deactivates neutrophil NLRP3 Inflammasome to relieve gout flares. *Cell Rep*. (2017) 18:2077–87. doi: 10.1016/j.celrep.2017.02.004

12. Yokose C, McCormick N, Choi HK. The role of diet in hyperuricemia and gout. *Curr Opin Rheumatol*. (2021) 33:135–44. doi: 10.1097/BOR.0000000000000779

13. Chiu THT, Liu C, Chang C, Lin M, Lin C. Vegetarian diet and risk of gout in two separate prospective cohort studies. *Clin Nutr*. (2020) 39:837–44. doi: 10.1016/j.clnu.2019.03.016

14. Koguchi T. Modification of dietary habits for prevention of gout in Japanese people: gout and the Japanese diet. *Am J Health Res*. (2021) 9:117. doi: 10.11648/j.ajhr.20210905.12

15. Shuiqing H, Qianqian X, Chong T, Li L, Jing Z, Xuechun L, et al. Inulin-type prebiotics reduce serum uric acid levels via gut microbiota modulation: a randomized, controlled crossover trial in peritoneal dialysis patients. *Eur J Nutr*. (2021) 61:665–77. doi: 10.1007/s00394-021-02669-y

16. Stamp LK, O'Donnell JL, Chapman PT. Emerging therapies in the long-term management of hyperuricaemia and gout. *Intern Med J*. (2007) 37:258–66. doi: 10.1111/j.1445-5994.2007.01315.x

17. R LiYu K, Li C. Dietary factors and risk of gout and hyperuricemia: a meta-analysis and systematic review. *Asia Pac J Clin Nutr*. (2018) 27:1344–56. doi: 10.6133/apjcn.201811_27(6).0022

18. Xianchun X. Accurate understanding of china's income, consumption and investment. *Soc Sci China*. (2014) 35:21–43. doi: 10.1080/02529203.2013.875659

19. Yang Y, Wang G, Pan X. *China food composition tables standard edition. National Institute for nutrition and health, Chinese Center for Disease Control and Prevention*. Beijing: Peking University Press (2018).

20. Rong S, Zou L, Zhang Y, Zhang G, Li X, Li M, et al. Determination of purine contents in different parts of pork and beef by high performance liquid chromatography. *Food Chem*. (2015) 170:303–7. doi: 10.1016/j.foodchem.2014.08.059

21. Leblanc JC, Guerin T, Noel L, Calamassi-Tran G, Volatier JL, Verger P. Dietary exposure estimates of 18 elements from the 1st French Total diet study. *Food Addit Contam*. (2005) 22:624–41. doi: 10.1080/02652030500135367

22. Kim HJ, Fay MP, Feuer EJ, Midthune DN. Permutation tests for joinpoint regression with applications to cancer rates. *Stat Med*. (2000) 19:335–51. doi: 10.1002/(SICI)1097-0258(20000215)19:3<335::AID-SIM336>3.0.CO;2-Z

23. Heng Q. Navigating China's economic development in the new era: from high-speed to high-quality growth. *China Q Int Strateg*. (2018) 4:177–92. doi: 10.1142/S2377740018500161

24. Ma G. Food, eating behavior, and culture in Chinese society. *J Ethn Foods*. (2015) 2:195–9. doi: 10.1016/j.jef.2015.11.004

25. Jiang S, Wang J, Zhao Y, Shang Y, Gao X, Li H, et al. Sustainability of water resources for agriculture considering grain production, trade and consumption in China from 2004 to 2013. *J Clean Prod*. (2017) 149:1210–8. doi: 10.1016/j.jclepro.2017.02.103

26. Wang Z, Zeng Q, Wu S, Guo M, Li Z, Xue Y, et al. Effects of curcumin-based photodynamic treatment combined with low-temperature storage on shelf life and purine content of Litopenaeus vannamei. *J Food Process Preserv*. (2022) 46:e16863. doi: 10.1111/jfpp.16863

27. Hou C, Xiao G, Amakye WK, Sun J, Xu Z, Ren J. Guidelines for purine extraction and determination in foods. *Food Nutr*. (2021) 2:557–73. doi: 10.1002/fft2.100

28. Xiao L, Sha W, Tao C, Hou C, Xiao G, Ren J. Effect on purine releasement of Lentinus edodes by different food processing techniques. *Food Chem X*. (2022) 13:100260. doi: 10.1016/j.fochx.2022.100260

29. Kaneko K, Takayanagi F, Fukuuchi T, Yamaoka N, Yasuda M, Mawatari K, et al. Determination of total purine and purine base content of 80 food products to aid nutritional therapy for gout and hyperuricemia. *Nucleosides Nucleotides Nucleic Acids*. (2020) 39:1449–57. doi: 10.1080/15257770.2020.1748197

30. Monika S, Martin K, Dora P, Kateina K, Lenka K. Changes in purine and uric acid content in edible insects during culinary processing. *Food Chem*. (2023) 403:134349. doi: 10.1016/j.foodchem.2022.134349

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EDITED BY
Abdelrahim H. A. Hassan,
Nile University, Egypt

REVIEWED BY
Raul Dominguez-Perles,
Spanish National Research Council (CSIC), Spain
Seda Ciftci,
Izmir Democracy University, Türkiye
Diego A. Moreno,
Spanish National Research Council (CSIC), Spain

*CORRESPONDENCE
Hidde P. van Steenwijk
h.vansteenwijk@maastrichtuniversity.nl

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Sulforaphane as a potential modifier of calorie-induced inflammation: a double-blind, placebo-controlled, crossover trial

Hidde P. van Steenwijk[1]*, Anna Vinken[1], Frits H. M. van Osch[2,3], Herman Peppelenbos[4], Freddy J. Troost[5,6], Aalt Bast[7,8], Khrystyna O. Semen[7] and Alie de Boer[1]

[1]Food Claims Centre Venlo, Campus Venlo, Faculty of Science and Engineering, Maastricht University, Venlo, Netherlands, [2]Department of Clinical Epidemiology, VieCuri Medical Center Venlo, Venlo, Netherlands, [3]Department of Epidemiology, NUTRIM, Faculty of Health, Medicine and Life Sciences, Maastricht University, Maastricht, Netherlands, [4]Department of Food Innovation, HAS University of Applied Sciences, 's-Hertogenbosch, Netherlands, [5]Division of Gastroenterology-Hepatology, Department of Internal Medicine, School of Nutrition and Translational Research in Metabolism, Maastricht University Medical Center+, Maastricht, Netherlands, [6]Food Innovation and Health, Center for Healthy Eating and Food Innovation, Maastricht University, Maastricht, Netherlands, [7]Faculty of Science and Engineering, University College Venlo, Campus Venlo, Maastricht University, Venlo, Netherlands, [8]Department of Pharmacology and Toxicology, Faculty of Health, Medicine and Life Sciences, Maastricht University, Maastricht, Netherlands

Background and aims: Observational data indicate that diets rich in fruits and vegetables have a positive effect on inflammatory status, improve metabolic resilience and may protect against the development of non-communicable diseases. Nevertheless, experimental evidence demonstrating a causal relationship between nutrient intake (especially whole foods) and changes in metabolic health is scarce. This study investigated the pleiotropic effects of sulforaphane from broccoli sprouts, compared to pea sprouts, on biomarkers of endothelial function, inflammation and metabolic stress in healthy participants subjected to a standardized caloric challenge.

Methods: In this double-blind, crossover, randomized, placebo-controlled trial 12 healthy participants were administered 16 g broccoli sprouts, or pea sprouts (placebo) followed by the standardized high-caloric drink PhenFlex given to disturb healthy homeostasis. Levels of inflammatory biomarkers and metabolic parameters were measured in plasma before and 2 h after the caloric overload.

Results: Administration of broccoli sprouts promoted an increase in levels of CCL-2 induced by caloric load ($p = 0.017$). Other biomarkers (sICAM-1, sVCAM-1, hs-CRP, and IL-10) individually showed insignificant tendencies toward increase with administration of sulforaphane. Combining all studied biomarkers into the systemic low-grade inflammation score further confirmed upregulation of the inflammatory activity ($p = 0.087$) after sulforaphane. No significant effects on biomarkers of metabolic stress were detected.

Conclusion: This study has demonstrated that sulforaphane facilitated development of a mild pro-inflammatory state during the caloric challenge, which could be suggestive of the onset of the hormetic response induced by this phytonutrient. The use of integrative outcomes measures such as the systemic low-grade inflammation score can be viewed as a more robust approach to study the subtle and pleiotropic effects of phytonutrients.

Clinical trial registration: www.clinicaltrials.gov, identifier NCT05146804.

KEYWORDS

sulforaphane, glucoraphanin, phenotypic flexibility, hormesis, biomarkers, nutrients, diet, inflammation

1 Introduction

Non-communicable diseases (NCDs) are the leading cause of death worldwide, accounting for 71% of total deaths each year (1). Chronic low-grade inflammation (CLGI) plays a crucial role in the pathology of NCDs, but also appears to affect apparently healthy people as a consequence of poor lifestyle choices, e.g., overeating, smoking and excessive alcohol consumption (2–4). A wealth of observational data indicates that healthy lifestyle choices, such as moderate exercise and diets rich in fruits and vegetables, have a particularly positive effect on inflammatory status and the development of various NCDs (5–7). Nevertheless, randomized placebo-controlled trials frequently fail to demonstrate causal relationships between nutrient intake (especially whole foods) and changes in metabolic health (8–11).

With an increasing understanding of disease, health is no longer seen as simply a fixed entity of complete physical, mental, and social well-being, but redefined as our body's ability to cope with everyday challenges (11–15). The concept of this phenotypic flexibility implies that health can be measured by the ability to adapt to conditions of temporary stress. Challenge testing, which may involve exercise or caloric overload, is often used in practice to assess phenotypic flexibility. This may be a more sensitive way of assessing the effects of fruits and vegetables on the health status of the healthy low-risk population (14–21). Unlike drugs, food-derived compounds exert subtle effects in the general population rather than treating specific disease states in patients (12, 13). While pharmacology is still dominated by the "one disease—one target—one drug" paradigm, nutritional interventions frequently work on many pathways involved in the development of chronic diseases, with hormetic principles at its heart (11–14, 22, 23). Moreover, nutritional science (and within claims substantiation) often still focuses on this more pharmacological approach, so that it is only considered 'effective' if one nutrient affects one target (24). All things considered, it is challenging to measure beneficial effects in healthy people, and especially when you are trying to see the effect of one nutrient on one target. It is assumed that any intervention works via a hormetic mechanism if the final beneficial effect on phenotypic flexibility is in fact achieved through initial structural damage or functional overstrain, which is ultimately responsible for the activation of the protective mechanisms (25–37). For example, physical activity and mild stress-inducing phytonutrients called hormetins are known to increase levels of oxidative stress, but this appears to be beneficial for health (11, 22, 38–41). Most dietary hormetins are known to induce the expression of antioxidant enzymes by triggering a pro-oxidant response via activation of the nuclear factor E2-related factor 2 (Nrf2)-pathway (42–50). While the degree of immediate hormetic effects following exposure to a particular stress may be only moderate, the chain of events following the initial phase leads to biologically amplified effects that are much larger, synergistic, and pleiotropic and therefore require integrative approach to assessment of the outcomes (12, 13, 16, 34, 38, 40). Norde et al. (51) proposed a novel approach to measure CLGI by combining multiple biomarkers into a systemic low-grade inflammation score.

Glucoraphanin, the biogenic precursor of sulforaphane, is present in large amounts in broccoli sprouts (52, 53). After damage to plant tissue, e.g., through chewing, glucoraphanin comes into contact with the enzyme myrosinase, which is separated from its substrate in the intact vegetable, and subsequently is converted to sulforaphane (53–55). Sulforaphane is the most potent naturally occurring inducer of Nrf2 (42–48). Previous studies showed that long-term consumption of broccoli sprouts improved fasting blood glucose levels and stabilization of insulin response in type 2 diabetic patients, particularly obese patients (56, 57). To our knowledge, no experimental study has been conducted to investigate the effects of broccoli sprouts on integrative outcome measures. In the current study, we investigate the pleiotropic effects of broccoli sprouts, compared to pea sprouts, on biomarkers of endothelial function, inflammation and metabolic stress in healthy participants subjected to a standardized caloric challenge.

2 Methods

We have conducted a randomized, placebo-controlled, double-blind study with a cross-over design (58, 59). The study protocol (NL77272.068.21) was approved by the Medical Ethics Review Committee of Maastricht University Medical Centre+ (MUMC+) and Maastricht University, Maastricht, the Netherlands, and performed in full accordance with the declaration of Helsinki of 1975 as revised in 2013, Fortaleza, Brazil (60). The trial registration number within ClinicalTrials.gov is NCT05146804. All subjects provided written informed consent.

2.1 Subjects

Twelve healthy participants (11 males and one female) were recruited by local and social media advertisements. Inclusion criteria were that participants were between 18 and 50 years old, had a body mass index (BMI) between 18.5 and 30 kg/m^2, with a stable weight (<5% body weight change) and constant eating habits over the past 3 months. Exclusion criteria were the previous diagnosis of an inflammatory condition or disease or a history of hypothyroidism, chronic kidney or/and liver disorders, coronary artery disease, malignant hypertension, seizures, involved in intensive sports activities more than four times a week or at top sport level, regular intake of medication that may affect inflammatory response including NSAIDs, psychotic, addictive, or other mental disorders, aversion, intolerance or allergy to cruciferous vegetables and/or palm olein, dextrose, protein supplement, vanilla aroma, the use of dietary supplements with potential effects on antioxidant or inflammatory status and/or viral or bacterial infections requiring the use of

antibiotics, laxatives and anti-diarrheal drugs 4 weeks prior to inclusion, excessive alcohol consumption (≥28 consumptions approx. 250 g alcohol per week), pregnancy and/or breastfeeding, reported slimming or medically prescribed diet, as well as adhering to a vegetarian or vegan lifestyle. The sample size calculation is based on a crossover study by Meijer et al. (61) [Trial NL3290 (NTR3435)] in which they examined whether broccoli seedlings could reduce glucose-induced postprandial inflammation in healthy male participants. Meijer et al. measured plasma concentrations of sVCAM-1 and sICAM-1 (primary outcomes) at different timepoints in healthy men after consumption of broccoli seedlings or lettuce (placebo). The detectable difference used for the sample size calculation is calculated based on the mean concentrations of sVCAM-1 (ng/mL). Variance = Mean difference/SD Variance = (26.7–2.4)/14.20 = 1.711.

'Variance explained by special effect' was set to 0.5. We calculated an effect size of 0.54. A power of 80% was implemented, the chance of having a type I error was 5% and an effect size of 0.54 (medium effect size). In present study, we have two groups (two repeated measures, within-between interaction) yielding 10 participants, and considering a 20% dropout, which results in the final sample size of 12 participants.

2.2 Study design and procedures

Commercially available broccoli sprouts BroccoCress®, a rich source of glucoraphanin (the parental glucosinolate of sulforaphane), were used as the experimental product. In total, 16 g of sprouts were utilized, equivalent to one serving (portion) of microgreens. Sulforaphane (BroccoCress®) and the placebo (Affilla Cress®) were randomly administered to each participant on separate testing days (as detailed in section 2.3). The period between two visits was 7 ± 3 days. Information about demographics, alcohol consumption, and anthropometric data were assessed on the first visit. Body mass index (BMI), total body fat and visceral fat were measured using the Omron BF511R® monitor. The same testing scheme was applied during two visits (Figure 1), i.e., each participant received a single serving of intervention/placebo, which after 90 min was followed by oral administration of the PhenFlex challenge. Blood samples were collected twice, just before intake of PhenFlex and 120 min after. All participants were instructed to come fasted to each visit, to avoid consumption of broccoli or other cruciferous vegetables 2 days before the visit and to restrain from intense physical activity on the day of the visit. During the visit, participants remained in the testing location and were allowed to drink water *ad libitum*. No food intake was permitted during the visit.

2.3 Intervention and caloric challenge (PhenFlex)

The broccoli sprouts were cut approximately 1 cm below the leaves (with the hypocotyl being cut below the cotyledons), weighed, and mashed with a small amount of tap water (approximately 13°C) in a kitchen blender for 30s at room temperature immediately before administration (Premium Impuls Blender Smoothiemaker; Impuls; 180 W). Subsequently, tap water (approximately 13°C) was added to the mixture to bring the total amount to 250 mL and participants were instructed to drink the entire mixture immediately. Commercially available pea sprouts (Affilla Cress®) were used as a placebo in this study since pea sprouts do not contain glucoraphanin/sulforaphane. Affilla Cress (16 g) was prepared and administered in a similar fashion. Blinding of participants was ensured by the even appearance of both drinks and the use of nasal plugs during the consumption of the investigational products. The placebo or intervention product was prepared by a researcher who was not involved in any other study procedures and data analysis. Ninety minutes after administration of the investigational products, participants were asked to drink the high-fat, high-glucose, high-caloric product (PhenFlex) (62). For the preparation of the PhenFlex (400 mL, 950 kcal) 60 g palm olein, 75 g dextrose, 20 g protein, 0.5 g artificial vanilla aroma and 320 mL tap water were used (62). In all cases, PhenFlex mixtures were freshly prepared, and the participants were instructed to consume the drink within 5 min.

2.4 Blood sampling and assessment of biomarkers

Samples of venous blood were taken twice per visit from the antecubital vein for measurement of inflammatory and metabolic biomarkers. Samples were collected in 4 mL BD tubes containing K2EDTA as anticoagulant, and centrifuged for 5 min (at 3,000 g, 4°C) within 30 min after collection. Plasma was stored at ≤−80°C until the day of analysis.

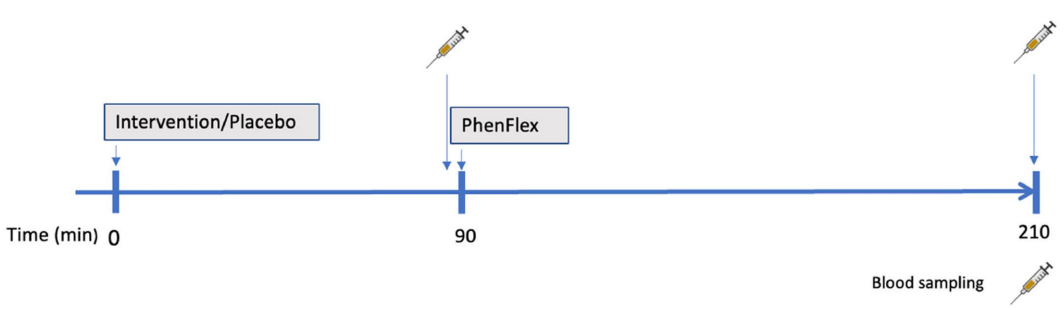

FIGURE 1
Schematic presentation of a study visit. Administration of intervention (sulforaphane/placebo) was followed in 90 min by administration of standardized caloric challenge PhenFlex. Blood samples were obtained before (90 min) and 2 h after (210 min) PhenFlex challenge.

Plasma samples were analyzed for inflammatory biomarkers, sVCAM-1, sICAM-1, IL-1β, IL-6, TNF-α, CCL-2, IL-8, IL-10, adiponectin, hs-CRP, and IL-12 p70 using Enzyme-linked immunosorbent assays (R&D Systems Netherlands; Supplementary Table 1). A systemic low-grade inflammation score was generated summing the z-score log-transformed inflammatory biomarkers plasma concentration (sICAM-1, sVCAM-1, IL-6, TNF-α, CRP, IL-12, CCL-2, IL-1β, and IL-8). The z-score log-transformed plasma adiponectin and IL-10 levels were subtracted from the systemic low-grade inflammation score due to their anti-inflammatory properties (51). Plasma samples were analyzed for glucose via colorimetric assay (Cayman Chemical; Glucose Colorimetric Assay Kit, Item No. 10009582) and lipoprotein A using Enzyme-linked immunosorbent assay (Abcam®; Human Lipoprotein A SimpleStep ELISA® Kit, ab212165).

2.5 Statistical analysis

All normally distributed data are presented as mean ± standard deviation (SD). The non-normally distributed data are shown as median (interquartile range). For categorical variables, frequency and/or percentages are presented. Differences between the groups were assessed by paired sample T-tests for normally distributed parameters, or Wilcoxon signed-rank tests for the data that was not normally distributed. Wilcoxon signed-rank tests were performed to check for potential carryover effects. Associations between clinical features and biomarkers were assessed using Pearson (normally distributed parameters) or Spearman's rank (non-normally distributed) correlation coefficient. All analyses were performed, two-tailed with a $p \leq 0.05$ considered statistically significant.

3 Results

3.1 Subject characteristics

Between November 2021 and January 2022, a total of 12 subjects were enrolled into the study and randomly allocated to either initial administration of sulforaphane or placebo. Baseline characteristics of the study population ($n = 12$) are summarized in Table 1. All participants completed the study and were included in the data analysis for biochemical testing (Figure 2). The pre-test (Wilcoxon rank sum test) revealed no differences between treatment allocations for all parameters (all $z < 0.00$, $p > 0.14$). Absence of sulforaphane in placebo was verified by testing urine samples which showed significantly higher concentration of the total metabolites of sulforaphane after intake of broccoli sprouts (8.2 vs. 0.4 μmol, $p < 0.001$).

3.2 The effect of sulforaphane on endothelial activation

The single serving of sulforaphane or placebo induced no significant changes in concentrations of sICAM-1 and sVCAM-1 in healthy participants before and 2 h after the PhenFlex challenge. A general trend was seen in the enhancement of endothelial activation in the sulforaphane group, however not significant; sICAM-1 (sulforaphane 1.5 ± 10.1 vs. placebo 3.1 ± 7.8 ng/mL, $p = 0.696$) and sVCAM-1 (sulforaphane 3.1 ± 5.2 vs. placebo 0.9 ± 4.5 ng/mL, $p = 0.431$; Table 2; Figure 3).

3.3 The effect of sulforaphane on inflammatory biomarkers

Eleven inflammatory biomarkers, sICAM-1, sVCAM-1, IL-6, TNF-α, hs-CRP, adiponectin, IL-12 p70, CCL-2, IL-10, IL-1β, and IL-8, were measured in plasma before and 2 h after the PhenFlex challenge. Levels of sICAM-1, sVCAM-1, hs-CRP, adiponectin, CCL-2, and IL-10 and changes are listed in Table 2. Changes in CCL-2, measured as differential concentrations before and 2 h after caloric load, showed a significant change between groups, with sulforaphane causing a significant increase in this biomarker compared to placebo [1.9 (3.3) vs. 0.0 (4.8) pg./mL, $p = 0.017$; Figure 4]. Changes in sICAM-1, sVCAM-1, hs-CRP (sulforaphane 2.2 ± 4.3 vs. placebo −0.5 ± 2.9 pg./mL, $p = 0.275$), and IL-10 [sulforaphane −0.6 (26.3) vs. placebo 4.4 (5.1) pg./mL, $p = 0.715$] revealed an overall slight and statistically non-significant pro-inflammatory effect of sulforaphane (Table 2). No detectable levels of IL-6, TNF-α, IL-12, IL-1β, and IL-8 were quantified.

3.4 The effect of sulforaphane on the systemic low-grade inflammation score

Aside from CCL-2, a more robust change was observed with the integration of the individual biomarkers in the composite systemic low-grade inflammation score (Table 3; Figure 4). In the sulforaphane group the composite score revealed a pro-inflammatory trend after caloric challenge [−0.092 (1.06) before vs. after 0.018 (1.06), $p = 0.087$] which was less prominent in the placebo group [−0.001 (0.81) before vs. after 0.014 (0.81), $p = 0.251$].

TABLE 1 Characteristics of the study participants.

Characteristics	Population ($n = 12$)
Sex (*n*, %)	
Female	1 (8.3)
Male	11 (91.7)
Age [years, mean (SD)]	26.9 (3.6)
BMI (kg/m^2)	23.1 (1.6)
Body fat (%)	
Female	28.9 (n/a)
Male	21.4 (3.1)
All	22.0 (3.6)
Visceral fat level [mean (SD)]	5.17 (1.57)
Alcohol consumption, *n* (%)	
Moderate	0 (0)
Heavy	9 (75)
Very heavy	3 (25)
Smoking status, *n* (%)	
Smoker	5 (42)
Non-smoker	7 (58)

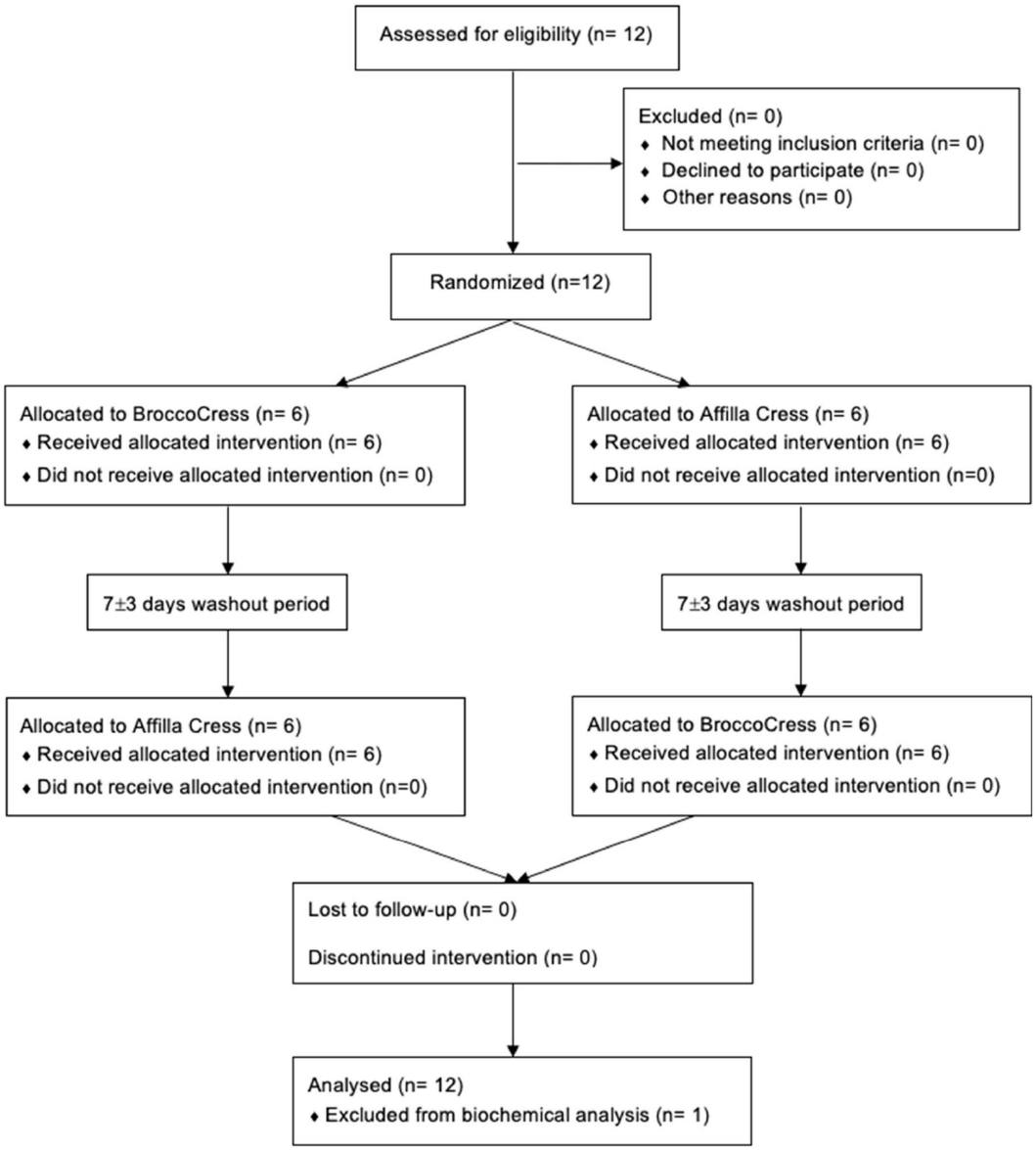

FIGURE 2
CONSORT flow diagram.

Before the PhenFlex challenge, no significant correlations between inflammation biomarkers and demographic, anthropometric, and lifestyle data were observed, with the exemption of IL-10 and smoking ($p = 0.042$), and the number of cigarettes per day ($p = 0.042$). Moreover, moderate strength correlations between the systemic low-grade inflammation score and fat percentage ($r_s = -0.551$, $p = 0.079$) and the number of cigarettes per day ($r_s = 0.557$, $p = 0.075$) were observed.

3.5 The effect of sulforaphane on glucose and lipid metabolism during caloric overload

The single serving of sulforaphane or placebo induced no significant changes in concentrations of glucose and lipoprotein A in healthy participants before and 2 h after the PhenFlex challenge (Table 4). Before the PhenFlex challenge, fasting glucose levels correlated negatively with age ($p = 0.003$), BMI ($p = 0.046$) and fat percentage ($p = 0.034$), and positively with adiponectin concentrations ($p = 0.015$). Fasting lipoprotein A concentrations correlated positively with CCL-2 ($p = 0.049$) and IL-10 levels ($p = 0.005$; Table 5). Moreover, administration of sulforaphane caused changes in glucose levels in response to the caloric load which correlated positively with changes in sICAM-1 ($p = 0.006$), adiponectin ($p = 0.048$) and lipoprotein A ($p = 0.005$). Changes in lipoprotein A concentrations in response to the challenge after sulforaphane administration correlated positively with changes in sICAM-1 levels ($p = 0.004$). In the placebo group, changes in glucose levels in response to caloric loading only negatively correlated with changes in IL-10 ($p < 0.001$; Table 6).

TABLE 2 The plasma concentrations of sICAM-1, sVCAM-1, hs-CRP, Adiponectin, CCL-2, and IL-10 before (min 90) and after (min 210) the PhenFlex challenge, Median (IQR).

Inflammatory biomarker		Sulforaphane		Placebo	
sICAM-1* (ng/mL)	Before	56.6 ± 25.6	$^{\#}p = 0.428$	63.9 ± 23.3	$^{\#}p = 0.513$
	After	59.0 ± 22.3		65.8 ± 20.0	
Δ sICAM-1* (ng/mL)		1.5 ± 10.1		3.1 ± 7.8	$^{\#\#}p = 0.696$
sVCAM-1* (ng/mL)	Before	50.5 ± 5.6	$^{\#}p = 0.128$	51.5 ± 7.9	$^{\#}p = 0.659$
	After	54.5 ± 10.2		52.1 ± 7.9	
Δ sVCAM-1* (ng/mL)		3.1 ± 5.2		0.9 ± 4.5	$^{\#\#}p = 0.431$
hs-CRP* (ng/mL)	Before	50.9 ± 9.0	$^{\#}p = 0.277$	53.4 ± 10.8	$^{\#}p = 0.466$
	After	52.4 ± 8.7		52.7 ± 10.7	
Δ hs-CRP* (ng/mL)		2.2 ± 4.3		−0.5 ± 2.9	$^{\#\#}p = 0.275$
Adiponectin^ (ng/mL)	Before	50.4 (6.9)	$^{\#}p = 0.799$	51.1 (0.8)	$^{\#}p = 0.767$
	After	50.9 (3.7)		52.5 (3.8)	
Δ Adiponectin^ (ng/mL)		0.5 (4.1)		0.5 (3.7)	$^{\#\#}p = 0.779$
CCL-2 (pg/mL)	Before	16.9 (25.6)	$^{\#}p = 0.314$	18.2 (24.2)	$^{\#}p = 0.374$
	After	19.1 (24.1)		18.3 (23.5)	
Δ CCL-2 (pg/mL)		1.9 (3.3)		0.0 (4.8)	$^{\#\#}p = 0.017$
IL-10^ (pg/mL)	Before	55.7 (213.4)	$^{\#}p = 0.893$	51.4 (178.8)	$^{\#}p = 0.225$
	After	51.2 (231.9)		56.1 (184.1)	
Δ IL-10^ (pg/mL)		−0.6 (26.3)		4.4 (5.1)	$^{\#\#}p = 0.715$

Significance for $^{\#}$within and $^{\#\#}$between group comparison; ^Anti-inflammatory biomarker; *Normally distributed (mean ± SD).

4 Discussion

4.1 The PhenFlex challenge did not unbalance endothelial homeostasis in young healthy participants

In the present study, metabolic overload did not significantly affect plasma adhesion marker levels in healthy participants, as measured 2 h after caloric overload. In contrast, previous research has shown that a single administration of the PhenFlex challenge increased the levels of sVCAM-1 and sICAM-1 after 2 h in healthy volunteers (18). Additionally, Derosa et al. demonstrated significant increases in these plasma adhesion markers within 2 h after an oral glucose tolerance test (OGTT) (63). A possible explanation for the lack of effect found on these markers in our study is the fact that the subjects in the current study were given whole food products (sprouts of broccoli or pea) before undergoing the challenge. Both products contain retinol, vitamin E and ascorbic acid, which could have counteracted the expected transient disruption of post-exposure endothelial homeostasis observed in other studies. This hypothesis is supported by findings of Nappo et al., who showed that supplementation with vitamin C and E prevented an increase in sICAM-1 and sVCAM-1 in healthy middle-aged subjects after a high-fat meal (64). Furthermore, Rubin et al. found no changes in plasma adhesion markers in young participants (25 years on average), after a standardized lipid-rich meal which contained retinol (65). Thus, in this study, the effect of sulforaphane on endothelial homeostasis may have been influenced by the other nutrients present in the whole food product. Nonetheless, the associations between metabolic parameters and inflammatory biomarkers during the PhenFlex challenge, particularly between plasma sICAM-1, and glucose and lipoprotein A concentrations in the sulforaphane group, provided relevant information on the modulation of endothelial function and metabolic homeostasis by sulforaphane in response to a high-glucose, high-fat product. Consistent with our findings, Chen et al. demonstrated significant relationships between the plasma glucose and insulin responses to an OGTT and plasma sICAM-1 concentrations in healthy participants (66). The fact that these correlations were not observed in the pea sprouts group (placebo) supports the hypothesis that the other bioactive compounds in the whole food products may have blunted the transient disruption of post-exposure homeostasis expected after caloric load. This is also revealed in part by the only correlation between circulating IL-10 and plasma glucose. The presence of other nutrients in broccoli sprouts may have interfered with the strong effects of sulforaphane, which, however, were still evident as more correlations were shown in this group.

4.2 An integrative measure to investigate the pleiotropic effects of phytonutrients is superior to single biomarkers

In this study, sulforaphane facilitated the development of a mild pro-inflammatory state during caloric challenge, as evidenced by a moderate increase in sICAM-1, sVCAM-1, hs-CRP, CCL-2 and decrease in IL-10. The effects of dietary intervention on chronic inflammation in other studies that used the PhenFlex challenge are inconsistent (62, 67). Kim et al. examined the effect of a single-intake microencapsulated garlic powder and/or tomato extract in healthy

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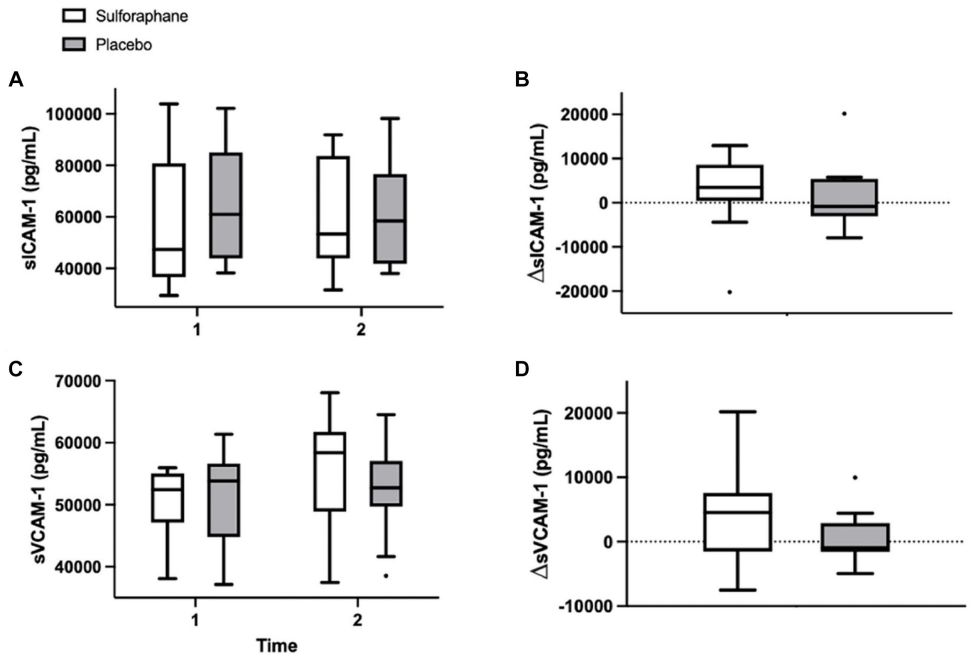

FIGURE 3
Plasma concentrations of sICAM-1 (pg/mL) and sVCAM-1 (pg/mL) in the sulforaphane and placebo groups before, after, and the changes during the PhenFlex challenge **(A-D)**. Data are presented as boxplots [median, interquartile range, outliers (circles)]. Timepoints: 1—before administration of PhenFlex (90 min); 2—2 h after PhenFlex (210 min). Comparison between timepoints in sulforaphane/placebo.

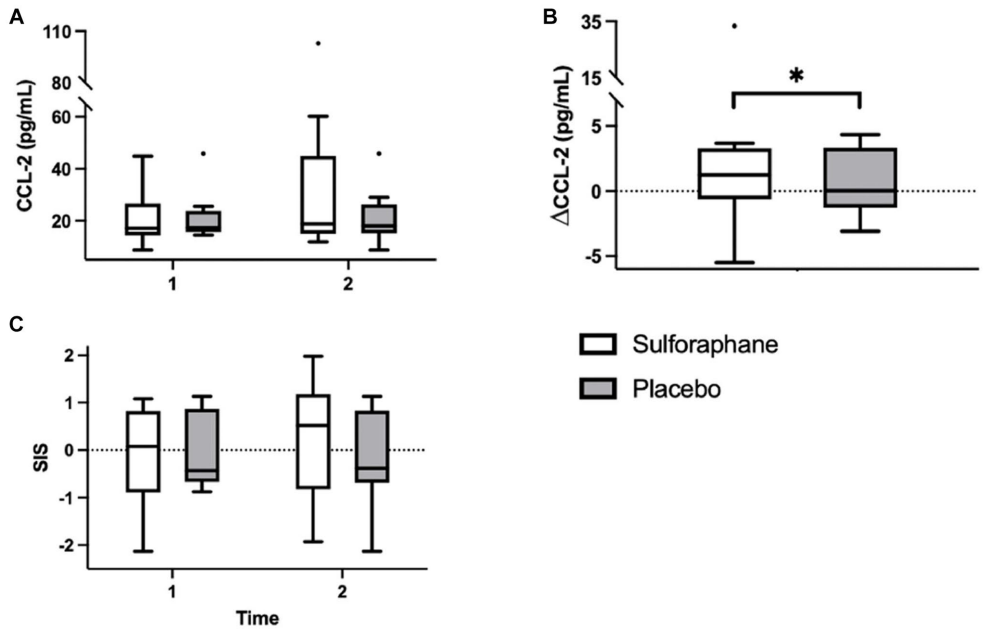

FIGURE 4
Plasma concentrations of CCL-2 (pg/mL) in the sulforaphane and placebo groups before, after, and the changes during the PhenFlex challenge **(A,B)**. The systemic low-grade inflammation score (SIS) in the sulforaphane and placebo before and after the PhenFlex challenge **(C)**. Data are presented as boxplots [median, interquartile range, outliers (circles)]. Timepoints: 1—before administration of PhenFlex (90 min); 2—2 h after PhenFlex (210 min). Comparison between timepoints in sulforaphane/placebo, $*p < 0.05$.

male smokers during the metabolic challenge. Consumption of tomato extract elicited a differential response, increasing CCL-2 and decreasing sVCAM-1 6 h after PhenFlex compared to placebo. When garlic powder was consumed, IL-13 levels decreased after 2 h and IL-1α increased 6 h after the challenge, indicating a pro-inflammatory effect of the food product. The combination of interventions elicited

TABLE 3 The systemic low-grade inflammation score (SIS) before (min 90) and after (min 210) the PhenFlex challenge, Mean ± SD.

Systemic low-grade inflammation Score (SIS)		Sulforaphane		Placebo	
SIS	Before	−0.092 ± 1.06	$^{\#}p = 0.087$	−0.001 ± 0.81	$^{\#}p = 0.251$
	After	0.018 ± 1.06		0.014 ± 0.81	

[#]Significance for within group comparison.

TABLE 4 The plasma concentrations of glucose and lipoprotein A before (min 90) and after (min 210) the PhenFlex challenge, Mean ± SD.

Parameter		Sulforaphane		Placebo	
Glucose* (mg/dL)	Before	73.8 ± 7.8	$^{\#}p = 0.363$	76.8 ± 5.2	$^{\#}p = 0.133$
	After	66.8 ± 11.5		67.0 ± 16.7	
Δ Glucose* (mg/dL)		−6.9 ± 17.8		−9.8 ± 18.5	$^{\#\#}p = 0.589$
Lipoprotein A* (μg/mL)	Before	166 ± 128	$^{\#}p = 0.214$	189 ± 144	$^{\#}p = 0.269$
	After	172 ± 132		199 ± 130	
Δ Lipoprotein A* (μg/mL)		3.8 ± 14.6		10.7 ± 26.3	$^{\#\#}p = 0.580$

Significance for [#]within and [##]between group comparison; *Normally distributed.

a mixed response, with IL-10 and CCL-7 being reduced 6 h after the metabolic challenge (67). Hoevenaars et al. investigated the effects of a 12-week whole grain wheat (WGW) intervention compared to refined wheat (RW) and observed increased CRP, IL-6, IL-8, and decreased IL-1β in RW and decreased CRP, serum amyloid A, IL-8, and IL-10 in WGW, indicating pro- and anti-inflammatory effects in respective groups (62). These inconclusive results highlight the importance of implementing integrative outcome measures to unravel the subtle, pleiotropic effects of phytonutrients. As an illustration, the study of Weseler et al. testing the effects of grape seed extract on multiple biomarkers reflecting vascular health integrated them into a vascular health index, which unveiled an improvement in overall vascular health from flavanols, which was less clear from the analysis of individual outcomes (13, 16). In addition, previous cross-sectional studies evaluated CLGI through an index that pools multiple indicators to provide a better overall picture of the synergistic changes of inflammatory biomarkers (51, 68–72).

To the best of our knowledge, this experimental study is the first to examine the effects of phytonutrients on calorie-induced inflammation as measured by a composite scoring system. Intriguingly, the score more accurately reflected the pro-inflammatory effect of broccoli sprouts than single biomarkers during phasic response. In addition, the relationships between risk factors for the development of NCDs such as high visceral fat and smoking, and inflammation became more apparent through the use of the score. Specifically, fat percentage and smoking showed a moderate inverse relationship with the score. Using the systemic low-grade inflammation score also led to another finding; five of the 11 inflammatory biomarkers were undetectable in the blood of our young and healthy population. These findings suggest that even a well-characterized scoring system may show limitations. For future research, assessing the health status or risk profile of the test population and adjusting the scoring system could be beneficial. The six biomarkers detectable under basal conditions in our study may be more suitable for challenge testing in young healthy subjects (19, 36, 73–76).

4.3 Metabolic challenge studies may reveal the beneficial effects of phytonutrients in multiple ways depending on mechanism of action in the body

Over the past few decades, research has increasingly focused on antioxidants as the main health-promoting compounds in fruits and vegetables, leading to a gigantic array of antioxidant supplements on the market today (5–7, 12, 77–79). However, initial excitement regarding the potential health benefits of antioxidants failed to be confirmed by clinical evidence (12). There is quite a bit of debate about whether supplementing with antioxidants is healthy, ineffective, or even harmful (5, 11, 12, 22, 28, 36, 77, 78, 80–86). So far, whole foods and fresh produce have not shown a clear protective effect against the PhenFlex challenge (62, 67). In fact, dietary interventions high in hormetins facilitated the development of a mild pro-inflammatory state during caloric overload, e.g., broccoli sprouts and garlic extract (67). We hypothesize that this moderate pro-inflammatory state in the sprouts containing sulforaphane may be due to the initial pro-oxidative action (to activate Nrf2) of hormetins present in fresh produce. As a result, the exogenous antioxidant capacity of the direct antioxidants present in both sprouts is blunted in the broccoli sprouts compared to the pea sprouts. This, in turn, led to a reduced initial integrative anti-inflammatory capacity against the caloric overload demonstrated by the broccoli sprouts compared to the placebo. However, we speculate that because sulforaphane also enhances endogenous antioxidant defenses via Nrf2 activation at a later stage, whole foods that increase both exogenous and endogenous antioxidants may have more significant effects on phenotypic flexibility (Figure 5). This may explain why supplementation of direct antioxidants such as ascorbic acid and vitamin E attenuates the metabolic stress of caloric loads (64, 65), while hormetins in fresh produce, e.g., sulforaphane, diallyl sulfide, withaferin A and rutin, induce a mild pro-inflammatory effect via activation of the Nrf2-pathway (38, 49, 50, 67, 87). We hypothesize that the health effects of fruit and vegetable consumption are due to the wide variety of bioactive compounds in the food matrices and the synergy between the different mechanisms of action of these phytonutrients in the body, rather than just antioxidants (36). One aspect of synergy may be a buffering effect (88, 89). The effect of a large intake of a given nutrient may vary depending on whether it is taken in concentrated form or as part of a food matrix, e.g., the matrix may slow down the absorption of the nutrient, which lowers the likelihood of a bolus effect (89).

The limitations of our study include a small sample size and a short observation period. A longer time of observation (6, 8, 12, or even 24 h) could have helped to demonstrate that the increase in inflammatory activity caused by sulforaphane represents the initial part of the hormetic response. In fact, previous research, conducted with larger sample sizes, has demonstrated the sustained anti-inflammatory effects of sulforaphane (56, 90). At the same time, biotechnological advancements that allow continuous monitoring of certain functions (e.g., glucose) and innovative designs (e.g., n-of-1 trials) may enable more accurate research into personalized nutrition

TABLE 5 Univariate correlates of demographic parameters with fasting metabolic and inflammatory parameters.

Parameter		Demographic					Inflammatory		
		Age	BMI	FP	Smo	Cig	Adi	CCL-2	IL-10
Metabolic	Glucose	−0.81	−0.61	−0.64	-	-	0.71	-	-
	Lp(a)	-	-	-	-	-	-	0.61	0.94
Inflammatory	IL-10	-	-	-	−0.83	−0.83			

Variables were correlated using Pearson (normally distributed parameters) or Spearman's rank (non-normally distributed) correlation. Only significant correlations are shown. FP, Fat percentage; Smo, Smoking; Cig, Cigarettes per day; Lp(a), Lipoprotein A; Adi, Adiponectin; IL-10, Interleukin-10; CCL-2, Chemokine ligand 2; SIS, Systemic low-grade inflammation score.

TABLE 6 Univariate correlates of changes in metabolic parameters (glucose and lipoprotein A) with inflammatory parameters during the PhenFlex challenge.

	Sulforaphane			Placebo
Parameter	sICAM-1	Adi	Lp(a)	IL-10
Glucose	0.79	0.64	0.81	−1.00
Lipoprotein A	0.82	-	n/a	-

Variables were correlated using Pearson (normally distributed parameters) or Spearman's rank (non-normally distributed) correlation. Only significant correlations are shown. Lp(a), Lipoprotein A; Adi, Adiponectin.

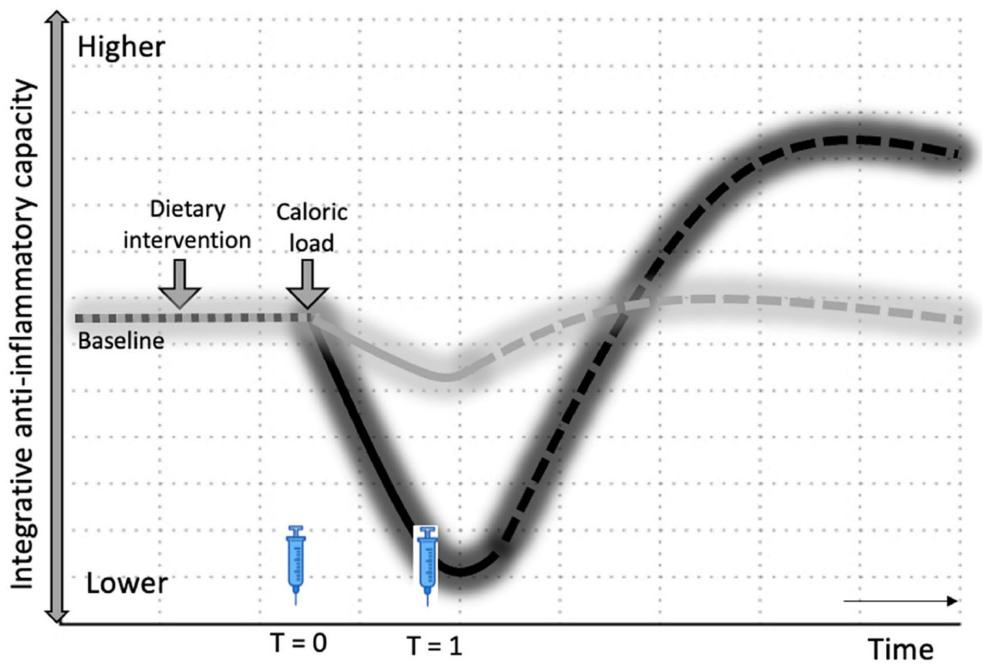

FIGURE 5
Hormesis hypothesis on health effects of fruits and vegetables. Changes in integrative anti-inflammatory capacity in response to intervention, followed by caloric load. The dotted lines represent the expected sustained effects on integrative anti-inflammatory potential through an increase in endogenous antioxidants via Nrf2 activation by hormetins. Black: Broccoli sprouts (with sulforaphane); Gray: Pea sprouts (without sulforaphane); T0 and T1—time points of blood sampling.

strategies in the future (91–94). Learning more about the interplay between phytonutrients may eventually reveal whether "an apple a day can keep the doctor away"—at least for a while.

5 Conclusion

This study has shown that the subtle and pleiotropic effects of phytonutrients can be studied in a short time by challenging the resilience and efficacy of adaptive mechanisms of healthy participants. Broccoli sprouts containing sulforaphane facilitated the development of a mild pro-inflammatory state during the caloric challenge, which suggests the onset of a hormetic response and became more evident when applying integrative outcome measures. The multifaceted approach allowed for more accurate quantification of the effects of phytonutrients in relation to inflammation and metabolic processes. Considering innovative integrative research approaches (e.g., composite scores, wearables, n-of-1 designs) would enhance our

understanding of the hormetic principles of phytonutrients and stimulate research into the health effects of food.

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 humans were approved by Medical Ethics Review Committee of Maastricht University Medical Centre+ (MUMC+) and Maastricht University, Maastricht, the Netherlands. The studies were conducted in accordance with the local legislation and institutional requirements. The participants provided their written informed consent to participate in this study.

Author contributions

HS: conceptualization, investigation, formal analysis, visualization, and writing—original draft. AV: investigation and formal analysis. FO: methodology and writing—review and editing. HP and FT: writing—review and editing. ABa: conceptualization, writing—review and editing, and supervision. KS: conceptualization, data curation, methodology, visualization, writing—review and editing, and supervision. ABo: conceptualization, writing—review and editing, funding acquisition, and supervision. All authors contributed to the article and approved the submitted version.

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Conflict of interest

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

Publisher's note

Supplementary material

The Supplementary material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fnut.2023.1245355/full#supplementary-material

References

1. Phillips CM, Chen L-W, Heude B, Bernard JY, Harvey NC, Duijts L, et al. Dietary inflammatory index and non-communicable disease risk: a narrative review. *Nutrients*. (2019) 11:1873. doi: 10.3390/nu11081873

2. Elisia I, Lam V, Cho B, Hay M, Li MY, Yeung M, et al. The effect of smoking on chronic inflammation, immune function and blood cell composition. *Sci Rep*. (2020) 10:19480. doi: 10.1038/s41598-020-76556-7

3. Wang HJ, Zakhari S, Jung MK. Alcohol, inflammation, and gut-liver-brain interactions in tissue damage and disease development. *World J Gastroenterol*. (2010) 16:1304–13. doi: 10.3748/wjg.v16.i11.1304

4. Ellulu MS, Patimah I, Khaza'ai H, Rahmat A, Abed Y. Obesity and inflammation: the linking mechanism and the complications. *Arch Med Sci*. (2017) 4:851–63. doi: 10.5114/aoms.2016.58928

5. Lapuente M, Estruch R, Shahbaz M, Casas R. Relation of fruits and vegetables with major cardiometabolic risk factors, markers of oxidation, and inflammation. *Nutrients*. (2019) 11:2381. doi: 10.3390/nu11102381

6. Bosma-Den Boer MM, Van Wetten ML, Pruimboom L. Chronic inflammatory diseases are stimulated by current lifestyle: how diet, stress levels and medication prevent our body from recovering. *Nutr Metab*. (2012) 9:32. doi: 10.1186/1743-7075-9-32

7. de Boer A, van de Worp WRPH, Hageman GJ, Bast A. The effect of dietary components on inflammatory lung diseases–a literature review. *Int J Food Sci Nutr*. (2017) 68:771–87. doi: 10.1080/09637486.2017.1288199

8. Vitolins MZ, Case TL. What makes nutrition research so difficult to conduct and interpret? *Diabetes Spectr*. (2020) 33:113–7. doi: 10.2337/ds19-0077

9. Heaney RP, Weaver CM, Blumberg J. EBN (evidence-based nutrition) Ver. 2.0. *Nutr Today*. (2011) 46:22–6. doi: 10.1097/NT.0b013e3182076fdf

10. Heaney RP. Nutrients, endpoints, and the problem of proof. *J Nutr*. (2008) 138:1591–5. doi: 10.1093/jn/138.9.1591

11. Hanekamp JC, Bast A, Calabrese EJ. Nutrition and health – transforming research traditions. *Crit Rev Food Sci Nutr*. (2015) 55:1074–80. doi: 10.1080/10408398.2012.680525

12. Bast A, Haenen GRMM. misconceptions about antioxidants. *Trends Pharmacol Sci*. (2013) 34:430–6. doi: 10.1016/j.tips.2013.05.010

13. Weseler AR, Bast A. Pleiotropic-acting nutrients require integrative investigational approaches: the example of flavonoids. *J Agric Food Chem*. (2012) 60:8941–6. doi: 10.1021/jf3000373

14. Witkamp RF, van Norren K. Let thy food be thy medicine….When possible. *Eur J Pharmacol*. (2018) 836:102–14. doi: 10.1016/j.ejphar.2018.06.026

15. Stroeve JHM, van Wietmarschen H, Kremer BHA, van Ommen B, Wopereis S. Phenotypic flexibility as a measure of health: the optimal nutritional stress response test. *Genes Nutr*. (2015) 10:13. doi: 10.1007/s12263-015-0459-1

16. Weseler AR, Ruijters EJB, Drittij-Reijnders M-J, Reesink KD, Haenen GRMM, Bast A. Pleiotropic benefit of monomeric and oligomeric Flavanols on vascular health—a randomized controlled clinical pilot study. *PLoS One*. (2011) 6:e28460. doi: 10.1371/journal.pone.0028460

17. van Ommen B, van der Greef J, Ordovas JM, Daniel H. Phenotypic flexibility as key factor in the human nutrition and health relationship. *Genes Nutr*. (2014) 9:423. doi: 10.1007/s12263-014-0423-5

18. Wopereis S, Stroeve JHM, Stafleu A, Bakker GCM, Burggraaf J, van Erk MJ, et al. Multi-parameter comparison of a standardized mixed meal tolerance test in healthy and type 2 diabetic subjects: the PhenFlex challenge. *Genes Nutr*. (2017) 12:21. doi: 10.1186/s12263-017-0570-6

19. Wopereis S, Wolvers D, van Erk M, Gribnau M, Kremer B, van Dorsten FA, et al. Assessment of inflammatory resilience in healthy subjects using dietary lipid and glucose challenges. *BMC Med Genet*. (2013) 6:44. doi: 10.1186/1755-8794-6-44

20. van den Broek TJ, Bakker GCM, Rubingh CM, Bijlsma S, Stroeve JHM, van Ommen B, et al. Ranges of phenotypic flexibility in healthy subjects. *Genes Nutr*. (2017) 12:32. doi: 10.1186/s12263-017-0589-8

21. van den Brink W, van Bilsen J, Salic K, Hoevenaars FPM, Verschuren L, Kleemann R, et al. Current and future nutritional strategies to modulate inflammatory dynamics in metabolic disorders. *Front Nutr*. (2019) 6:129. doi: 10.3389/fnut.2019.00129

22. Sthijns MMJPE, Weseler AR, Bast A, Haenen GRMM. Time in redox adaptation processes: from evolution to Hormesis. *Int J Mol Sci*. (2016) 17:649. doi: 10.3390/ijms17101649

23. Peluso I. Diet and exercise in lifestyle medicine: the hormetic effects of bioactive compounds on human health. *Curr Opin Toxicol*. (2022) 30:100342. doi: 10.1016/j.cotox.2022.03.003

24. Lenssen KGM, Bast A, de Boer A. Clarifying the health claim assessment procedure of EFSA will benefit functional food innovation. *J Funct Foods*. (2018) 47:386–96. doi: 10.1016/j.jff.2018.05.047

25. Di Pierro F. Antioxidants and cancer: a debate on prevention, progression, hormesis, and cruciferous vegetables. *Forum Nutr*. (2015) 14:175–9. doi: 10.1007/s13749-015-0064-3

26. Tiwari A. The antioxidant paradox. *Pharmacogn Mag*. (2019) 15:173. doi: 10.4103/0973-1296.265039

27. Biswas SK. Does the interdependence between oxidative stress and inflammation explain the antioxidant paradox? *Oxidative Med Cell Longev*. (2016) 2016:5698931–9. doi: 10.1155/2016/5698931

28. Halliwell B. The antioxidant paradox: less paradoxical now? *Br J Clin Pharmacol*. (2013) 75:637–44. doi: 10.1111/j.1365-2125.2012.04272.x

29. Mattson MP. Hormesis defined. *Ageing Res Rev*. (2008) 7:1–7. doi: 10.1016/j.arr.2007.08.007

30. Calabrese EJ. Hormesis: path and progression to significance. *Int J Mol Sci*. (2018) 19:871. doi: 10.3390/ijms19102871

31. Calabrese EJ, Agathokleous E, Kapoor R, Dhawan G, Calabrese V. Luteolin and hormesis. *Mech Ageing Dev*. (2021) 199:111559. doi: 10.1016/j.mad.2021.111559

32. Calabrese EJ. Hormetic mechanisms. *Crit Rev Toxicol*. (2013) 43:580–606. doi: 10.3109/10408444.2013.808172

33. Calabrese EJ, Kozumbo WJ. The phytoprotective agent sulforaphane prevents inflammatory degenerative diseases and age-related pathologies via Nrf2-mediated hormesis. *Pharmacol Res*. (2021) 163:105283. doi: 10.1016/j.phrs.2020.105283

34. Leak RK, Calabrese EJ, Kozumbo WJ, Gidday JM, Johnson TE, Mitchell JR, et al. Enhancing and extending biological performance and resilience. *Dose-Response*. (2018) 16:1559325818784501. doi: 10.1177/1559325818784501

35. Calabrese EJ, Baldwin LA. Chemical hormesis: its historical foundations as a biological hypothesis. *Hum Exp Toxicol*. (2000) 19:2–31. doi: 10.1191/096032700678815585

36. van Steenwijk HP, Bast A, de Boer A. The role of circulating lycopene in low-grade chronic inflammation: a systematic review of the literature. *Molecules*. (2020) 25:25194378. doi: 10.3390/molecules25194378

37. Yelisyeyeva O, Semen K, Zarkovic N, Kaminskyy D, Lutsyk O, Rybalchenko V. Activation of aerobic metabolism by Amaranth oil improves heart rate variability both in athletes and patients with type 2 diabetes mellitus. *Arch Physiol Biochem*. (2012) 118:47–57. doi: 10.3109/13813455.2012.659259

38. Rattan SIS. Hormesis in aging. *Ageing Res Rev*. (2008) 7:63–78. doi: 10.1016/j.arr.2007.03.002

39. Rattan SIS. Physiological hormesis and hormetins in biogerontology. *Curr Opin Toxicol*. (2022) 29:19–24. doi: 10.1016/j.cotox.2022.01.001

40. Rossnerova A, Izzotti A, Pulliero A, Bast A, Rattan SIS, Rossner P. The molecular mechanisms of adaptive response related to environmental stress. *Int J Mol Sci*. (2020) 21:7053. doi: 10.3390/ijms21197053

41. Semen KO, Yelisyeyeva OP, Kaminskyy DV, Cherkas AP, Zarkovic K, Lutsyk O, et al. Interval hypoxic training in complex treatment of *Helicobacter pylori*-associated peptic ulcer disease. *Acta Biochim Pol*. (2010) 57:199–208. doi: 10.18388/abp.2010_2395

42. Hu R, Xu C, Shen G, Jain MR, Khor TO, Gopalkrishnan A, et al. Identification of Nrf2-regulated genes induced by chemopreventive isothiocyanate PEITC by oligonucleotide microarray. *Life Sci*. (2006) 79:1944–55. doi: 10.1016/j.lfs.2006.06.019

43. Bryan HK, Olayanju A, Goldring CE, Park BK. The Nrf2 cell defence pathway: Keap1-dependent and -independent mechanisms of regulation. *Biochem Pharmacol*. (2013) 85:705–17. doi: 10.1016/j.bcp.2012.11.016

44. Valgimigli L, Iori R. Antioxidant and pro-oxidant capacities of ITCs. *Environ Mol Mutagen*. (2009) 50:222–37. doi: 10.1002/em.20468

45. Wei L-Y, Zhang J-K, Zheng L, Chen Y. The functional role of sulforaphane in intestinal inflammation: a review. *Food Funct*. (2022) 13:514–29. doi: 10.1039/D1FO03398K

46. Dinkova-Kostova AT, Talalay P. Direct and indirect antioxidant properties of inducers of cytoprotective proteins. *Mol Nutr Food Res*. (2008) 52:S128–38. doi: 10.1002/mnfr.200700195

47. Joko S, Watanabe M, Fuda H, Takeda S, Furukawa T, Hui S-P, et al. Comparison of chemical structures and cytoprotection abilities between direct and indirect antioxidants. *J Funct Foods*. (2017) 35:245–55. doi: 10.1016/j.jff.2017.05.039

48. Jiao Z, Chang J, Li J, Nie D, Cui H, Guo D. Sulforaphane increases Nrf2 expression and protects alveolar epithelial cells against injury caused by cigarette smoke extract. *Mol Med Rep*. (2017) 16:1241–7. doi: 10.3892/mmr.2017.6700

49. Heyninck K, Sabbe L, Chirumamilla CS, Szarc Vel Szic K, Vander Veken P, Lemmens KJA, et al. Withaferin a induces heme oxygenase (HO-1) expression in endothelial cells via activation of the Keap1/Nrf2 pathway. *Biochem Pharmacol*. (2016) 109:48–61. doi: 10.1016/j.bcp.2016.03.026

50. Sthijns MMJPE, Schiffers PM, Janssen GM, Lemmens KJA, Ides B, Vangrieken P, et al. Rutin protects against H(2)O(2)-triggered impaired relaxation of placental arterioles and induces Nrf2-mediated adaptation in human umbilical vein endothelial cells exposed to oxidative stress. *Biochim Biophys Acta Gen Subj*. (2017) 1861:1177–89. doi: 10.1016/j.bbagen.2017.03.004

51. Norde MM, Fisberg RM, Marchioni DML, Rogero MM. Systemic low-grade inflammation–associated lifestyle, diet, and genetic factors: a population-based cross-sectional study. *Nutrition*. (2020) 70:110596. doi: 10.1016/j.nut.2019.110596

52. Murillo G, Mehta RG. Cruciferous vegetables and cancer prevention. *Nutr Cancer*. (2001) 41:17–28. doi: 10.1080/01635581.2001.9680607

53. Fahey JW, Wehage SL, Holtzclaw WD, Kensler TW, Egner PA, Shapiro TA, et al. Protection of humans by plant glucosinolates: efficiency of conversion of glucosinolates to isothiocyanates by the gastrointestinal microflora. *Cancer Prev Res*. (2012) 5:603–11. doi: 10.1158/1940-6207.CAPR-11-0538

54. Fahey JW, Zhang Y, Talalay P. Broccoli sprouts: an exceptionally rich source of inducers of enzymes that protect against chemical carcinogens. *Proc Natl Acad Sci U S A*. (1997) 94:10367–72. doi: 10.1073/pnas.94.19.10367

55. Posner GH, Cho CG, Green JV, Zhang Y, Talalay P. Design and synthesis of bifunctional Isothiocyanate analogs of Sulforaphane: correlation between structure and potency as inducers of Anticarcinogenic Detoxication enzymes. *J Med Chem*. (1994) 37:170–6. doi: 10.1021/jm00027a021

56. López-Chillón MT, Carazo-Díaz C, Prieto-Merino D, Zafrilla P, Moreno DA, Villaño D. Effects of long-term consumption of broccoli sprouts on inflammatory markers in overweight subjects. *Clin Nutr*. (2019) 38:745–52. doi: 10.1016/j.clnu.2018.03.006

57. Bahadoran Z, Tohidi M, Nazeri P, Mehran M, Azizi F, Mirmiran P. Effect of broccoli sprouts on insulin resistance in type 2 diabetic patients: a randomized double-blind clinical trial. *Int J Food Sci Nutr*. (2012) 63:767–71. doi: 10.3109/09637486.2012.665043

58. van Steenwijk HP, Winter E, Knaven E, Brouwers JF, van Baardwijk M, van Dalum JB, et al. The beneficial effect of sulforaphane on platelet responsiveness during caloric load: a single-intake, double-blind, placebo-controlled, crossover trial in healthy participants. *Front Nutr*. (2023) 10:561. doi: 10.3389/fnut.2023.1204561

59. van Steenwijk HP, van Osch FHM, Troost FJ, Bast A, de Boer A, Semen KO. Heart rate variability correlates with the effect of sulforaphane on calorie-induced inflammation in healthy participants: a randomized placebo-controlled study. *Clin Nutr Open Sci*. (2023) 49:140–56. doi: 10.1016/j.nutos.2023.05.002

60. World Medical Association. World medical association declaration of Helsinki: ethical principles for medical research involving human subjects. *JAMA*. (2013) 310:2191. doi: 10.1001/jama.2013.281053

61. Meijer K. *Metabolic inflammation: Mechanistic views and preventive nutritional strategies*; (2014).

62. Hoevenaars FPM, Esser D, Schutte S, Priebe MG, Vonk RJ, Van Den Brink WJ, et al. Whole grain wheat consumption affects postprandial inflammatory response in a randomized controlled trial in overweight and obese adults with mild hypercholesterolemia in the Graandioos study. *J Nutr*. (2019) 149:2133–44. doi: 10.1093/jn/nxz177

63. Derosa G, D'Angelo A, Salvadeo SAT, Ferrari I, Fogari E, Gravina A, et al. Oral glucose tolerance test effects on endothelial inflammation markers in healthy subjects and diabetic patients. *Horm Metab Res*. (2010) 42:8–13. doi: 10.1055/s-0029-1237728

64. Nappo F, Esposito K, Cioffi M, Giugliano G, Molinari AM, Paolisso G, et al. Postprandial endothelial activation in healthy subjects and in type 2 diabetic patients: role of fat and carbohydrate meals. *J Am Coll Cardiol*. (2002) 39:1145–50. doi: 10.1016/s0735-1097(02)01741-2

65. Rubin D, Claas S, Pfeuffer M, Nothnagel M, Foelsch UR, Schrezenmeir J. S-ICAM-1 and s-VCAM-1 in healthy men are strongly associated with traits of the metabolic syndrome, becoming evident in the postprandial response to a lipid-rich meal. *Lipids Health Dis*. (2008) 7:32. doi: 10.1186/1476-511X-7-32

66. Chen NG, Azhar S, Abbasi F, Carantoni M, Reaven GM. The relationship between plasma glucose and insulin responses to oral glucose, LDL oxidation, and soluble intercellular adhesion molecule-1 in healthy volunteers. *Atherosclerosis*. (2000) 152:203–8. doi: 10.1016/s0021-9150(99)00460-8

67. Kim Y, Kim KJ, Park SY, Lim Y, Kwon O, Lee JH, et al. Differential responses of endothelial integrity upon the intake of microencapsulated garlic, tomato extract or a mixture: a single-intake, randomized, double-blind, placebo-controlled crossover trial. *Food Funct*. (2018) 9:5426–35. doi: 10.1039/c8fo01431k

68. Shivappa N, Bonaccio M, Hebert JR, Di Castelnuovo A, Costanzo S, Ruggiero E, et al. Association of proinflammatory diet with low-grade inflammation: results from the Moli-sani study. *Nutrition*. (2018) 54:182–8. doi: 10.1016/j.nut.2018.04.004

69. Pounis G, Bonaccio M, Di Castelnuovo A, Costanzo S, de Curtis A, Persichillo M, et al. Polyphenol intake is associated with low-grade inflammation, using a novel data analysis from the Moli-sani study. *Thromb Haemost*. (2016) 115:344–52. doi: 10.1160/TH15-06-0487

70. Bonaccio M, Di Castelnuovo A, Pounis G, De Curtis A, Costanzo S, Persichillo M, et al. A score of low-grade inflammation and risk of mortality: prospective findings from the Moli-sani study. *Haematologica*. (2016) 101:1434–41. doi: 10.3324/haematol.2016.144055

71. Winther-Larsen A, Aggerholm-Pedersen N, Sandfeld-Paulsen B. Inflammation-scores as prognostic markers of overall survival in lung cancer: a register-based study of 6,210 Danish lung cancer patients. *BMC Cancer*. (2022) 22:63. doi: 10.1186/s12885-021-09108-5

72. Shi H, Schweren LJS, ter Horst R, Bloemendaal M, van Rooij D, Vasquez AA, et al. Low-grade inflammation as mediator between diet and behavioral disinhibition: a UK biobank study. *Brain Behav Immun*. (2022) 106:100–10. doi: 10.1016/j.bbi.2022.07.165

73. Li Y-F, Chang Y-Y, Huang H-C, Wu Y-C, Yang M-D, Chao P-M. Tomato juice supplementation in young women reduces inflammatory adipokine levels independently of body fat reduction. *Nutrition*. (2015) 31:691–6. doi: 10.1016/j.nut.2014.11.008

74. Ouchi N, Walsh K. Adiponectin as an anti-inflammatory factor. *Clin Chim Acta*. (2007) 380:24–30. doi: 10.1016/j.cca.2007.01.026

75. Eboka-Loumingou Sakou RF, Longo-Mbenza B, Nkalla-Lambi M, Mokondjimobe E, Monabeka HG, Moukassa D, et al. Inflammatory biomarkers and prediction of insulin resistance in Congolese adults. *Heliyon*. (2021) 7:e06139. doi: 10.1016/j.heliyon.2021.e06139

76. Krause K, Sabat R, Witte-Händel E, Schulze A, Puhl V, Maurer M, et al. Association of CCL2 with systemic inflammation in Schnitzler syndrome. *Br J Dermatol*. (2019) 180:859–68. doi: 10.1111/bjd.17334

77. Bagetta D, Maruca A, Lupia A, Mesiti F, Catalano R, Romeo I, et al. Mediterranean products as promising source of multi-target agents in the treatment of metabolic syndrome. *Eur J Med Chem*. (2020) 186:111903. doi: 10.1016/j.ejmech.2019.111903

78. Kashi DS, Shabir A, Da Boit M, Bailey SJ, Higgins MF. The Effcacy of administering fruit-derived polyphenols to improve health biomarkers, exercise performance and related physiological responses. *Nutrients*. (2019) 11:389. doi: 10.3390/nu11102389

79. Bast A. Antioxidant pharmacotherapy. *Drug News Perspect*. (1994) 7:465–72.

80. Bjelakovic G, Nikolova D, Gluud LL, Simonetti RG, Gluud C. Mortality in randomized trials of antioxidant supplements for primary and secondary prevention: systematic review and Meta-analysis. *JAMA*. (2007) 297:842–57. doi: 10.1001/jama.297.8.842

81. Biesalski HK, Grune T, Tinz J, Zöllner I, Blumberg JB. Reexamination of a Meta-analysis of the effect of antioxidant supplementation on mortality and health in randomized trials. *Nutrients*. (2010) 2:929–49. doi: 10.3390/nu2090929

82. Watson J. Oxidants, antioxidants and the current incurability of metastatic cancers. *Open Biol*. (2013) 3:120144. doi: 10.1098/rsob.120144

83. Minihane AM, Vinoy S, Russell WR, Baka A, Roche HM, Tuohy KM, et al. Low-grade inflammation, diet composition and health: current research evidence and its translation. *Br J Nutr*. (2015) 114:999–1012. doi: 10.1017/S0007114515002093

84. Lacourt TE, Vichaya EG, Chiu GS, Dantzer R, Heijnen CJ. The high costs of low-grade inflammation: persistent fatigue as a consequence of reduced cellular-energy availability and non-adaptive energy expenditure. *Front Behav Neurosci*. (2018) 12:78. doi: 10.3389/fnbeh.2018.00078

85. van Steenwijk HP, Bast A, de Boer A. Immunomodulating effects of fungal Beta-glucans: from traditional use to medicine. *Nutrients*. (2021) 13:1333. doi: 10.3390/nu13041333

86. Halliwell B. The antioxidant paradox. *Lancet*. (2000) 355:1179–80. doi: 10.1016/S0140-6736(00)02075-4

87. Son TG, Camandola S, Mattson MP. Hormetic dietary phytochemicals. *NeuroMolecular Med*. (2008) 10:236–46. doi: 10.1007/s12017-008-8037-y

88. Jacobs DRJ, Gross MD, Tapsell LC. Food synergy: an operational concept for understanding nutrition. *Am J Clin Nutr*. (2009) 89:1543S–8S. doi: 10.3945/ajcn.2009.26736B

89. Mulholland CA, Benford DJ. What is known about the safety of multivitamin-multimineral supplements for the generally healthy population? Theoretical basis for harm. *Am J Clin Nutr*. (2007) 85:318S–22S. doi: 10.1093/ajcn/85.1.318S

90. Mirmiran P, Bahadoran Z, Hosseinpanah F, Keyzad A, Azizi F. Effects of broccoli sprout with high sulforaphane concentration on inflammatory markers in type 2 diabetic patients: a randomized double-blind placebo-controlled clinical trial. *J Funct Foods*. (2012) 4:837–41. doi: 10.1016/j.jff.2012.05.012

91. Lillie EO, Patay B, Diamant J, Issell B, Topol EJ, Schork NJ. The n-of-1 clinical trial: the ultimate strategy for individualizing medicine? *Perinat Med*. (2011) 8:161–73. doi: 10.2217/pme.11.7

92. Bapir M, Campagnolo P, Rodriguez-Mateos A, Skene SS, Heiss C. Assessing variability in vascular response to cocoa with personal devices: a series of double-blind randomized crossover n-of-1 trials. *Front Nutr*. (2022) 9:886597. doi: 10.3389/fnut.2022.886597

93. Rodrigues E, Lima D, Barbosa P, Gonzaga K, Guerra RO, Pimentel M, et al. HRV monitoring using commercial wearable devices as a health Indicator for older persons during the pandemic. *Sensors*. (2022) 22:2001. doi: 10.3390/s22052001

94. Georgiou K, Larentzakis AV, Khamis NN, Alsuhaibani GI, Alaska YA, Giallafos EJ. Can wearable devices accurately measure heart rate variability? A systematic review. *Folia Med*. (2018) 60:7–20. doi: 10.2478/folmed-2018-0012

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EDITED BY
Tingting Ju,
University of Alberta, Canada

REVIEWED BY
Ines Banjari,
University of Osijek, Croatia

*CORRESPONDENCE
Aalt Bast
✉ a.bast@maastrichtuniversity.nl

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Exploring health and toxicity in food choices: 10 examples navigating the gray area

Aalt Bast[1,2]* and Khrystyna O. Semen[1]

[1]University College Venlo, Maastricht University, Venlo, Netherlands, [2]Department of Pharmacology and Toxicology, Faculty of Health, Medicine and Life Sciences, Maastricht University, Maastricht, Netherlands

People's perception on what is healthy and what is toxic food, determines food preferences and eating behavior. The difference between heathy and toxic food and food ingredients is however not always clear. This is illustrated with 10 examples. Unjustly, all-natural food is regarded as safe. Regulation on health claims on food and food risks is not balanced. Biphasic responses of the physiological effect of food ingredients show that mild toxicity of these substances results in health promotion. Nutritional substances with drugs may have either a negative or a positive effect on health. New toxicological methodologies can be brought into play, to better understand the dynamics of health and disease. Unfortunately, we still cannot taste toxicity.

KEYWORDS

hormesis, toxicity, health, nutrition, flavonoid, taste, botanicals, vitamin B6

1 Introduction

Tasters were important in ancient history. With some imagination, one might say that tasters can be regarded as early toxicologists. Tasters had to protect Egyptian pharaohs, and Roman emperors. To prevent poisoning of these rulers with food by competitors, tasters were hired. The bible tells a famous story of the Pharaoh who was angry with two of his servants, and put them in prison. One of them was the Pharaoh's cupbearer. The other was the Pharaoh's chief baker. On Pharaoh's birthday,

> "He [the Pharaoh] restored the chief cupbearer to his former position so that he placed the cup in Pharaoh's hand, but the chief baker he impaled" [Genesis 40:1–23].

The chief baker and chief cupbearer were probably high priests and as servants of the Pharaoh of high social standing. The latter makes it attractive to compare them with modern toxicologists [smile authors]. The comparison can be extended. Food was regarded as the bearer of divinity and of life and was therefore given in the hand of the Pharaoh by these high priests. Our current view on food also changes from merely a necessity to survive to something that is the bearer of health. We now use fortified foods, food supplements and even nutraceuticals to boost our health. However, when this area is considered more closely, the distinction between healthy and toxic is far from clear. This is illustrated with 10 random examples.

2 Regulatory inconsistency for health promoting and toxic aspects of food

Regulators frequently use the precautionary principle to handle putative food risks. A well-known definition of precaution is the triple-negative definition, which reads "not having scientific certainty is not a justification for not regulating." The principle reflects the impossibility to provide absolute proof of safety. The precautionary principle seems to offer guidance on what to regulate (1). However, by definition it does not. In order to implement this regulatory framework on toxic risks, random choices on risks to be tackled are made. Moreover, it blinds regulators for external effects of these choices. This way of regulation emphasizes risk. There is always a toxic risk of food.

Health promoting effects of food are regulated differently from toxic risks. A legal framework used to highlight a particular beneficial effect of a food product should ensure that a health claim is clear, accurate and based on scientific evidence. Information that is misleading to consumers is prohibited. The roadmap to a health claim automatically entails that well-designed placebo controlled double blind studies are necessary to authorize a health claim for a food product.

This shows a regulatory inconsistency in dealing with toxic aspects of food vs. a health claim on food. To balance this better, it should be considered to introduce the concept health risk, a graded health promoting response. This would be in alignment with the way in which toxic risks are presented (2).

3 Natural safety vs. chemical danger: biophilia and chemophobia

There is a wide spread belief that compounds derived from nature are not really chemicals. Or at least they do not count as a chemical, because the word "chemical" has a negative connotation. Substances that are made in plants, microbes or animals are just there by nature. We are part of nature and natural equals beneficial. Natural is frequently marketed with the prefix "bio." Marketing uses this prefix eagerly. Biovitamin C sells better than just vitamin C (3). Conversely, synthetic substances are inherently regarded unhealthy. It is implied that the body is not equipped to cope with these man-made compounds. The fear or aversion to chemicals is called chemophobia and the intuitive love of all-natural, biophilia. The public sense of healthy food vs. toxic food is strongly influenced by these emotional responses.

A seminal paper documenting that natural is not synonymous with safety has been written by Ames et al. already in 1990 (4). The paper indicated that 99.99% (by weight) of the pesticides in the American diet are chemicals that plants produce to defend themselves. Comparative hazards of human exposures to synthetic pesticide residues are insignificant.

Statements that organic food is healthier are false. In fact there are many other examples illustrating that biophobia would be more appropriate than biophilia (5).

Unceasing information on chemicals (and it is without saying both of natural and of man-made origin) will aid general understanding of the role and use of these ingredients in food. Demystifying ingredients in food products will ultimately help to rationalize healthy and safety aspects of food.

4 New concepts of health: toxic changes lead to health

The WHO definition of health dates from 1948 and reads "A state of complete physical, mental and social well-being and not merely the absence of disease or infirmity." It is not surprising that this all-encompassing definition emerged directly after the horrors of World War II. At that moment, everything should get better, including all aspects of health. Following this definition, powerful drugs were designed in the 20th century. Drugs with a specific action on a single molecular target, thus minimalizing side effects. Pharmacology, the science of drug action, was precisely described as a form of "selective toxicity" (6). Apparently, even in that period, a selective form of toxicity was regarded as a stimulating factor for health.

Increasing knowledge on molecular processes that define health suggests that health is not a stagnant condition, and the original definition of health has become flawed. Health forms a dynamic condition rather than a fixed state of perfection and can be seen as "the ability to adapt." Obviously, this has consequences for preventing or treating diseases. Health promoting approaches should aim for increasing the aptitude to adapt.

Moreover, it is increasingly recognized that a shortcoming of a healthy homeostasis can involve various physiological pathways simultaneously, not just one specific molecular system. An imbalanced homeostasis may be the result of an intrinsic or extrinsic stressor. The increased ability to withstand such a stressor can then be regarded as a marker for health. To measure the health promoting effects of food, new methods should be developed. It is suggested that a mild aberrant physiology induced via an intrinsic stressor (e.g., a slight metabolic disturbance) or via an extrinsic stressor (e.g., a high caloric meal) might be used to investigate whether the intervention is able to increase the ability to adapt.

The other notion is that food in contrast to selectively acting drugs, works via a multitude of targets. In addition, the effects of food are generally less strong than those of drugs. This so-called pleiotropic action of food requires integrative methodologies for determining activity (7). A combination of markers could be envisioned to characterize a food effect. Several attempts have undertaken in this respect. Unfortunately, these new concepts are not yet incorporated in regulatory documents.

In conclusion, moderate toxic changes might shift the physiological balance in such a way that a beneficial health promoting increase in adaptation ability results.

5 Lessons from toxicology for health effects of food

In an inspiring paper, Langley et al. argued that current and future biomedical knowledge in the 21st century will ultimately lead to understanding of the dynamics of human disease (8). Human specific models understanding disease pathways comparable to unraveling adverse outcome pathways (AOPs) in toxicology will aid to comprehend cause and progression of pathophysiology. AOPs describe how interactions of compounds with biological systems cause injury and thus AOPs are thought to construct non-animal testing strategies as predictive models for toxicity of compounds. Similarly, unraveling pathways of positive effects of food constituents will lead

to understanding how these will lead to health promoting effect of food ingredients.

6 Hormesis: a biphasic response to toxic compounds

Following examples in paragraphs 4 and 5, automatically the process of hormesis comes to mind. Hormesis is best described as an adaptive response to low levels of stress or damage by, for example compounds, resulting in enhanced robustness of some physiological systems for a finite period (9). The dose–response relationship is one of the most important aspects in toxicology. Safety regulation is built around the theory of linear dose response relationships. Risk predictions based on animal experiments using high doses still are mainstay in toxicology. Extrapolation to lower dose, the dose of exposure, is subsequently performed. The assumption of a linear relationship between dose and response ignores that our cells have developed mechanisms to detoxify harmful chemicals. In fact, low doses of these chemicals may even trigger beneficial responses. These adaptive or biphasic responses are also known as hormetic responses. Although there are numerous examples of compounds that follow this hormetic response, toxicological thinking is still hesitant to apply it and to use the beneficial hormetic stimulus of "toxic" compounds to our advantage.

This hesitation is understandable since there are still many questions to be solved (9). Such as (i) what is the optimal dose, frequency, duration and timing of exposure; (ii) what are synergistic stimuli; (iii) what is the kinetics of the hormetic response; (iv) how is the response influenced by age or gender etc.; (v) are there any adverse effects. For now, this will hamper direct application of the hormetic response to promote health.

7 The example of flavonoids

In the beginning of the 20th century, several vitamins were discovered. From citrus fruits not only vitamin C but also vitamin P was isolated. The chemical characterization of vitamin P appeared to be difficult. Moreover, no deficiency disease could be linked to this component from citrus extract. It appeared however that this yellow colored pigment "vitamin P" had protective effects on vascular permeability and could enforce the effect of vitamin C. The citrus extract components were then named flavonoids [*flavus* (Latin) means yellow]. More specifically the major components of the citrus extract could be identified as oligomers of flavan-3-ol units, i.e., (+)-catechin or (−)-epicatechin (10). The average total flavan-3-ol intake in Europe has been reported to be 369 mg/d. There are many other flavonoids. Quercetin, commonly found in apples, onions and green tea forms 70% of the total flavonoid intake (11).

When the timeline of major achievements in the field of molecular biology, medicine and nutritional science is plotted, it is remarkable that flavonoids follow that line perfectly. This indicates the multitude of effect these flavonoids have. It started with the discovery of vitamins, and currently flavonoids are investigated as modulators of epigenetic processes.

A general comment on the use of these flavonoids is their apparent low bioavailability. However, there are indications that flavan-3-ols, known for their effect on microcirculatory vessels, have strong affinity for the vascular wall. Moreover, quercetin seems to cumulate to some extent in lung tissue (12). It is not surprising therefore that a beneficial effect of quercetin has been established in the lung disease sarcoidosis (13). Besides distribution, further selectivity in action is reached by the process of flavonoid regeneration from the glucuronide metabolites. The liberation of the parent flavonoid molecule seems possible locally at the site where they should act, viz., a spot of inflammation where the beta-glucuronidase of neutrophils deals with the local de-conjugation of the glucuronide (14).

Flavonoids are well-known for their antioxidant action (15). It has even been reported that flavonoids can take over the role of physiological antioxidants like vitamin E (16). This general broad mode of action of flavonoids remarkably aligns with specificity, which is further illustrated by the exiting recent finding that flavonoids after being oxidized activate the transcription factor Nrf2. This is probably due to the thiol reactivity of the oxidized form of flavonoids (17). Toxicologists would in general classify thiol reactivity as a toxic process. However, in this case, it lengthens and intensifies the protective effect of flavonoids because Nrf2 activation gives induction of various protective cellular factors.

Flavonoids are regarded as widely available bioactives in the diet. They have been studied for decades and their multitude of activities is remarkable. Recent data indicate that their activity is at least partly due to their (toxic) reactivity. A clear example of the overlapping areas of toxicity and health.

8 Dietary components in combination with drugs

The focus in literature on food-drug interactions is the notion that these interactions result in negative effects in safety and efficacy of drug therapy, as well as in the nutritional status of the patient. Authors advise urging patients to inform their doctors and pharmacists about their food intake and dietary supplements so that these negative interactions can be avoided (18). Failure to identify and properly manage drug-nutrient interactions can lead to serious consequences (19). There is concern about interactions between herbal medicines or dietary supplements with conventional cytostatics in cancer patients (20).

In contrast to these negative aspects there are also examples of positive interactions between food or food components with drugs. Doxorubicin is a widely used anti-tumor drug. Its major side effect is a dose dependent cardiotoxicity. The flavonoid 7-mono-O-(β-hydroxyethyl)-rutoside (monoHER) can completely prevent this cardiotoxicity without interfering with the antitumor effect of doxorubicin in mice (21). The extrapolation of these data to humans is probably hampered because of different metabolism in mouse and human (22).

Besides this example in which a toxic side effect of drug is prevented with a food derived compound, it is also possible to stimulate the action of drugs. The efficacy of corticosteroids can be enhanced with flavonoids. This has broad clinical implications because desensitization to corticosteroids is a well-known phenomenon and might thus be prevented (23, 24). Moreover, a recent case report describes the facilitating effect of grapefruit juice in cortisol replacement therapy via modulation of drug metabolizing enzymes (25).

9 Vitamin B6 deficiency by high dose B6 supplementation

The water-soluble vitamin B6 functions as a coenzyme in many physiological reactions (26). Severe vitamin B6 deficiency is not very common. Alcoholics are at risk of deficiency because of low dietary intake and impaired metabolism of the vitamin. Neurologic symptoms might occur due to vitamin B6 deficiency. Paradoxically, supplementation with high doses of vitamin B6 may also lead to polyneuropathy (27). This rather enigmatic observation was recently explained (27). The major forms of vitamin B6 (also called B6 vitamers) are pyridoxine, pyridoxal, pyridoxamine, and their phosphorylated derivatives pyridoxine 5′-phosphate, pyridoxal 5′-phosphate, pyridoxamine 5-phosphate. Supplementation frequently occurs with pyridoxine, which has to be converted in the body into pyridoxal-phosphate, which is the active form. It was recently suggested that high levels of pyridoxine inhibit pyridoxal-phosphate dependent enzymes by competing with the active vitamer pyridoxal-phosphate. A high level of vitamin B6 in the form of pyridoxine thus inhibits the action of the active form of vitamin B6, i.e., pyridoxal-phosphate. A high dose of pyridoxine may give symptoms comparable to a vitamin B6 deficiency.

10 Botanicals: toxic or healthy?

Botanicals are substances derived from plants, algae, fungi or lichens. They are also called herbal dietary supplements. Although the consumer perception is that they are safer than conventional medicines, many adverse reactions by the products are described yearly. Worldwide discussion is ongoing which level of evidence is needed in order to provide market authorization for these products (28). In the USA these products are regulated by the "Dietary Supplement and Health Education Act" whereas in the European Union botanicals are sometimes brought to the food and sometimes to the pharmaceutical market. In the EU, pharmaceutical regulations have a provision that occasionally permits to include data on traditional use of botanicals in the approval process (28). In contrast, substantiation of health benefits of botanicals in the food market with evidence based on traditional use is complex. Authorization of a health claim in the EU is possible following the "Nutrition and Health Claim Regulation" (NHCR), which includes two randomized controlled trials Recognition to evidence based on traditional use, supporting a botanical health claim is still under debate (29, 30). Difficulties in using traditional data on heath promoting aspects is that modern botanicals should be used in a similar way with similar purpose as in traditional use. Extraction methods might be modernized yielding a more concentrated form of the botanical which may coincide with higher concentrations of potentially toxic contaminants. Moreover, simultaneous use with modern medicine may be different from the traditional situation lading to safety problems.

In general verification of safety steps like (i) characterization of the product; (ii) collection of bibliographic data; (iii) information from traditional use; (iv) data interpretation; (v) identification, interpretation and management control of risks, are important for botanicals (31).

11 The taste of healthy and toxic food

We started this reflection on heathy and toxic food, with tasters. Originally five basic tastes are discerned, viz., bitter, salty, sour, sweet and umami. It is generally thought that the bitter taste signals toxicity, alerting animals not to consume these bitter molecules. Unfortunately, detailed analysis showed that bitterness is not a very reliable marker for toxicity (32). In fact, extra-oral bitter taste receptors have other functions. Agonists for bitter taste receptors in lung tissue have for example been suggested to have therapeutic potential in the treatment of asthma (33).

Recently, a new model to describe mouthfeel has been presented (34). This model might be useful in situations where taste is distorted because of disease, age or drug use to optimize food perception. Although taste might not be the optimal tool to discern toxicity, it certainly is pivotal for good appetite and health.

12 Discussion

Some overarching concluding remarks on the notion of a sliding scale of healthy and toxic food based on these examples are possible. The awareness that a strict distinction between healthy and toxic food is not always possible will help the public to have less emotional fear for man-made chemicals. Simultaneously, the unsubstantiated dangerous believe in the safety of natural compounds will be challenged.

Hormesis, a biphasic response to toxic substances, needs more attention. This is not only important in risk assessment but also in explaining the health benefit of food ingredients like flavonoids.

Interaction of food with drugs should not be regarded from a negative viewpoint only. The positive aspects, i.e., protection against side effects of drugs or enhancing the efficacy of drugs needs receiving more attention.

The new definition of health, ability to adapt, leads to a paradigm shift in research on the influence of food on health. Recent developments in toxicology like the "adverse outcome pathways" encompassing novel molecular biology knowledge, may be helpful in understanding health and disease.

The "bitter taste" of toxins becomes sweeter if toxicity leads to healthiness.

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References

1. Hanekamp JC, Bast A. Hormesis in precautionary regulatory culture: models preferences and advancement of science. *Hum Exp Toxicol*. (2007) 26:855–73. doi: 10.1177/0960327107083414

2. Hanekamp JC, Bast A. Food supplements and European regulation within a precautionary context: a critique and implications for nutritional, toxicological and regulatory consistency. *Crit Rev Food Sci*. (2007) 47:267–85. doi: 10.1080/10408390600737748

3. Bast A. The risk of eating: natural versus man-made toxins In: G Voss and G Ramos, editors. *Chemistry of crop protection*. Weinheim: Wiley-VCH (2003). 63–8.

4. Ames BN, Profet M, Gold LS. Dietary pesticides (99.99% all natural). *Proc Natl Acad Sci U S A*. (1990) 87:7777–81. doi: 10.1073/pnas.87.19.7777

5. Bast A, Hanekamp JC. *Toxicology. What everyone should know*. London (UK), San Diego (USA), Cambridge (USA), Oxford (UK): Academic press (2017).

6. Albert A. *Selective toxicity. The Physico-chemical basis of therapy*. *7th* ed. London, U.K.: Chapman and Hall (1985).

7. Weseler AR, Bast A. Pleiotropic-acting nutrients require integrative investigational approaches: the example of flavonoids. *J Agric Food Chem*. (2012) 60:8941–6. doi: 10.1021/jf3000373

8. Langley G, Austin CP, Balapure AK, Birnbaum LS, Bucher JR, Fentem J, et al. Lessons from toxicology: developing a 21st-century paradigm for medical research. *Environ Health Perspect*. (2015) 123:A268–72. doi: 10.1289/ehp.1510345

9. Leak RH, Calabrese EJ, Kozumbo WJ, Gidday JM, Johnson TE, Mitchell JR, et al. Enhancing and extending biological performance and resilience. *Dose Response*. (2018) 16:1559325818784501. doi: 10.1177/1559325818784501

10. Weseler AR, Bast A. Masquelier's grape seed extract: from basic flavonoid research to a well-characterized food supplement with health benefits. *Nutr J*. (2017) 16:5. doi: 10.1186/s12937-016-0218-1

11. Boots AW, Haenen GRMM, Bast A. Health effects of quercetin: from antioxidant to nutraceutical. *Eur J Pharmacol*. (2008) 585:325–37. doi: 10.1016/j.ejphar.2008.03.008

12. De Boer VCJ, Dihal AA, van der Woude H, Arts ICW, SiegfriedW AGM, Rietjens IMCM, et al. Tissuie distribution of quercetin in rats and pigs. *J Nutr*. (2005) 135:1718–25. doi: 10.1093/jn/135.7.1718

13. Boots AW, Drent W, de Boer VC, Bast A, Haenen GRMM. Quercetin reduces markers of oxidative stress and inflammation in sarcoidosis. *Clin Nutr*. (2011) 30:506–12. doi: 10.1016/j.clnu.2011.01.010

14. Bartholomé R, Haenen GRMM, Hollman CH, Bast A, Dagnelie PC, Roos D, et al. Deconjugation kinetics of glucuronidated phase II flavonoid metabolites by beta-glucuronidase from neutrophils. *Drug Metab Pharmacokinet*. (2010) 25:379–87. doi: 10.2133/dmpk.DMPK-10-RG-002

15. van Acker SABE, van den Berg D-J, Tromp MNJ, Griffioen DH, van Bennekom WP, van der Vijgh WJF, et al. A quantum chemical explanation for the antioxidant activity of flavonoids. *Chem Res Toxicol*. (1996) 9:1305–12. doi: 10.1021/tx9600964

16. van Acker FAA, Schouten O, Haenen GRMM, van der Vijgh WJF, Bast A. Flavonoids can replace alpha-tocopherol as an antioxidant. *FEBS Lett*. (2000) 473:145–8. doi: 10.1016/S0014-5793(00)01517-9

17. Lemmens KJA, Sthijns MMJPE, van der Vijgh WJF, Bast A, Haenen GRMM. The antioxidant flavonoid monoHER provides efficient protection and includes the innate Nrf2 mediated adaptation in endothelial cells subjected to oxidative stress. *PharmaNutrition*. (2014) 2:69–74. doi: 10.1016/j.phanu.2014.05.003

18. Bushra R, Aslam N, Khan AY. Food-drug interactions. *Oman Med J*. (2011) 26:77–83. doi: 10.5001/omj.2011.21

19. Genser D. Food and drug interaction: consequences for the nutritional/health status. *Ann Nutr Metab*. (2008) 52:29–32. doi: 10.1159/000115345

20. Alsanad SM, Williamson EM, Howard RL. Cancer patients at risk of herb/food supplement-drug interactions: a systematic review. *Pytotherapy Res*. (2014) 28:1749–55. doi: 10.1002/ptr.5213

21. van Acker SABE, Kramer K, Grimbergen JA, van den Berg D-J, van der Vijgh WJF, Bast A. Monohydroxyethylrutoside as protector against chronic doxorubicin-induced cardiotoxicity. *Br J Pharmacol*. (1995) 115:1260–4. doi: 10.1111/j.1476-5381.1995.tb15034.x

22. Jacobs H, Koek GH, Peters R, Moalin M, Tack J, van der Vijgh WJ, et al. Differences in pharmacological activities of the antioxidant flavonoid monoHER in humans and mice are caused by variations in its metabolic profile. *Clin Pharmacol Ther*. (2011) 90:852–9. doi: 10.1038/clpt.2011.200

23. Verissimo G, Bast A, Weseler AR. Monomeric and oligomeric flavanols maintain the endogenous glucocorticoid response in human macrophages in pro-oxidant conditions in vitro. *Chem Biol Interact*. (2018) 291:237–44. doi: 10.1016/j.cbi.2018.06.024

24. Ruijters EJB, Haenen GRMM, Weseler AR, Bast A. The cocoa flavanol (−)-epicatechin protects the cortisol response. *Pharmacol Res*. (2014) 79:28–33. doi: 10.1016/j.phrs.2013.11.004

25. Drent M, Wijnen P, Bekers O, Bast A. Grapefruit juice facilitates cortisol replacement therapy: role of CYP3A variant alleles. *Arch Clin Med Case Rep*. (2021) 5:656–64. doi: 10.26502/acmcr.96550405

26. Reddy P. Preventing vitamin B6-related neurotoxicity. *Am J Ther*. (2022) 29:e637–43. doi: 10.1097/MJT.0000000000001460

27. Vrolijk MF, Opperhuizen A, Jansen EHJM, Hageman GJ, Bast A, Haenen GRMM. The vitamin B6 paradox: supplementation with high concentrations of pyridoxine leads to decreased vitamin B6 function. *Toxicol In Vitro*. (2017) 44:206–12. doi: 10.1016/j.tiv.2017.07.009

28. Lenssen K, Bast A, de Boer A. International perspectives on substantiating the efficacy of herbal dietary supplements and herbal medicines through evidence on traditional use. *Compr Rev Food Sci Food Saf*. (2019) 18:910–22. doi: 10.1111/1541-4337.12446

29. Anton R, Serafini M, Delmulle L. The substantiation of claims for botanical food supplements in relation to traditional use. *Eur Food Feed Law Rev*. (2013) 8:321–8.

30. Lenssen KGM, Bast A, de Boer A. The complexity of proving health effects with data on 'traditional use': a critical perspective on supporting botanical health claims. *Trends Food Sci Technol*. (2022) 120:338–43. doi: 10.1016/j.tifs.2021.12.030

31. Anton R, Serafini M, Delmulle L. The role of traditional knowledge in the safety assessment of botanical food supplements. Requirements for manufacturers. *Eur Food Feed Law Rev*. (2013) 8:241–328.

32. Nisim I, Dagan-Wiener A, Niv MY. The taste of toxicity: a quantitative analysis of bitter and toxic molecules. *IUBM Life*. (2017) 69:938–46. doi: 10.1002/iub.1694

33. Conaway S, Nayak AP, Deshpande DA. Therapeutic potential and challenges of bitter taste receptors on lung cells. *Curr Opin Pharmacol*. (2020) 51:43–9. doi: 10.1016/j.coph.2020.07.004

34. Agorastos G, van Halsema E, Bast A, Klosse P. Review of mouthfeel classification. A new perspective of food perception. *J Food Sci Nutr*. (2020):1–10. doi: 10.46715/jfsn2020.09.1000107

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EDITED BY
Siddika Yalcin,
Hacettepe University, Türkiye

REVIEWED BY
Francesk Mulita,
General University Hospital of Patras, Greece
Ahmed Madar,
University of Oslo, Norway

*CORRESPONDENCE
Xin Fan
✉ drfanxin@163.com

†These authors have contributed equally to this work

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The correlation between urinary iodine levels and gallstone risk: elevated iodine intake linked to gallstone occurrence

Yunfan Li[1†], Minchen Wang[1†], Wenyi Du[2†], Liuyao Qi[1], Xiaopeng Liu[1] and Xin Fan[1*]

[1]Department of General Surgery, Affiliated Hospital of Jiangsu University, Zhenjiang, China, [2]Department of General Surgery, The Affiliated Wuxi People's Hospital of Nanjing Medical University, Wuxi, China

Background: Essential trace elements are vital for human growth and development. Nevertheless, excessive intake can pose risks. As of yet, no research has looked at the possibility of a relationship between the prevalence of gallstones and urinary concentrations of nickel, molybdenum, and iodine.

Objectives: The purpose of this study was to examine the correlation between urinary levels of iodine, molybdenum, and nickel and the occurrence of gallstones in a U.S. population and to verify whether excessive iodine intake is associated with the occurrence of gallstones.

Methods: Data from 2,734 participants that were gathered between 2017 and 2020 were examined. Employing inductively coupled plasma mass spectrometry (ICP-MS), the levels of nickel (Ni), iodine (I), and molybdenum (Mo) in the urine were determined. Gallstones presence was determined using a standardized questionnaire. Restricted cubic spline analysis, subgroup analysis, and logistic regression analysis were used to evaluate the relationship between the occurrence of gallstones and urinary essential trace elements.

Results: The logistic regression analysis indicated an increased risk of gallstone development in Quartiles 2, Quartiles 3, and Quartiles 4 groups in comparison to the Quartiles 1 group, based on urinary iodine levels (OR = 1.69, 95% CI: 1.11–2.56; OR = 1.68, 95% CI: 1.10–2.55; OR = 1.65, 95% CI: 1.09–2.51). Urinary iodine levels were nonlinearly positively linked with the development of gallstones, according to restricted cubic spline analysis (*P*-Nonlinear = 0.032). Subgroup analyses showed that high levels of urinary iodine were associated with a high risk of gallstones in different populations, and were more pronounced in adults aged 60 years and older, in women, with a BMI ≥ 25, and in diabetic patients.

Conclusion: Our research revealed a correlation between an increased risk of gallstones and increasing urinary iodine levels. Urinary iodine levels serve as indicators of the body's iodine status, thus suggesting that excessive iodine intake may be linked to an elevated risk of gallstone formation.

KEYWORDS

trace elements, iodine intake, NHANES, urinary iodine, gallstones

1 Introduction

The primary cause of gallstones, a common digestive ailment marked by the production of stones in the bile ducts or gallbladder, is unusually high cholesterol levels in the bile. About 10–20% of persons worldwide suffer from gallstones, which has a substantial financial impact on individuals (1–4). Even though 80% of individuals with gallstones may not display any signs, the disease can progress from carriers with no symptoms to individuals with symptoms and complicated issues such as acute pancreatitis, cholangitis, and acute cholecystitis if prompt treatment is not obtained (5, 6). Obesity is recognized as an important risk factor for the development of gallstones (1). Literature shows that obesity is strongly associated with complications such as cardiovascular disease, type 2 diabetes mellitus, malignant tumors, asthma, osteoarthritis, chronic back pain, obstructive sleep apnea, nonalcoholic fatty liver disease, and gallbladder disease (7). In addition, previous studies have shown that the occurrence of gallstones is strongly associated with age, gender (female), pregnancy, hypertension, diabetes, and hyperlipidemia (1, 8–10). Also, among different ethnicities, the prevalence of gallstones is higher in Hispanic populations in the Americas and South America (1). As for the income level of the country, the prevalence of gallstones is also higher in low-income countries and upper-middle-income countries (11).

The human body requires around 20 elements, including both metallic and non-metallic elements, to maintain normal physiological functions. Among these essential elements, there are certain trace elements that are vital to the human body, despite being present in very small quantities. Examples of these essential trace elements include iodine molybdenum, and nickel (12, 13). It is important to note that excessive consumption of these trace elements can have detrimental effects on human health. For instance, an excessive intake of iodine has been identified as a risk factor for autoimmune thyroiditis and thyroid cancer (14, 15). Furthermore, excessive intake of nickel has been linked to the development of tumors and damage to the immune system (16, 17).

Previous studies have suggested a possible association between particular elements and gallstones development. More precisely, some researchers have found that dietary magnesium intake could decrease the risk of gallstone formation, while others have postulated that elevated blood levels of selenium may be a risk factor for gallstone formation (18, 19). After examining pertinent information, we did not discover any research examining the possible correlation between urinary iodine, nickel, and molybdenum and gallstone incidence. However, previous studies have found that excessive iodine intake may lead to hyperthyroidism and hypothyroidism, both of which promote the development of gallstones (14, 20, 21). The purpose of this study was to examine the correlation between urinary levels of iodine, molybdenum, and nickel and the occurrence of gallstones in a U.S. population and to verify whether excessive iodine intake is associated with the occurrence of gallstones.

2 Methods

2.1 Design of the study

A recurrent study, the NHANES is carried out by the National Center for Health Statistics (NCHS). The purpose of this extensive national survey is to assess the nutritional and general health of Americans of all ages. The survey utilizes an extensive methodology that involves health assessments carried out at mobile health clinics, health surveys conducted at participants' residences, as well as the extensive gathering of demographic information, medical tests, lab analyses, illnesses occur surveys, and documents of prescribed medications. This varied strategy guarantees a comprehensive assessment of multiple health-related parameters, enabling a comprehensive comprehension of the participants' well-being. The National Center for Health Statistics' Ethical Review Board has approved the NHANES program in its entirety, and each participant voluntarily gave informed consent. Usually, NHANES data are released every 2 years. Regretfully, the COVID-19 epidemic forced a temporary end to the program in March 2020. Consequently, a nationally representative sample that includes pre-pandemic data up until March 2020 was created by combining the data obtained between 2019 and March 2020 with the NHANES 2017–2018 cycle. Participants in this trial cycle were particularly questioned about their history of gallstones. In the beginning, the study had 15,560 participants. However, 11,000 people had missing data on urinary essential trace elements, 1,813 participants did not finish the gallstone questionnaire, and 13 participants declined to answer or did not know the questionnaires on hypertension, diabetes mellitus, and education level. The final analysis comprised 2,734 people in total, and Figure 1 presents a flow chart that details the screening procedure. The sample size formula for a cross-sectional study with qualitative data is as follow: $n = Z^2_{1-\alpha/2} * P * (1-P)/d^2$, where n is the sample size, $Z_{1-\alpha/2} = 1.96$ when α is taken as 0.05, P is the predicted prevalence of the disease, and d is the permissible error, which is generally taken as $d = \alpha/2 = 0.025$ (22). When $p = 0.1$ (1), we can calculate that the minimum sample size needed for this cross-sectional study is 554. Therefore, the inclusion of 2,734 participants in our study is meeting the design requirements for a cross-sectional study.

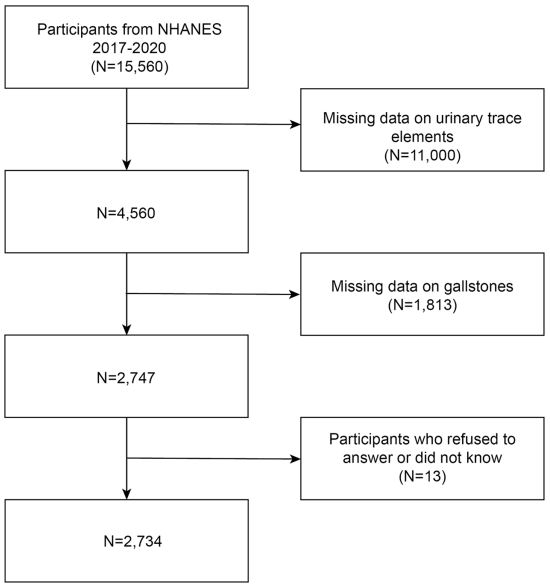

FIGURE 1
Study flowchart: NHANES enrolled a total of 15,560 participants from 2017 to 2020. Out of these individuals, 2,734 met the inclusion and exclusion criteria and were included in the analysis.

2.2 How to define gallstones

In order to determine whether gallstones were present or not, we used a questionnaire called "Has your doctor ever diagnosed you with gallstones?" Gallstones were categorized as present in participants who responded positively, whereas their absence was classified in those who did not.

2.3 Quantification of urinary essential trace elements

Participants were asked to provide random urine by voiding into specimen cups. Urine samples were prepared, stored, and sent to the National Center for Environmental Health, Atlanta, GA, Division of Laboratory Sciences, for examination. Using inductively coupled plasma mass spectrometry (ICP-MS), the quantification of iodine (I), nickel (Ni), and molybdenum (Mo) in the urine were determined. The mass spectrometer receives liquid samples via the ICP ionization source. A nebulizer reduces the liquid samples to tiny droplets in an argon aerosol, which are subsequently injected into the ICP. Individual isotopes of an element can be identified by first allowing the ions to pass through a focusing zone, followed by the dynamic reaction cell (DRC), the quadrupole mass filter, and finally, a quick series of selective counting at the detector. For I, Mo, and Ni, the corresponding lower limits of detection (LLOD) were 2.4 μg/L, 0.80 μg/L, and 0.31 μg/L, respectively. All urinary trace element values below the limit of detection (LLOD) were substituted with the LLOD divided by $\sqrt{2}$.

2.4 Covariate identification

Gender, age, race, educational status, waist circumference, BMI, family income to poverty ratio, serum triglyceride concentration, and history of diabetes and hypertension were all used as covariates in the statistical model. The "Your doctor informed you that you have diabetes" questionnaire was used to ascertain if diabetes was present or not. Individuals who gave a positive response were identified as having diabetes. In a similar vein, people who answered affirmatively on the questionnaire "Your doctor informed you that you have high blood pressure" were classified as having hypertension.

2.5 Statistical analyses

Sample size (percentage) was used to represent categorical variables, while the mean (standard deviation) was used to express continuous variables. Two Sample *t*-test was utilized for continuous variables and chi-square tests were employed for categorical variables to evaluate differences between groups. The odds ratios (OR) and their corresponding 95% confidence intervals (CI) were determined using logistic regression to evaluate the association between every quartile of urinary levels of iodine (I), molybdenum (Mo), and nickel (Ni) with the occurrence of gallstones. Analysis involved the development of three logistic regression models. Model 1 failed to include covariate improvements, whereas Model 2 considered age, gender, and race. Model 3, after making complete adjustments, involved factors like race, age, gender, education level, family income, BMI, waist size, serum triglyceride levels, as well as the presence of hypertension and diabetes history. The relationship between the likelihood of gallstones development and urinary iodine level was examined using a restricted cubic spline analysis. Covariates such as age, gender, educational attainment, family income, waist size, BMI, and serum triglyceride concentration were taken into account while adjusting the model. Subgroup analyses were used to explore whether high levels of urinary iodine associated with a high risk of gallstones differed across subgroups of BMI, age, gender, serum triglyceride levels, diabetes, and hypertension. A random forest approach is used to fill in the missing values for the covariates. The statistical studies were conducted utilizing EmpowerStats and R 4.2.2 software.[1]

3 Results

3.1 Participants' fundamental traits

Table 1 includes the fundamental traits and other variables of the research participants, categorized based on the existence or non-existence of gallstones. Urinary iodine, molybdenum, and nickel were grouped into quartiles based on concentration. Participants with gallstones were older, predominantly female, and of predominantly Non-Hispanic White ethnicity. In addition, they had larger BMI and waist circumference, higher rates of hypertension and diabetes, and higher serum triglyceride levels. However, there were no significant differences in HDL-C, LDL-C and Serum total cholesterol levels between participants with and without gallstones. In the quartile groups of urinary iodine concentration levels, the proportion of patients with gallstones showed a wavy increase from Quartile 1 to Quartile 4 groups. The proportion of patients with gallstones in the urinary iodine concentration Quartile 3 group was slightly lower than that in Quartile 2, while the highest proportion of participants was found in the Quartile 4 group (29.88%, $p = 0.009$). However, there was no statistically significant change in the proportion of patients with gallstones in the quartile groups of urinary molybdenum, and nickel concentration levels.

3.2 Relationship between the risk of gallstones and urinary essential trace elements

The relationship between the risk of gallstones development and urinary essential trace elements is shown in Table 2. Participants were grouped into quartiles based on the levels of iodine, nickel, and molybdenum in their urine for analysis. The control group was defined as the quartile with the lowest concentration of each element. Urinary iodine levels ranged from 2.6ug/L to 70.8ug/L in Quartile 1, from 70.8ug/L to 124ug/L in Quartile 2, from 124ug/L to 230ug/L in Quartile 3 and from 230ug/L to 23637.3ug/L in Quartile 4. In the first model, which

1 https://www.empowerstats.com

TABLE 1 Baseline characteristics of participants.

Characteristics	Total	Gallstones		*p*-value
	n = 2,734	Yes (*n* = 251)	No (2,483)	
Age, years, M (SD)	51 (17)	59 (16)	50 (17)	<0.001
Gender, *n* (%)				<0.001
Male	1,341 (49.0%)	73 (29.1%)	1,268 (51.1%)	
Female	1,393 (51.0%)	178 (70.9%)	1,215 (48.9%)	
Race, *n* (%)				<0.001
Mexican American	327 (12.0%)	31 (12.4%)	296 (11.9%)	
Other Hispanic	273 (10.0%)	30 (12.0%)	243 (9.8%)	
Non-Hispanic White	905 (33.1%)	111 (44.2%)	794 (32.0%)	
Non-Hispanic Black	742 (27.1%)	49 (19.5%)	693 (27.9%)	
Non-Hispanic Asian	348 (12.7%)	17 (6.8%)	331 (13.3%)	
Other Race	139 (5.1%)	13 (5.2%)	126 (5.1%)	
Education, *n* (%)				0.314
<9th grade	198 (7.2%)	12 (4.7%)	186 (7.5%)	
9–11th grade	316 (11.6%)	29 (11.6%)	287 (11.5%)	
High school graduate	695 (25.4%)	75 (29.9%)	620 (25.0%)	
Some college or AA degree	862 (31.5%)	78 (31.1%)	787 (31.6%)	
College graduate or above	663 (24.3%)	57 (22.7%)	606 (24.4%)	
BMI, M (SD)	30 (7)	32 (8)	30 (7)	<0.001
Waist circumference, cm, M (SD)	101 (17)	107 (16)	100 (17)	<0.001
Ratio of family income to poverty, M (SD)	2.59 (1.53)	2.60 (1.43)	2.59 (1.54)	0.932
Serum triglyceride, mg/dL, M (SD)	113 (88)	122 (53)	115 (92)	0.043
HDL-C, mg/dL, M (SD)	54 (15)	54 (14)	54 (16)	0.808
LDL-C, mg/dL, M (SD)	109 (31)	110 (24)	111 (26)	0.432
Serum total cholesterol, mg/dL, M (SD)	185 (39)	185 (38)	185 (39)	0.936
I, ug/L, *n* (%)				0.009
Q1 [2.6, 70.8]	684 (25.0%)	41 (16.3%)	643 (25.9%)	
Q2 [70.8, 124]	681 (24.9%)	68 (27.1%)	613 (24.7%)	
Q3 [124, 230]	684 (25.0%)	67 (26.7%)	617 (24.8%)	
Q4 [230, 23637.3]	685 (25.1%)	75 (29.9%)	610 (24.6%)	
Mo, ug/L, *n* (%)				0.268
Q1 [0.93, 18.8]	684 (25.0%)	74 (29.5%)	610 (24.5%)	
Q2 [18.8, 36.5]	683 (25.0%)	61 (24.3%)	622 (25.1%)	
Q3 [36.5, 63.2]	683 (25.0%)	53 (21.1%)	630 (25.4%)	
Q4 [63.2, 665]	684 (25.0%)	63 (25.1%)	621 (25.0%)	
Ni, ug/L, *n* (%)				0.545
Q1 [0.22, 0.65]	671 (24.6%)	56 (22.3%)	615 (24.8%)	
Q2 [0.65, 1.17]	687 (25.1%)	68 (27.1%)	619 (24.9%)	
Q3 [1.17, 1.95]	690 (25.2%)	58 (23.1%)	632 (25.5%)	
Q4 [1.95, 64.1]	686 (25.1%)	69 (27.5%)	617 (24.8%)	
Rate of hypertension, *n* (%)	1,069 (39.1%)	126 (50.2%)	943 (38.0%)	<0.001
Rate of diabetes, *n* (%)	496 (18.1%)	67 (26.7)%	429 (17.2%)	<0.001

M, mean values; SD, standard deviation; *n*, sample size; BMI, body mass index; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoproteins cholesterol; I, iodine; Mo, molybdenun; Ni, nikel.

TABLE 2 Association between trace elements and gallstones.

	OR (95% CI), *p*-value		
	Model 1	Model 2	Model 3
I, ug/L			
Q1 [2.6, 70.8]	1.00 (reference)	1.00 (reference)	1.00 (reference)
Q2 [70.8, 124]	1.73 (1.16, 2.61), 0.007	1.76 (1.17, 2.68), 0.007	1.69 (1.11, 2.56), 0.014
Q3 [124, 230]	1.70 (1.14, 2.57), 0.010	1.74 (1.15, 2.65), 0.009	1.68 (1.10, 2.55), 0.016
Q4 [230, 23637.3]	1.93 (1.30, 2.88), 0.001	1.75 (1.16, 2.66), 0.008	1.65 (1.09, 2.51), 0.018
Mo, ug/L			
Q1 [0.93, 18.8]	1.00 (reference)	1.00 (reference)	1.00 (reference)
Q2 [18.8, 36.5]	0.81 (0.56, 1.15), 0.239	0.84 (0.58, 1.21), 0.342	0.78 (0.53, 1.13), 0.189
Q3 [36.5, 63.2]	0.69 (0.48, 1.00), 0.051	0.80 (0.55, 1.17), 0.258	0.73 (0.49, 1.08), 0.118
Q4 [63.2, 665]	0.83 (0.58, 1.19), 0.318	1.10 (0.76, 1.59), 0.601	1.00 (0.69, 1.46), 0.987
Ni, ug/L			
Q1 [0.22, 0.65]	1.00 (reference)	1.00 (reference)	1.00 (reference)
Q2 [0.65, 1.17]	1.21 (0.83, 1.75), 0.321	1.25 (0.85, 1.83), 0.257	1.14 (0.77, 1.68), 0.521
Q3 [1.17, 1.95]	1.01 (0.69, 1.48), 0.968	1.11 (0.75, 1.65), 0.610	1.04 (0.70, 1.56), 0.838
Q4 [1.95, 64.1]	1.23 (0.85, 1.79), 0.272	1.26 (0.86, 1.86), 0.229	1.17 (0.80, 1.74), 0.418

Model 1: no adjustment for covariates.
Model 2: covariates such as gender, age, and race were included.
Model 3: a comprehensive model incorporating age, gender, race, educational level, family income, BMI, waist circumference, Serum triglyceride level, and a history of diabetes and hypertension.

was the initial model without adjusting for covariates, the likelihood of developing gallstones was found to be linked to urinary iodine levels. Participants in Quartiles 2–4 showed a 73, 70, and 93% increased risk of getting gallstones, respectively, in comparison to those in Quartiles 1. Within the second model, those in Quartiles 2–4 had a 76, 74, and 75% greater risk of getting gallstones, respectively, in comparison to those in Quartile 1, after controlling for gender, age, and race. In adjusted model 3 controlling for gender, age, race, education, household income, BMI, waist size, Serum triglyceride concentration, as well as previous diagnoses of diabetes and high blood pressure, an elevated level of urinary iodine was correlated with a higher risk of getting gallstones. Individuals in Quartiles 2–4 displayed a 69, 68, and 65% rise in gallstone risk, respectively, in comparison to those in Quartile 1. Neither Model 1, Model 2, nor Model 3 showed any correlation between the risk of gallstones and urinary nickel and molybdenum levels. Furthermore, restricted cubic spline analysis revealed what Figure 2 showed that urinary iodine levels were nonlinearly positively correlated with the development of gallstones (*P*-Nonlinear = 0.032). When the urinary iodine concentration was less than 203 ug/L, the curve of OR was steeper than the curve when the urinary iodine concentration was greater than 203 ug/L.

3.3 Subgroup analyses

Studies were performed on subgroups to explore if various factors such as gender, age, BMI, serum triglyceride concentration, diabetes, and hypertension influenced the connection between urinary iodine levels and the likelihood of gallstone formation (Figure 3). Upon controlling for pertinent variables, our study revealed variations in participants based on age, gender, BMI, and diabetes status. Notably, Quartiles 2–4 of urinary iodine concentration exhibited a strong correlation with the risk of developing gallstones among individuals aged 60 and above, female, had a BMI of 25 or higher, and were diagnosed with diabetes.

4 Discussion

Using information from the 2017–2020 NHANES, this cross-sectional study looked into any possible relationship between the occurrence of gallstones and essential trace elements in urine. Even after controlling for relevant variables, the results point to a substantial correlation between higher urinary iodine concentration and an elevated risk of gallstones. Notably, those 60 years of age or older, women, those with a BMI of 25 or above, and those with a diagnosis of diabetes showed the strongest correlation with this association. However, elevated levels of nickel, and molybdenum in the urine were not associated with an increased risk of gallstones.

To the best of our knowledge, there have been no previous studies specifically examining the correlation between urinary trace elements levels and gallstones, but our results indicate that urinary iodine levels are positively associated with the risk of getting gallstones, whereas elevated levels of nickel, and molybdenum are not. In the context of China, Mei-Hsuan Lee et al. collected data on serum metal levels in a study covering 701 patients with gallstones and concluded that serum levels of cadmium, chromium, copper, molybdenum, and vanadium were associated with the development of gallstones (23). Additionally, blood selenium was reported by Wang et al. as a separate risk indicator

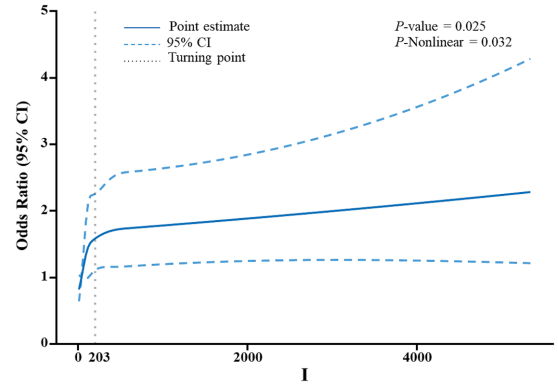

FIGURE 2
An analysis utilizing a restricted cubic spline was conducted to evaluate the correlation between levels of iodine in urine and the risk of developing gallstones. The adjustment of the model included factors such as age, gender, educational attainment, household income, body mass index (BMI), waist circumference, and serum triglyceride levels. The depiction of odds ratios (OR) was presented as a continuous solid blue line, with the incorporation of 95% confidence intervals displayed as blue dashed lines. Furthermore, the inflection point was visually indicated by gray dotted lines.

for gallstones in the USA using data from the NHANES database (18). Currently, no definitive conclusions have been drawn regarding the effects of trace elements (both metallic and nonmetallic) on the occurrence of gallstones in humans. Therefore, it is noteworthy that our research indicates a link between increased iodine levels in urine and a higher risk of gallstone development.

Iodine is an important trace element that is closely related to human growth, development and metabolism (24). Iodine is mainly consumed through the diet and is rapidly and almost completely absorbed in the stomach and duodenum (>90%). More than 90% of the iodine ingested is ultimately excreted in the urine, with the remaining portion excreted in the feces or sweat (5–10%). Urinary iodine concentration is therefore a sensitive indicator of recent iodine intake (25–28). Iodine deficiency is usually caused by a lack of iodine in the diet and is particularly common in inland areas. Iodine deficiency is the main cause of diseases such as stunting, developmental delay, and endemic goiter (29). For this reason, in 1994, the WHO proposed a global strategy to add potassium iodate to salt to ensure adequate iodine in the diet. This strategy to control iodine deficiency has yielded tremendous results, with approximately 70% of households worldwide having access to adequately iodized salt. However, in order to ensure adequate iodine intake, iodine overdose should also be avoided, and data from the WHO show that adequate or excessive iodine intake has been observed in many countries (30, 31). In addition to the overuse of salt iodization, there are other factors that may contribute to excessive iodine intake. For example, the daily consumption of certain iodine-rich foods such as seaweeds (32), and the use of iodine-containing water purification tablets lead to excessive iodine in drinking water (33), in addition to the medical use of iodine-containing medications such as amiodarone and iodine-containing contrast media, which are also important contributors to excessive iodine intake (34, 35).

Although there have been no definitive experimental studies linking excessive iodine intake to the development of gallstones, previous studies have found that excessive iodine intake may lead to a variety of disorders, including hypothyroidism, hyperthyroidism, and autoimmune thyroid disease (ATD) (14). Hypothyroidism and hyperthyroidism may play an important role in the association between excessive iodine intake and the development of gallstones. Kube I et al. found that hypothyroidism increases the hydrophobicity of primary bile acids and thus increases the incidence of gallstones (36). Wang Y et al. found that both hypothyroidism and hyperthyroidism can contribute to the development of gallstones through different mechanisms (20). Nakano S et al. concluded that rapid weight loss due to hyperthyroidism can lead to the development of gallstones (37). In addition, high levels of iodine intake have effects on blood glucose, blood pressure and lipid metabolism (38, 39), and may contribute to the development of gallstones (1, 9). The main components of bile include bile acids, cholesterol and phospholipids, which are excreted in certain proportions. When a component is oversaturated, stones are formed, the most common of which are cholesterol stones (1). CYP7A1, also known as cholesterol 7α-hydroxylase, is the key enzyme that determines the rate at which cholesterol is converted to bile acids. Thyroid hormone enhances the expression of CYP7A1 mRNA, which is essential for the role of thyroid hormone in regulating cholesterol levels (40, 41). In hypothyroidism, cholesterol levels are elevated, which ultimately leads to the formation of gallstones. In addition, in hypothyroidism, the number of LDL receptors in the liver decreases, resulting in the failure to remove some of the LDL, a decrease in bile flow and the development of sphincter of Oddi disfunction, which ultimately leads to the formation of gallstones as well (20). Hepatic secretion of bile salts, phosphatidylcholine and cholesterol is mainly determined by ATP-binding cassette (ABC) transporters on the apical membrane of hepatocytes (20, 42). Multiple receptors, namely the intrahepatocytic nuclear retinoid X receptor (RXR), and liver X receptor (LXR), have been discovered to govern the regulation of these ABC transporters. Hyperthyroidism is induced by up-regulation of the expression of the liver nuclear receptor genes LXRα and RXR expression induces cholesterol gallstone formation (20). Therefore, we believe that hypothyroidism and hyperthyroidism due to excessive iodine intake are the main causes of gallstone formation.

Subgroup analyses showed differences in urinary iodine levels and gallstone risk in specific populations. Obesity is an important risk factor for the development of gallstones, mainly because obesity leads to abnormalities including hepatic and dyslipidemia, which are manifested by excessive bile secretion by the liver, as well as the development of hyperlipidemia. In addition, obesity leads to insulin resistance, which has been shown to be associated with gallbladder stone formation (43, 44). Estrogen increases the risk of gallstone formation by stimulating the hepatic synthesis and secretion of cholesterol while blocking the synthesis of bile salts. In addition, the elderly and diabetes are also recognized as independent risk factors for gallstone formation (1, 8, 44, 45). This may explain the fact that the correlation between high urinary iodine levels and a high risk of gallstones is particularly pronounced in people aged 60 and older, women, had a BMI of 25 or more, and those diagnosed with diabetes mellitus.

Restricted cubic spline analysis showed that urinary iodine levels were nonlinearly positively correlated with the occurrence of gallstones (*P*-Nonlinear = 0.032). When the urinary iodine concentration was less than 203 ug/L, the curve of OR was steeper than the curve when the

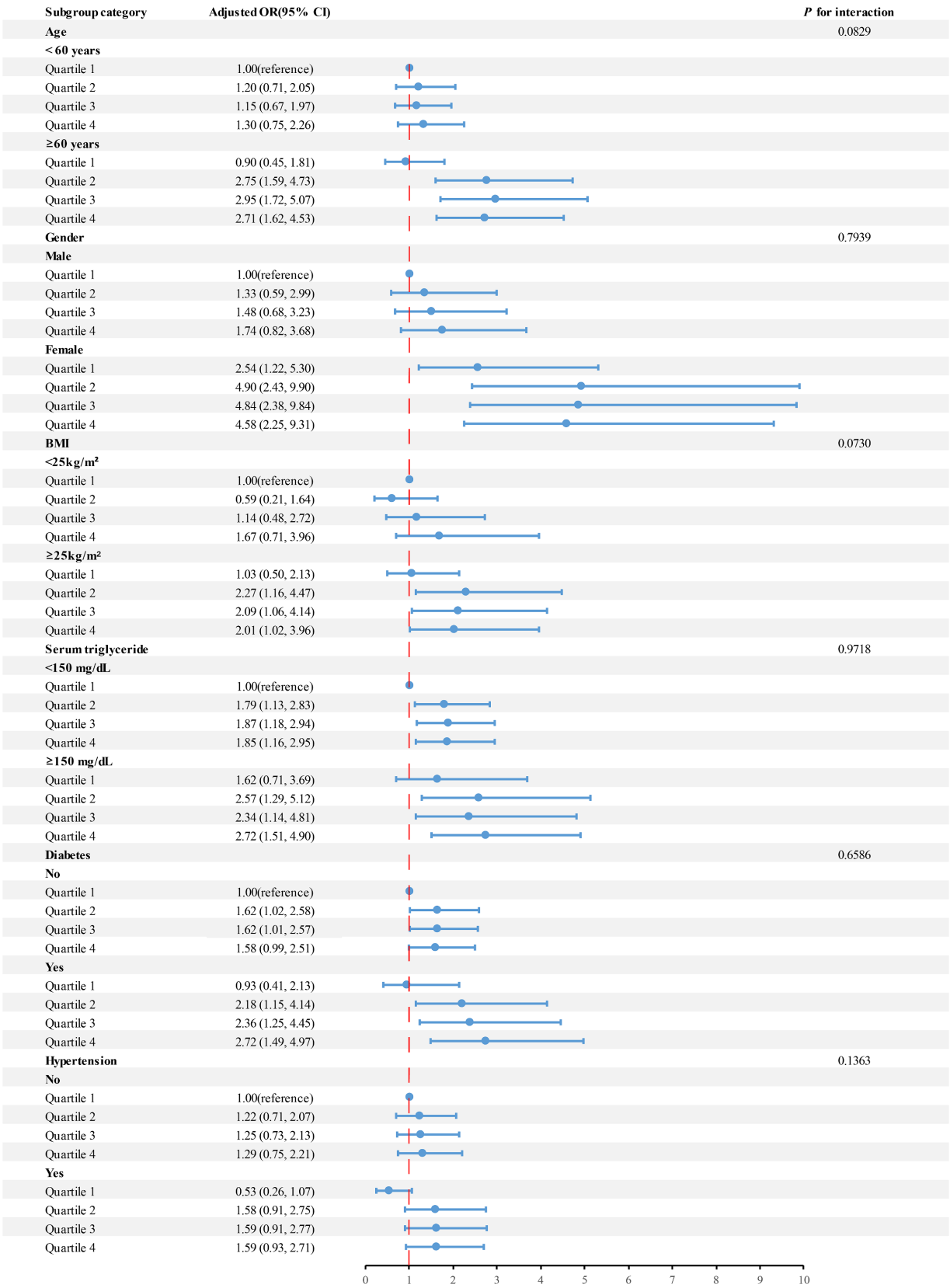

FIGURE 3
Analyses were performed on subgroups to explore if various factors such as age, gender, BMI, serum triglyceride levels, hypertension, and diabetes influenced the connection between urinary iodine levels and the risk of developing gallstones.

urinary iodine concentration was greater than 203 ug/L. Unfortunately we did not find a mechanism that causes this phenomenon, and the relevant mechanisms need to be further investigated.

The following are the strengths of this study. First off, the study offers valuable guidance for avoiding and decreasing the incidence of gallstones in Americans. Second, the study's data came from NHANES, which employed a standardized experimental testing technique and a nationally representative sample to successfully minimize study mistakes. This study has various restrictions. First, the diagnosis of gallstones was obtained through a self-report questionnaire, which may have led to inaccurate diagnoses and errors in participant recall. Second, iodine levels in urine were obtained from a single sample and were not sampled multiple times over a period of time, which does not reflect participants' long-term iodine intake levels. In addition, in this cross-sectional study, a large number of participants were not tested for iodine levels in urine and did not provide self-reports of gallstones, resulting in a reduced number of participants and reducing the validity of the experimental results.

To summarize, our research revealed a correlation between high levels of iodine in the urine and a heightened risk of developing gallstones. It is worth mentioning that this connection was especially noticeable in individuals aged 60 and above, females, individuals with a body mass index of 25 or greater, and those diagnosed with diabetes. Urinary iodine levels serve as indicators of the body's iodine status, thus suggesting that excessive iodine intake may be linked to an elevated risk of gallstone formation. There is no correlation between elevated urinary levels of nickel and molybdenum and a higher risk of gallstones. In the next step of our research, we will use cohort studies, case–control studies, and animal experiments to discover the causal relationship between urinary iodine levels and the occurrence of gallstones. These studies will deepen our understanding of iodine intake and the etiology of gallstone disease and help us to properly use iodine supplements and prevent gallstones.

Data availability statement

Publicly available datasets were analyzed in this study. This data can be found at: https://www.cdc.gov/nchs/nhanes/index.htm.

Ethics statement

The studies involving humans were approved by the Ethical Review Board of the National Center for Health Statistics. The studies were conducted in accordance with the local legislation and institutional requirements. The participants provided their written informed consent to participate in this study.

Author contributions

YL: Conceptualization, Investigation, Software, Writing – original draft, Writing – review & editing, Formal analysis, Methodology. MW: Data curation, Formal analysis, Investigation, Writing – original draft. WD: Conceptualization, Data curation, Methodology, Writing – original draft. LQ: Data curation, Formal analysis, Supervision, Writing – review & editing. XL: Data curation, Formal analysis, Writing – review & editing. XF: Conceptualization, Data curation, Formal analysis, Funding acquisition, Investigation, Project administration, Resources, Validation, Writing – original draft, Writing – review & editing.

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Conflict of interest

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

Publisher's note

References

1. Lammert F, Gurusamy K, Ko CW, Miquel JF, Méndez-Sánchez N, Portincasa P, et al. Gallstones. *Nat Rev Dis Primers*. (2016) 2:16024. doi: 10.1038/nrdp.2016.24

2. Luo X, Yang W, Joshi AD, Wu K, Simon TG, Yuan C, et al. Gallstones and risk of cancers of the liver, biliary tract and pancreas: a prospective study within two U.S. Cohorts. *Br J Cancer*. (2022) 127:1069–75. doi: 10.1038/s41416-022-01877-5

3. Stinton LM, Shaffer EA. Epidemiology of gallbladder disease: Cholelithiasis and Cancer. *Gut Liver*. (2012) 6:172–87. doi: 10.5009/gnl.2012.6.2.172

4. Shabanzadeh DM. Incidence of gallstone disease and complications. *Curr Opin Gastroenterol*. (2018) 34:81–9. doi: 10.1097/mog.0000000000000418

5. Innes K, Hudson J, Banister K, Croal B, Ramsay C, Ahmed I, et al. Core outcome set for symptomatic uncomplicated gallstone disease. *Br J Surg*. (2022) 109:539–44. doi: 10.1093/bjs/znac095

6. Erichsen R, Frøslev T, Lash TL, Pedersen L, Sørensen HT. Long-term statin use and the risk of gallstone disease: a population-based case-control study. *Am J Epidemiol*. (2011) 173:162–70. doi: 10.1093/aje/kwq361

7. Mulita F, Lampropoulos C, Kehagias D, Verras GI, Tchabashvili L, Kaplanis C, et al. Long-term nutritional deficiencies following sleeve gastrectomy: a 6-year single-Centre retrospective study. *Menopause Rev*. (2021) 20:170–6. doi: 10.5114/pm.2021.110954

8. Weikert C, Weikert S, Schulze MB, Pischon T, Fritsche A, Bergmann MM, et al. Presence of gallstones or kidney stones and risk of type 2 diabetes. *Am J Epidemiol*. (2010) 171:447–54. doi: 10.1093/aje/kwp411

9. Zhang Y, Sun L, Wang X, Chen Z. The association between hypertension and the risk of gallstone disease: a cross-sectional study. *BMC Gastroenterol*. (2022) 22:138. doi: 10.1186/s12876-022-02149-5

10. Wang J, Shen S, Wang B, Ni X, Liu H, Ni X, et al. Serum lipid levels are the risk factors of gallbladder stones: a population-based study in China. *Lipids Health Dis*. (2020) 19:50. doi: 10.1186/s12944-019-1184-3

11. Wang X, Yu W, Jiang G, Li H, Li S, Xie L, et al. Global epidemiology of gallstones in the 21st century: a systematic review and meta-analysis. *Clin Gastroenterol Hepatol*. (2024). doi: 10.1016/j.cgh.2024.01.051

12. Zoroddu MA, Aaseth J, Crisponi G, Medici S, Peana M, Nurchi VM. The essential metals for humans: a brief overview. *J Inorg Biochem*. (2019) 195:120–9. doi: 10.1016/j.jinorgbio.2019.03.013

13. Davies NT. An appraisal of the newer trace elements. *Philos Trans R Soc Lond Ser B Biol Sci*. (1981) 294:171–84. doi: 10.1098/rstb.1981.0097

14. Luo Y, Kawashima A, Ishido Y, Yoshihara A, Oda K, Hiroi N, et al. Iodine excess as an environmental risk factor for autoimmune thyroid disease. *Int J Mol Sci*. (2014) 15:12895–912. doi: 10.3390/ijms150712895

15. Huang F, Cong W, Xiao J, Zhou Y, Gong M, Sun J, et al. Association between excessive chronic iodine exposure and the occurrence of papillary thyroid carcinoma. *Oncol Lett*. (2020) 20:1. doi: 10.3892/ol.2020.12051

16. Huang H, Zhu J, Li Y, Zhang L, Gu J, Xie Q, et al. Upregulation of Sqstm1/P62 contributes to nickel-induced malignant transformation of human bronchial epithelial cells. *Autophagy*. (2016) 12:1687–703. doi: 10.1080/15548627.2016.1196313

17. Troost RJ, Kozel MM, van Helden-Meeuwsen CG, van Joost T, Mulder PG, Benner R, et al. Hyposensitization in nickel allergic contact dermatitis: clinical and immunologic monitoring. *J Am Acad Dermatol*. (1995) 32:576–83. doi: 10.1016/0190-9622(95)90340-2

18. Wang J, Sun YX, Xiang S, Yang C, Li XJ, Zhang MQ, et al. The association between blood heavy metals and gallstones: a cross-sectional study. *Sci Total Environ*. (2023) 904:166735. doi: 10.1016/j.scitotenv.2023.166735

19. Du W, Yan C, Wang Y, Song C, Li Y, Tian Z, et al. Association between dietary magnesium intake and gallstones: the mediating role of Atherogenic index of plasma. *Lipids Health Dis*. (2024) 23:82. doi: 10.1186/s12944-024-02074-4

20. Wang Y, Yu X, Zhao QZ, Zheng S, Qing WJ, Miao CD, et al. Thyroid dysfunction, either hyper or hypothyroidism, promotes gallstone formation by different mechanisms. *J Zhejiang Univ Sci B*. (2016) 17:515–25. doi: 10.1631/jzus.B1500210

21. Ravi PC, Thugu TR, Singh J, Dasireddy RR, Kumar SA, Isaac NV, et al. Gallstone disease and its correlation with thyroid disorders: a narrative review. *Cureus*. (2023) 15:e45116. doi: 10.7759/cureus.45116

22. Poon CM, Chan DP, Lee SS, Wong NS. Seroepidemiology of hepatitis a and B in the general population in Hong Kong: protocol of a cross-sectional survey using spatial sampling in a highly Urbanised City. *BMJ Open*. (2021) 11:e042065. doi: 10.1136/bmjopen-2020-042065

23. Lee MH, Gao YT, Huang YH, McGee EE, Lam T, Wang B, et al. A Metallomic approach to assess associations of serum metal levels with gallstones and gallbladder Cancer. *Hepatology*. (2020) 71:917–28. doi: 10.1002/hep.30861

24. Andersson M, Braegger CP. The role of iodine for thyroid function in lactating women and infants. *Endocr Rev*. (2022) 43:469–506. doi: 10.1210/endrev/bnab029

25. Perry WF, Hughes JF. The urinary excretion and thyroid uptake of iodine in renal disease. *J Clin Invest*. (1952) 31:457–63. doi: 10.1172/jci102630

26. Vejbjerg P, Knudsen N, Perrild H, Laurberg P, Andersen S, Rasmussen LB, et al. Estimation of iodine intake from various urinary iodine measurements in population studies. *Thyroid*. (2009) 19:1281–6. doi: 10.1089/thy.2009.0094

27. Peniamina R, Skeaff S, Haszard JJ, McLean R. Comparison of 24-H diet records, 24-H urine, and duplicate diets for estimating dietary intakes of potassium, sodium, and iodine in children. *Nutrients*. (2019) 11:2927. doi: 10.3390/nu11122927

28. Zimmermann MB, Jooste PL, Pandav CS. Iodine-deficiency disorders. *Lancet*. (2008) 372:1251–62. doi: 10.1016/s0140-6736(08)61005-3

29. Delange F. The disorders induced by iodine deficiency. *Thyroid*. (1994) 4:107–28. doi: 10.1089/thy.1994.4.107

30. Pearce EN, Andersson M, Zimmermann MB. Global iodine nutrition: where do we stand in 2013? *Thyroid*. (2013) 23:523–8. doi: 10.1089/thy.2013.0128

31. Zimmermann MB. Iodine deficiency and excess in children: worldwide status in 2013. *Endocr Pract*. (2013) 19:839–46. doi: 10.4158/ep13180.Ra

32. Zava TT, Zava DT. Assessment of Japanese iodine intake based on seaweed consumption in Japan: a literature-based analysis. *Thyroid Res*. (2011) 4:14. doi: 10.1186/1756-6614-4-14

33. Georgitis WJ, McDermott MT, Kidd GS. An iodine load from water-purification tablets alters thyroid function in humans. *Mil Med*. (1993) 158:794–7. doi: 10.1093/milmed/158.12.794

34. Minelli R, Gardini E, Bianconi L, Salvi M, Roti E. Subclinical hypothyroidism, overt thyrotoxicosis and subclinical hypothyroidism: the subsequent phases of thyroid function in a patient chronically treated with amiodarone. *J Endocrinol Investig*. (1992) 15:853–5. doi: 10.1007/bf03348819

35. Padovani RP, Kasamatsu TS, Nakabashi CC, Camacho CP, Andreoni DM, Malouf EZ, et al. One month is sufficient for urinary iodine to return to its baseline value after the use of water-soluble iodinated contrast agents in post-thyroidectomy patients requiring radioiodine therapy. *Thyroid*. (2012) 22:926–30. doi: 10.1089/thy.2012.0099

36. Kube I, Tardio LB, Hofmann U, Ghallab A, Hengstler JG, Führer D, et al. Hypothyroidism increases cholesterol gallstone prevalence in mice by elevated hydrophobicity of primary bile acids. *Thyroid*. (2021) 31:973–84. doi: 10.1089/thy.2020.0636

37. Nakano S, Suzuki M, Haruna H, Yamataka A, Shimizu T. Gallstone formation due to rapid weight loss through hyperthyroidism. *J Pediatr Endocrinol Metab*. (2019) 32:1395–8. doi: 10.1515/jpem-2019-0149

38. Liu J, Liu L, Jia Q, Zhang X, Jin X, Shen H. Effects of excessive iodine intake on blood glucose, blood pressure, and blood lipids in adults. *Biol Trace Elem Res*. (2019) 192:136–44. doi: 10.1007/s12011-019-01668-9

39. Shen X, Yang L, Liu YY, Zhang XH, Cai P, Huang JF, et al. Associations between urinary iodine concentration and the prevalence of metabolic disorders: a cross-sectional study. *Front Endocrinol*. (2023) 14:1153462. doi: 10.3389/fendo.2023.1153462

40. Ness GC, Pendelton LC, Zhao Z. Thyroid hormone rapidly increases cholesterol 7 alpha-hydroxylase MRNA levels in Hypophysectomized rats. *Biochim Biophys Acta*. (1994) 1214:229–33. doi: 10.1016/0005-2760(94)90068-x

41. Crestani M, Karam WG, Chiang JY. Effects of bile acids and steroid/thyroid hormones on the expression of cholesterol 7 alpha-hydroxylase Mrna and the Cyp7 gene in Hepg2 cells. *Biochem Biophys Res Commun*. (1994) 198:546–53. doi: 10.1006/bbrc.1994.1080

42. Morita SY, Tsuda T, Horikami M, Teraoka R, Kitagawa S, Terada T. Bile salt-stimulated phospholipid efflux mediated by Abcb 4 localized in nonraft membranes. *J Lipid Res*. (2013) 54:1221–30. doi: 10.1194/jlr.M032425

43. Cruz-Monserrate Z, Conwell DL, Krishna SG. The impact of obesity on gallstone disease, acute pancreatitis, and pancreatic Cancer. *Gastroenterol Clin N Am*. (2016) 45:625–37. doi: 10.1016/j.gtc.2016.07.010

44. Nervi F, Miquel JF, Alvarez M, Ferreccio C, García-Zattera MJ, González R, et al. Gallbladder disease is associated with insulin resistance in a high risk Hispanic population. *J Hepatol*. (2006) 45:299–305. doi: 10.1016/j.jhep.2006.01.026

45. de Bari O, Wang TY, Liu M, Portincasa P, Wang DQ. Estrogen induces two distinct cholesterol crystallization pathways by activating Erα and Gpr30 in female mice. *J Lipid Res*. (2015) 56:1691–700. doi: 10.1194/jlr.M059121

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EDITED BY
Misha Vrolijk,
Maastricht University, Netherlands

REVIEWED BY
Zeng Shan,
People's Hospital of Xinjiang Uygur Autonomous Region, China
Aishanjiang Yusufujiang,
Xinjiang Medical University, China

*CORRESPONDENCE
Gwi Yeong Jang
janggy@korea.kr
Hyung Don Kim
radioleg@korea.kr

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Platycodon grandiflorum exhibits anti-neuroinflammatory potential against beta-amyloid-induced toxicity in microglia cells

Yun-Jeong Ji[1], Min Hye Kang[1,2], Geum-Soog Kim[1], Hyung Don Kim[1,2]* and Gwi Yeong Jang[1]*

[1]Department of Herbal Crop Research, National Institute of Horticultural Herbal Science, Rural Development Administration, Eumseong, Republic of Korea, [2]Department of Biochemistry, School of Life Sciences, Chungbuk National University, Cheongju, Republic of Korea

Background/objectives: *Platycodon grandiflorum* (PG) is used in traditional oriental medicine to treat several ailments.

Methods: The study investigated the anti-inflammatory and neuroprotective effects of PGW (*P. grandiflorum*) extract in Aβ25-35-induced inflammation in BV2 microglia cells.

Result: PGW demonstrated significant inhibition of nitric oxide (NO) production, with reductions of 30.4, 36.7, and 61.2% at concentrations of 50, 100, and 200 μg/mL, respectively. Moreover, PGW effectively suppressed the production of pro-inflammatory cytokines IL-1β and IL-6 and exhibited significant inhibitory activity against TNF-α at 200 μg/mL. Furthermore, PGW treatment mitigated apoptosis in Aβ-induced BV2 cells by modulating the mitochondrial apoptosis pathway, regulating Bcl-2 family protein synthesis, and inhibiting caspase activation. Mechanistically, PGW attenuated the activation of the MAPK (JNK, ERK, p38) pathway induced by Aβ, showing a concentration-dependent decrease in phosphorylation levels of these proteins. Additionally, PGW inhibited the NF-κB pathway activation by reducing the phosphorylation levels of p65 and IκBα in a concentration-dependent manner.

Conclusion: PGW demonstrated anti-inflammatory and neuroprotective effects in Aβ-induced neuronal cells, suggesting its potential as a therapeutic agent for neuroinflammatory associated with neurodegenerative diseases.

KEYWORDS

***Platycodon grandiflorum*, amyloid beta, microglia cells, neuro-inflammatory, NF-κB pathway**

1 Introduction

Platycodon grandiflorum (PG) is a medicinal plant with a long history in Korea, China, and Japan. It has been used for various traditional medical purposes (1). PG root is particularly effective in treating bronchial diseases, coughs, colds, and indigestion. These traditional effects are attributed to the plant's triterpenoid saponins, carbohydrates, and fiber, especially saponins and platycodins (2). PG can lower blood sugar (3), improves cholesterol metabolism (4), exerts anti-obesity (5), anti-inflammatory (6, 7), immunostimulatory (2), and antioxidant effects (8),

and ameliorates atopic dermatitis (9). Based on traditional efficacy and modern scientific research results, bellflower root extract's beneficial effects have been proven in the peripheral nervous system and the brain. PG extract showed a protective effect against Aβ25-35-induced neurotoxicity, which prevents neuronal cell death by inhibiting the production of reactive oxygen species. Based on this accumulated evidence, many studies have recently reported the efficacy of bellflower root extract in Alzheimer's disease. Our previous study reported that PGE reduces oxidative stress and inhibits Aβ deposition in the hippocampus of AD animal models. Additionally, saponin, an essential component of PGE, not only alleviated oxidative stress and prevented neuronal cell death in Aβ-treated hippocampal HT22 cells but also prevented AD-related pathologies such as Aβ deposition, oxidative damage, neuroinflammation, and neurodegeneration in AD animal models (10). Although PG has shown beneficial effects in AD, the mechanisms underlying PGE's effects in the brain remain unclear, particularly with regard to its effects on oxidative stress, neuroinflammation, and Aβ deposition.

Many studies have been conducted to elucidate the relationship between Aβ and neuroinflammation in the pathogenesis of Alzheimer's disease (11). Because inflammatory mechanisms are highly interactive and rarely occur in isolation, anti-inflammatory effects may alleviate various diseases or symptoms resulting from Alzheimer's disease (12). Although the mechanisms by which Aβ triggers inflammatory processes are complex, there is evidence that this peptide induces activation of the transcription factor NF-κB. In response to inflammatory stimuli, IκB kinase (IKK) phosphorylates IκB inhibitors and free NF-κB translocates to the nucleus and binds to κB binding sites in the promoter regions of target genes. This induces the transcription of proinflammatory mediators such as inducible nitric oxide synthase (iNOS), cyclooxygenase-2 (COX-2), tumor necrosis factor-α (TNF-α), and interleukin-1β (IL-1β) (13). Also, mitogen-activated protein kinases (MAPKs) regulate the production of key inflammatory mediators. Upon exposure to Aβ stimulation, c-Jun N-terminal kinase (JNK), extracellular signalregulated kinase (ERK), and p38 MAPK are activated by phosphorylation, which regulates the activation of the NF-κB signaling pathway (14). These findings suggest that Aβ-induced neuroinflammation is associated with activating NF-κB and MAPK signaling pathways, leading to the expression of inflammatory mediators and potentially contributing to AD pathogenesis (15, 16). As a result, increased Aβ accumulation stimulates the aging of neurons and microglia, which promotes neuroinflammation and neurodegeneration, resulting in a vicious cycle of Alzheimer's disease.

Most studies mainly focus on the neuroprotective effects of PG extracts on neurons, and little is known about the mechanisms by which PG extracts protect glial cells from Aβ-induced neurotoxicity. In this study, to investigate the anti-inflammatory and neuroprotective effects of PGW on BV2 microglial cells, oxidative stress, and inflammation were induced by treating BV2 microglial cells with Aβ25-35. We investigated whether PGW could inhibit ROS and nitric oxide (NO) production in BV2 microglia under oxidative stress. Specifically, we evaluated PGW's nitric oxide (NO) inhibitory effect in BV2 microglial cells treated with Aβ25-35. Additionally, we determined the impact of PGW on Aβ-induced ROS generation and toxicity in Aβ25-35-treated BV2 microglia. Next, we investigated the effect of PGE, which showed a nitric oxide (NO) inhibitory effect, on proinflammatory cytokine production in Aβ-treated BV2 microglial cells. Finally, we sought to demonstrate how PGW alleviates neuroinflammation and exerts neuroprotective effects by activating NF-κB and MAPK signaling pathways in Aβ-induced neurons.

2 Materials and methods

2.1 Sample preparation and extraction

The'Etteumbeak' PG used in this study was harvested in Boeun, Chungcheongbuk-do in 2022. 'Etteumbeak' PG has been altered from diploid to tetraploid by treating native PG with colchicine. 'Etteumbeak' PG grows rapidly and can be cultivated for 1–2 years, and its productivity is ~40% higher than native PG. Fifty liters of water (sample: solvent, 1:10) per 5 kg of PG sample were added, and hot-water extraction was performed at 90°C for 6 h. The extract was filtered and lyophilized to prepare a dry base. The extraction yield was 34%, the sample (PGW) was dissolved in dimethylsulfoxide (DMSO; Sigma-Aldrich, St. Louis, MO, United States) and stored at −80°C until needed.

2.2 Analysis of saponins from PGW by HPLC-UVD

After dissolving PG extract in 40 mL of distilled water, it was degreased with diethyl ether using a separatory funnel. Afterward, the separated aqueous layer was extracted three times using n-butanol saturated with water. The n-butanol layer was evaporated at 50°C to obtain a residue, which was dissolved in methanol and used for analysis. Saponin standards (Platycoside G1, Platycoside E, Deapio-platycodin D3, Platycodin D3, Deapio-platycodin D, Platyconic acid A, Platycodin D2, Platycodin D, Polygalacin D, Platycoside A, Platycodin A, Platycodigenin) were obtained from Chengdu Biopurify Phytochemicals Ltd. (Chengdu, Sichuan, China). HPLC-grade water and acetonitrile (ACN) were purchased from J.T. Baker (Phillipsburg, NJ, United States). All other chemicals were of reagent grade. Methanol (1 mL) was added to the PGW powder 100 mg, and the mixture was vortexed and filtered through a membrane filter (0.45 μm) for using an analytical sample. Saponins were analyzed by high-performance liquid chromatography (HPLC; 1,200 Series, Agilent Technologies, Santa Clara, CA, United States). Under separation conditions, the mobile phase consisted of 0.1% formic acid in water (solvent A) and 0.1% formic acid in ACN (solvent B), with gradient elution: 0–5 min, isocratic 10% A; 5–19 min, 10–22% B; 19–37 min, 22–28% B; 37–54 min, 28–35% B; 54–59 min, 35–60% B; 59–60 min, and 60–95% B. A Triart C18 column (100 × 4.6 mm, 3 μm i.d.; YMC Co., Kyoto, Japan) was used, with a temperature of 30°C and flow rate of 1 mL/min. The diode-array detection detector wavelength was 203 nm. The sample injection volume was 30 μL, and the column temperature was maintained at 40°C. The ELSD conditions were set to an atomizer temperature of 42°C, drift tube temperature of 85°C, and N2 gas pressure of 50 psi for the analysis.

2.3 Cell culture

Mouse brain-derived BV2 microglial cells were cultured in Dulbecco's modified Eagle's medium (DMEM; Gibco, Canada) containing 1%

penicillin/streptomycin and 10% fetal bovine serum (FBS) at 37°C in an atmosphere containing 5% CO2. Subcultures were performed every 2 days and cells at passages 5–10 were used in experiments.

2.4 Cell viability assay

BV-2 cells are a cell line that replaces microglia. They will reflect the characteristics of microglia and are suitable for evaluating the role of microglia in eliminating Aβ toxic accumulation in the early stages of Alzheimer's disease (17). In support, we assessed neuroinflammation in response to Aβ-induced toxicity in BV-2 cells (microglia). To investigate the effect of PGW on Aβ25-35-induced cytotoxicity, BV2 microglial cells were treated with Aβ25-35, alone (10 mM; Sigma-Aldrich) or with PGW for 24 h (alone) or 1 h (with PGW), followed by Aβ for 24 h. Next, the cells were treated with 3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium(MTS; Promega, Madison, WI, United States) and the change in absorbance at 490 nm was measured using a multiplate reader (Biotek).

2.5 Measurement of NO production

To assay NO production, BV2 microglia were transferred to 96-well plates at 1.0×10^4/well and, 24 h later, were pretreated with PGW extract (0–200 μg/mL) for 1 h. Following 18 h of treatment with 10 μM/mL Aβ, equal volumes of culture medium and Griess reagent (Promega) were added to the 96-well plate, followed by for 10 min. Absorbance at 540 nm was measured using a multiplate reader.

2.6 Enzyme-linked immunosorbent assay

BV2 microglial cells (1.0×10^5 cells/mL) were distributed on 60 mm plates, cultured for 24 h, and then pretreated with PGW (50,100 and 200 μg/mL). After 1 h, cells were treated with ab (10 μg/mL) and cultured for 24 h. Afterward, the culture medium was centrifuged at 10,000 rpm for 3 min to remove precipitates, and the supernatant was recovered. The levels of TNF-α, IL-6, and IL-1β in the recovered supernatant were measured using a mouse enzyme-linked immunosorbent assay (ELISA) kit (R&D Systems Inc., Minneapolis, MN, United States). Next, the membrane was blocked with 2% BCS for 1 h and washed three times with TBST solution, once every 10 min.

2.7 Preparation of cell lysates and western blotting

BV2 cells were seeded on a plate at 1.0 × 104/well, incubated for 24 h, treated with PGW at 50, 100, and 200 μg/mL for 1 h, and treated with Aβ25-35 at 10 μM for 48 h. Next, sample was washed twice with phosphate-buffered saline (PBS), dissolved in radioimmunoprecipitation assay (RIPA) buffer (Cell Signaling Technology, Danvers, MA, United States), and reacted on ice for 30 min. The cell lysis solvent was centrifuged at 4°C and 13,000 rpm for 20 min, and the protein concentration in the supernatant was quantified using Bradford's reagent (Bio-Rad, Hercules, CA, United States). The cell lysis solvent was mixed with 4× Laemmli buffer (iNtRON, Seongnam, South Korea) and heated at 95°C for 5 min, and samples corresponding to 10 μg of protein were separated by 10% SDS-PAGE. The proteins were transferred to a polyvinylidene difluoride membrane (PVDF; Millipore, Darmstadt, Germany) and blocked with 2% bovine serum albumin in TBST at room temperature for 30 min. Primary antibodies against tyrosinase (TRP-1 and TRP-2; Cell Signaling Technology) were diluted 1:1,000, added to the membrane, and incubated overnight at 4%. Subsequently, the membrane was washed thrice with TBST for 10 min each, and the secondary antibody (Cell Signaling Technology; 1:2,000), was added over 1 h. The membrane was washed three times with TBST for 10 min each. Proteins were detected using the Enhanced Chemiluminescence Western Blotting Detection Kit (Bio-Rad) and the ChemiDoc Imaging System (Bio-Rad).

2.8 Statistical analysis

All analyses were randomly conducted in a blinded manner. Statistical analyses were conducted using GraphPad Prism 7.0 software (GraphPad Software, La Jolla, CA, United States). Data are presented with error bars representing standard deviations (SD). Nonlinear regression analysis was performed to derive curves showing the relationship between each group. The significance of differences between groups was evaluated using an independent t-test and one-way ANOVA, followed by Tukey's *post-hoc* test or Fisher's LSD test for pairwise comparisons. Effect sizes were included to measure the magnitude of observed differences to provide a more comprehensive statistical analysis. For t-tests, Cohen's d was calculated, and for ANOVA, eta-squared (η^2) values were reported. Additionally, 95% confidence intervals (CIs) were provided for mean differences and effect sizes to assess the recision and reliability of the estimates. Statistical significance was considered at $p < 0.05$.

3 Results

3.1 HPLC analysis of *Platycodon grandiflorum* water extract

Chromatograms of PG saponin standards and PGW are depicted in Table 1 and Figure 1. By comparing retention times (RT) with 12 saponin standards, including major saponins such as platycoside E and platycodin D (commonly known as PG saponins), eight saponins were identified in PGW. The elution RT confirmed the presence of platycoside G1 (deapi-platycoside E), platycoside E, platycodin D3, deapio-platycodin D3, platyconic acid A, platycodin D2, platycodin D2, platycodin D, and polygalacin D. The contents of these saponins were measured at 292.56, 801.72, 270.99, 84.45, 194.65, 326.03, 381.77, and 337.76 μg/g, respectively, with Platycoside E exhibiting the highest concentration.

3.2 Cytotoxicity of PGW extract

To assess its impact on BV2 microglial cells, the cell culture medium was treated with PGW extract at 0, 50, 100, and 200 μg/mL concentrations, followed by a MTS assay. No cytotoxic effects were observed across all concentrations of PGW extract (Figure 2A). Therefore, 200 μg/mL PGW extract was selected for subsequent

experiments. In the next phase, BV2 cells were exposed to PGW at 50, 100, and 200 μM/mL concentrations for 1 h before being subjected to 10 μM of Aβ for 24 h. MTS assays revealed a reduction in cytotoxicity to approximately 46% following incubation with 10 μM amyloid beta for 24 h (Figure 2B). However, adding PGW effectively mitigated the cytotoxic effects induced by Aβ, indicating that PGW suppresses Aβ-induced cytotoxicity.

TABLE 1 Saponin composition of PGW.

No.	RT (min)	Compounds	Content (μg/g extract, d.b.)
1	22.37	Platycoside G1	292.56 ± 14.26
2	23.05	Platycoside E	801.72 ± 29.32
3	25.91	Deapio-platycodin D3	N.D.
4	26.82	Platycodin D3	270.99 ± 13.04
5	32.46	Deapio-platycodin D	84.45 ± 6.44
6	33.17	Platyconic acid A	194.65 ± 9.57
7	33.29	Platycodin D2	326.03 ± 23.28
8	33.63	Platycodin D	381.77 ± 13.68
9	34.81	Polygalacin D	337.76 ± 17.46
10	37.98	Platycoside A	N.D.
11	47.75	Platycodin A	N.D.
12	50.97	Platycodigenin	N.D.

Data are expressed as mean ± standard deviation of triplicate samples. N.D., not detected.

3.3 Inhibition of Aβ-induced ROS generation by PGW

ROS synthesis induced by Aβ in microglia is implicated in oxidative neuronal damage and neurodegeneration, contributing to the onset of neurological diseases. Hence, we explored whether the anti-inflammatory effect of PGW was attributable to decreased ROS production. As anticipated, treatment with 10 μM Aβ markedly increased ROS synthesis (Figure 3). However, pretreatment with PGW significantly attenuated ROS levels in a dose-dependent manner, reducing them to 30.4, 42.0, and 44.2%. This suggests that PGW exerts its anti-inflammatory action by suppressing ROS production in BV2 cells.

3.4 Inhibition of nitric oxide production by PGW extract

Nitric oxide (NO) radical, known for its inflammatory properties, contributes to oxidative neuronal damage by reacting

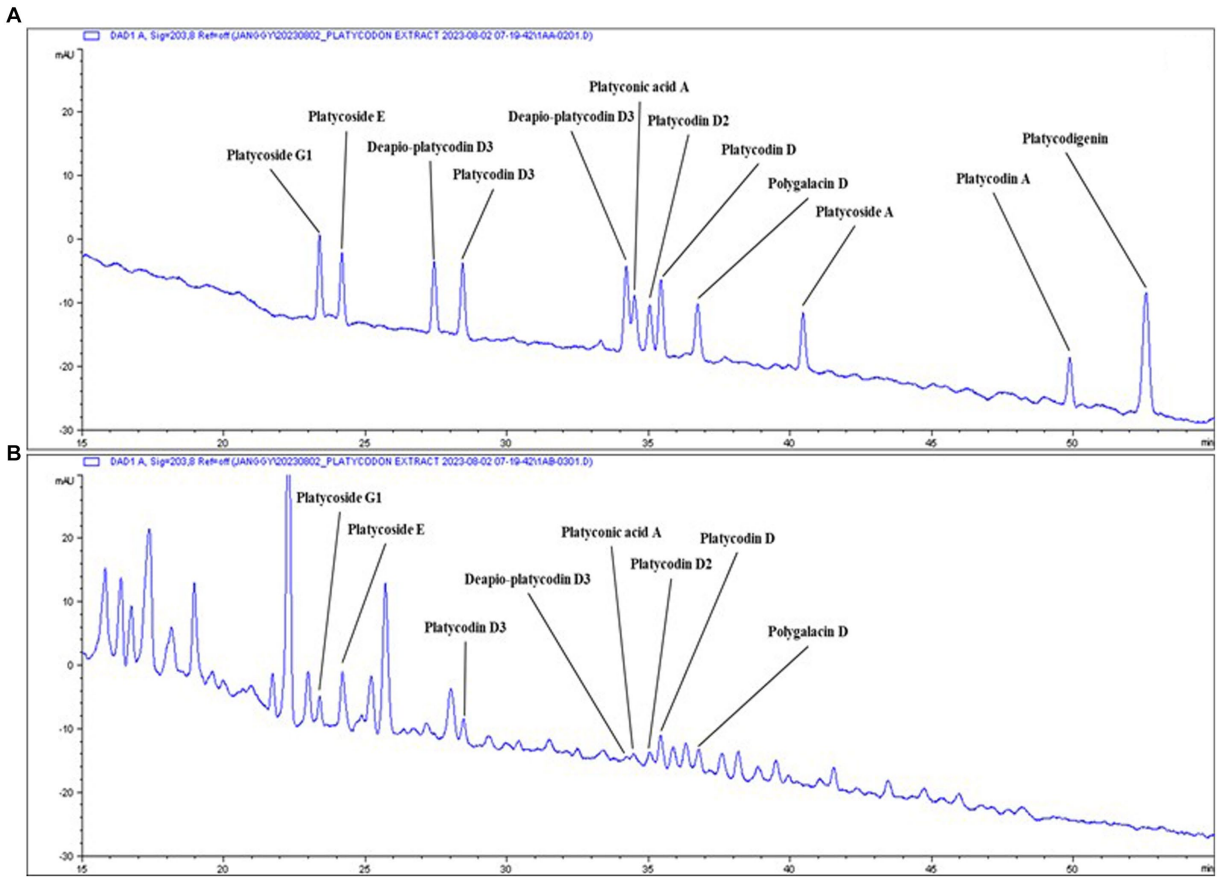

FIGURE 1
Typical chromatogram of PGW. Samples: **(A)** Saponin standards, **(B)** *Platycodon grandiflorum* water extract (PGW).

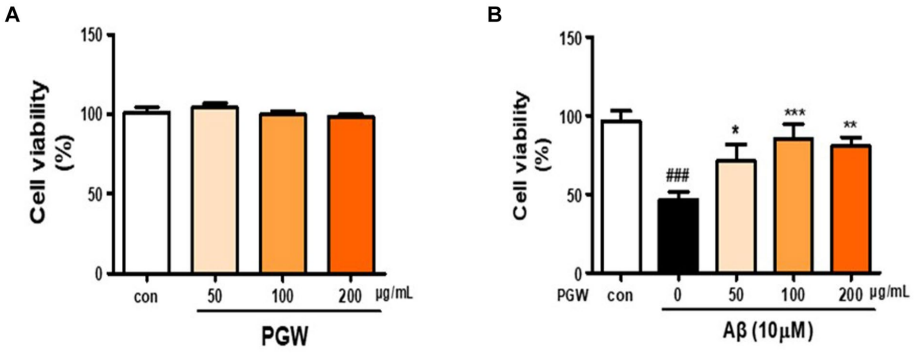

FIGURE 2
Inhibitory effect of PGW on Aβ25-35-induced oxidative stress in BV2 cells. **(A)** BV2 cells were treated with PGW (50, 100, and 200 μg/mL) for 24 h. Control groups were treated with the same volume of conditioned medium (0.2% DMSO). **(B)** BV2 cells were treated with PGW (50, 100, and 200 μg/mL) and incubated with 10 μM Aβ for 24 h Significance was determined by one-way ANOVA. *Post-hoc* analysis was performed using Tukey's multiple comparison test; ###$p < 0.001$ compared with the control (white bar). *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$ compared with Aβ treatment (black bar).

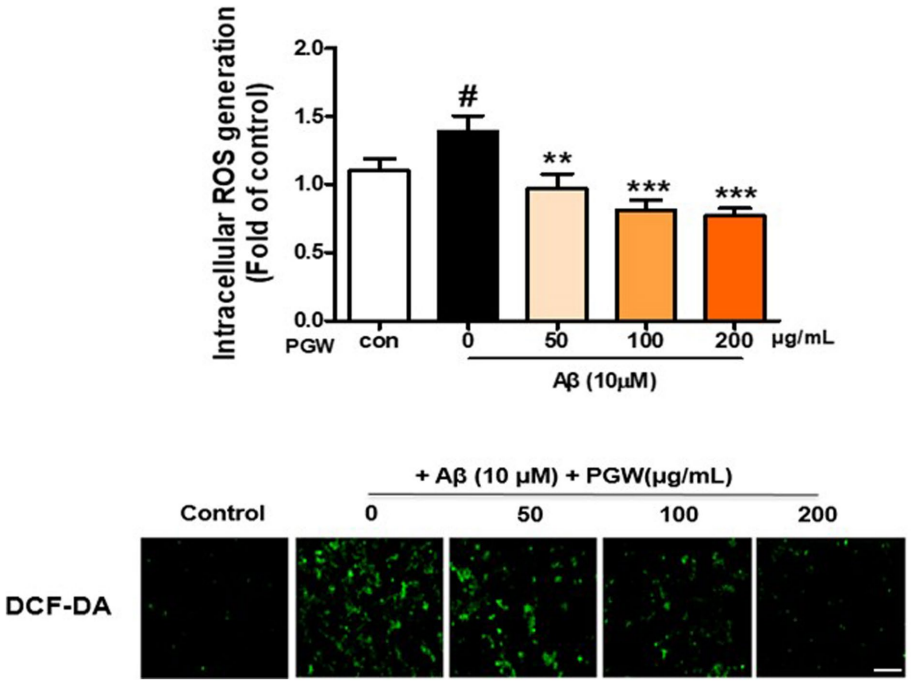

FIGURE 3
Inhibition of Aβ25-35-induced oxidative stress in BV2 cells by PGW. Cells were treated with PGW (50, 100, and 200 μg/mL) for 24 h and stimulated with Aβ25-35 (10 μM) for 30 min. Control groups were treated with the same volume of conditioned medium (0.2% DMSO). ROS generation in BV2 cells was visualized by confocal fluorescence microscopy. Absorbance was measured after DCF-DA staining of cells treated with Aβ25-35 alone, PGW (50, 100, 200 μg/mL) alone, or Aβ25-35 plus PGW. Scale bar = 50 μm. Significance was determined by one-way ANOVA. *Post-hoc* analysis was performed using Tukey's multiple comparison test; #$p < 0.05$ compared with the control (white bar); **$p < 0.01$, ***$p < 0.001$ compared with Aβ25-35 treatment (black bar).

with oxygen to form peroxynitrite (NO3-). Thus, a considerable amount of NO, a pro-oxidant molecule that exhibits potent cytotoxicity, is generated. To validate the anti-inflammatory potential of PGW, we assessed NO production. Treatment with PGW at concentrations of 50, 100, and 200 μg/mL significantly reduced NO production by 30.4, 36.7, and 61.2%, respectively (Figure 4).

3.5 Inhibits production of pro-inflammatory cytokines (TNF-α, IL-6, IL-1β)

Microglial activation by Aβ leads to the secretion of various inflammatory cytokines, contributing to neuronal damage and cell death. In our experiment, we investigated the effect of PGW on the

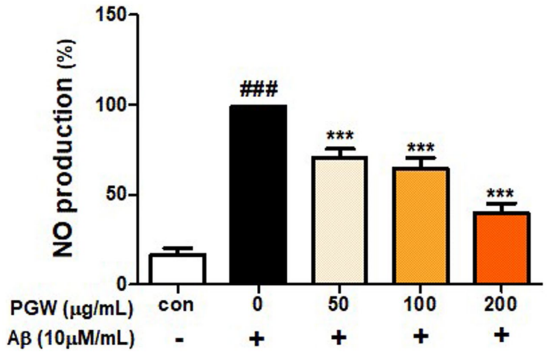

FIGURE 4
Inhibitory effects of PGW on nitric oxide production in BV2 microglia cells. The production of nitric oxide was assayed in the culture medium of cells stimulated with Aβ (10 μM) for 24 h in the presence of PGW (50, 100, and 200 μg/mL). Control groups were treated with the same volume of conditioned medium (0.2% DMSO). The result is expressed as percentages compared to the respective values obtained for the control. Data represent the means±SD with three separate experiments. Significance was determined by one-way ANOVA. *Post-hoc* analysis was performed using Tukey's multiple comparison tests; ###$p < 0.001$ compared with the control (white bar); ***$p < 0.001$ compared with Aβ treatment (black bar).

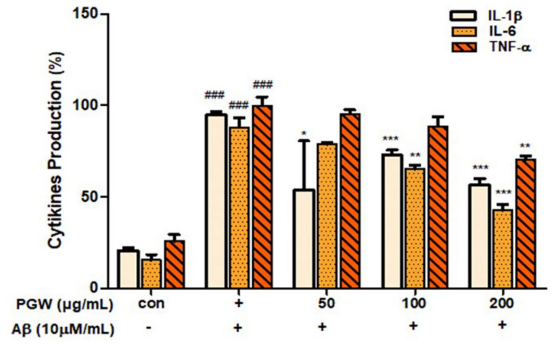

FIGURE 5
Inhibitory effects of PGW on producing pro-inflammatory cytokines in BV2 microglia cells. The production of IL-1β, IL-6, and TNF-α was assayed in the culture medium of cells stimulated with Aβ (10μM) for 24h in the presence of PGW (50, 100, and 200μg/mL). Control groups were treated with the same volume of conditioned medium (0.2% DMSO). Results are expressed as a percentage of the control. Data represent the means± SD with three separate experiments. Significance was determined by one-way ANOVA. *Post-hoc* analysis was performed using Tukey's multiple comparison tests; ###$p<0.001$ compared with the control (white bar); *$p<0.05$, **$p<0.01$, ***$p<0.001$ compared with Aβ treatment (black bar).

expression of cytokines IL-1β, IL-6, and TNF-α in BV2 cells induced by Aβ using an ELISA kit. Treatment with PGW at concentrations of 50, 100, and 200 μg/mL significantly suppressed the production of IL-1β by 20, 28, and 44%, respectively, and IL-6 production was significantly inhibited by 22, 35, and 58%. While TNF-α did not exhibit decrease, significant inhibitory activity was observed at 200 μg/mL (Figure 5).

3.6 Effect of PGW on apoptosis pathway-related factors in Aβ25-35-induced BV2 cells

We investigated whether Aβ treatment induces apoptosis in BV2 cells. As depicted in Figure 5, cells treated with Aβ exhibited a significant increase in apoptotic cells compared to control cells. Bax levels were elevated, and Bcl-2 and Bcl-xL levels were decreased in BV2 cells treated solely with Aβ. Caspase-3 activation was highest in the group administered Aβ alone (Figures 6A,B). Co-treatment of BV2 cells with Aβ and PGW significantly mitigated apoptosis compared to treatment with Aβ alone. PGW pretreatment restored Bcl-2 family protein levels similar to controls (Figure 6A). Additionally, PGW significantly inhibited caspase-3 activation in a dose-dependent manner, leading to cytochrome C release and reduced expression of caspase-9 and caspase-3 (Figure 6B). Therefore, PGW mediated inhibition of Aβ-induced apoptosis exerts neuroprotective effects by suppressing the mitochondrial apoptosis pathway and regulating Bcl-2 synthesis.

3.7 Effect of PGW on MAPK (JNK, ERK, p38) pathway activation in BV2 cells

To elucidate the link between PGW's anti-inflammatory activity and the MAPK pathway, we analyzed the phosphorylation of MAPK pathway proteins. Our findings demonstrated that Aβ significantly increased the phosphorylation levels of p38, JNK, and ERK in a concentration-dependent manner. PGW treatment mitigated this increase in phosphorylation concentration independently (Figure 7). Specifically, PGW significantly decreased p38 phosphorylation at a concentration of 200 μg/mL (Figure 7). Moreover, PGW reduced JNK phosphorylation in a concentration dependent manner and ERK phosphorylation starting from the 100 μg/mL treatment group. These results suggest that PGW attenuates the inflammatory response by inhibiting the MAPK pathway in Aβ-induced BV2 microglia cells.

3.8 Effect of PGW on NF-κB pathway activation in BV2 cells

We investigated the association between PGW's anti-inflammatory activity and the NF-κB pathway by analyzing the expression and phosphorylation of NF-κB pathway proteins. Aβ treatment increased the phosphorylation levels of p65 and IκBα, proteins of the NF-κB pathway, whereas PGW treatment decreased their phosphorylation levels in a concentration-dependent manner (Figure 8A). Specifically, p65 phosphorylation decreased concentration independently, with a significant decrease observed at 200 μg/mL. Similarly, IκBα phosphorylation decreased concentration-dependently, reaching levels higher than those of the vehicle-treated group at 200 μg/mL (Figure 8B). These findings suggest that PGW suppresses the expression of pro-inflammatory genes by inhibiting the NF-κB pathway.

3.9 Dose–response curves of PGW on protein expression

This graph shows the results of analyzing the effect of PGW on the expression of various proteins by concentration. The x-axis of

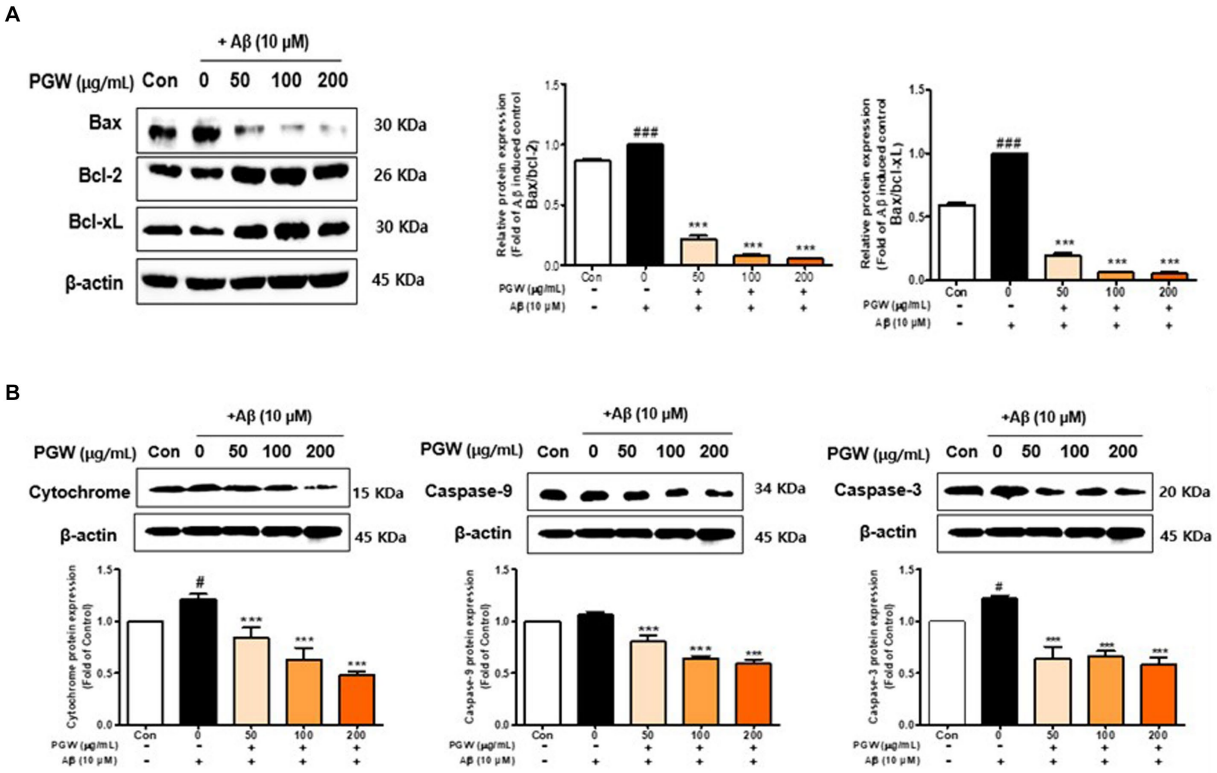

FIGURE 6
Effect of PGW on Aβ-induced Bcl-2 expression in BV2 cells: PGW protects BV2 cells from Aβ-induced oxidative apoptosis. Cells were treated with PGW (50, 100, and 200 μg/mL) for 24 h, followed by Aβ (10 μM) for 24 h. Control groups were treated with the same volume of conditioned medium (0.2% DMSO). Western blotting was performed with β-actin as the loading control. **(A)** Bax/Bcl-xL, Bax/Bcl-2, **(B)** cytochrome C, caspase-9, and caspase-3 levels in mitochondria. Values are means ± SD ($n = 3$). Significance was determined by one-way ANOVA. *Post-hoc* analysis was performed using Tukey's multiple comparison test; #$p < 0.05$, ###$p < 0.001$ compared with the control (white bar); **$p < 0.01$, ***$p < 0.001$ compared with Aβ treatment (black bar).

the graph represents the PGW concentration (μg/mL), the y-axis represents the protein expression level as a ratio compared to the control group, and each curve represents the change in expression of a specific protein.When PGW concentration exceeds 100 μg/mL, most proteins' expression levels either plateau or increase slightly. In research and clinical applications, a PGW concentration of approximately 100 μg/mL is most effective in maximally inhibiting protein expression. Proteins such as Bax/Bcl-2 and Bcl-xL have a more robust response to PGW, significantly reducing their expression levels. This means that these proteins are more sensitive to the effects of PGW. We show that PGW may be essential in suppressing the expression of proteins that cause inflammation and apoptosis. These properties of PGW have confirmed its potential as a neuroprotective and anti-inflammatory treatment.

4 Discussion

PG is used as a traditional herbal medicine to treat respiratory ailments, including cough, phlegm, sore throat, and lung abscess. The bioactive components responsible for these therapeutic effects have been identified, and the potential mechanisms include inhibition of airway mucus hypersecretion, relief of inflammatory responses, and inhibition of inflammatory cytokine secretion (18, 19). AD research has recently focused on inflammation-related processes. When inflammation or nerve damage occurs, microglia are activated to produce inflammatory mediators and cytokines, thereby promoting the inflammatory response (20). Activation of microglia can induce neurotoxicity by triggering the production of proinflammatory and cytotoxic factors in neuronal cell lines treated with LPS, β-amyloid, glutamate, and arachidonate (21). Therefore, drugs that promote the neuroinflammatory response of microglia or eliminate $A\beta_{25-35}$ may have therapeutic potential for neurodegenerative diseases. Our previous study showed that PG saponins effectively suppress Aβ-induced oxidative stress and apoptosis by activating the antioxidant pathway (Nrf2/ARE). In this study, we investigated the inhibitory effect of PGW on the production of $A\beta_{25-35}$-induced inflammatory mediators and cytokines in BV2 microglial cells. $A\beta_{25-35}$ is a short fragment of the amyloid precursor protein and has a neurotoxic effect similar to $A\beta_{1-40}$ or $A\beta_{1-42}$. Therefore, $A\beta_{25-35}$ is suitable for studying $A\beta_{25-35}$-induced cytotoxicity in mouse microglial cells (22). Oxidative stress is implicated in a variety of pathological processes, including cancer, diabetes, steatohepatitis, atherosclerosis, neurological degeneration, and inflammatory diseases, so we investigated the oxygen-radical scavenging activity of PG (23). There is a close relationship among oxidative stress, aging, and inflammation. Elevated levels of biomarkers for oxidative stress are

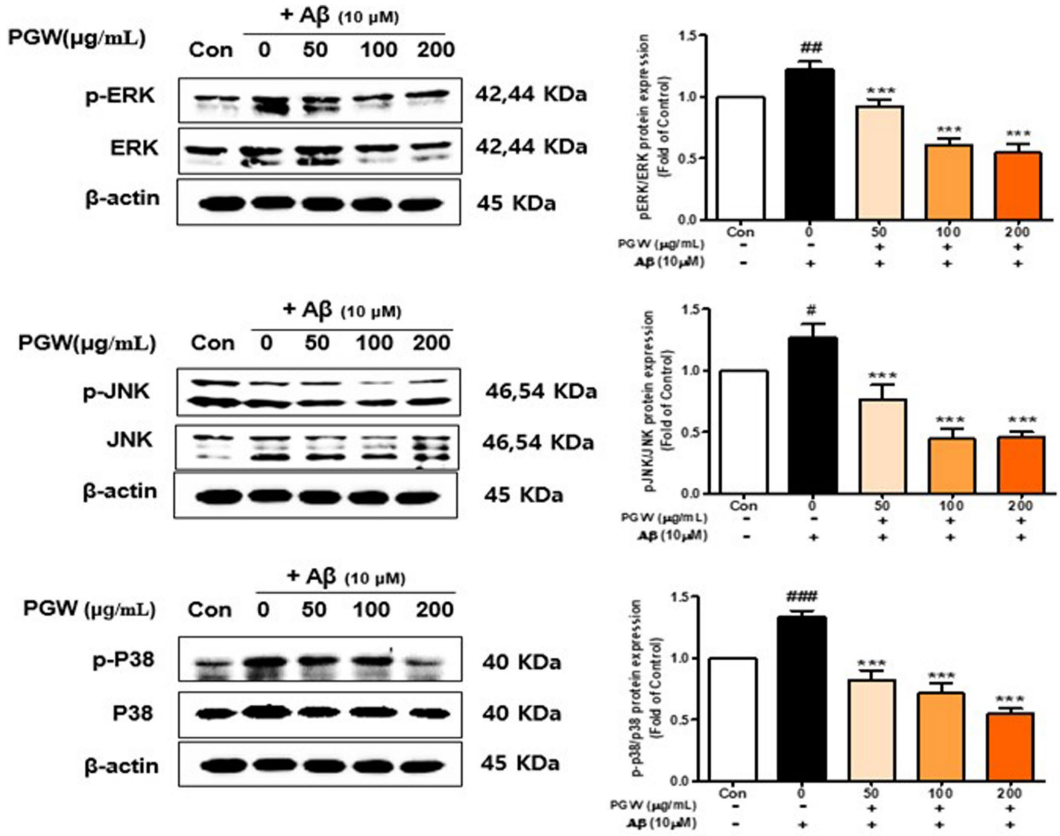

FIGURE 7
Effect of PGW on MAPK, ERK /p-ERK, JNK/p-JNK, and P38/p-P38 protein levels. Cells were treated with PGW (50, 100, and 200 μg/mL) for 24 h, followed by Aβ (10 μM) for 24 h. Control groups were treated with the same volume of conditioned medium (0.2% DMSO). Western blotting was performed using β-actin as the loading control. Values are means ± SD ($n = 3$). Significance was determined by one-way ANOVA. *Post-hoc* analysis was performed using Tukey's multiple comparison test; #$p < 0.05$, ###$p < 0.001$ compared with the control (white bar); *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$ compared with Aβ treatment (black bar).

associated with elevated levels of inflammatory cytokines. Calabrese et al. (24) showed that peripheral or central nerve stimulation induces inflammatory changes leading to PD symptoms and progression. Kim et al. (25) reported that an ethanol extract of PG showed significant DPPH and ABTS radical-scavenging activities and contained high levels of antioxidant phenolic compounds. Serafini suggested that the interactions of polyphenols, phenolic acids, saponins, and triterpenoids regulate NF-κB (26). Jang et al. (27) reported that several platycodin saponins from bellflower seeds inhibit the expression of proinflammatory genes by blocking NF-κB activation in LPS-induced RAW 264.7 cells. Our results show that PGW exerts an anti-inflammatory effect as a result of its antioxidant activity. In a follow-up study, we plan to investigate the anti-inflammatory activities of four selected indicators.

Early chemical studies on *P. grandiflorum* indicated that the triterpenoid saponins are the major active chemical constituents, and many previous reports suggested platycodin D and polygalacin D as the major compounds and a study by Lu et al., for individual saponin content of, platycoside E was reported to be the most prevalent compound in some provinces (28). Yoo et al., reported that platycoside E(α) was the most abundant, followed by polygalacin D2(β) and 3′-O-acetylplatyconic acid A as a result of the analysis of 18 platycosides in bellflower samples from 8 provinces in Korea (29). Our research results also showed that the content of platycoside E was the highest, followed by platycodin D, polygalacin D, platycodin D2, platycoside G1, platycodin D3, platyconic acid A, and deapio-platycodin D (Figure 1; Table 1). Choi et al. reported that bellflower saponin platicoside E effectively improved ethanol-induced cognitive dysfunction in rats (30).

Polygalacin D2 and platycodin D have been reported to reduce Neuroinflammation (31). It has been reported that platycodin D2 is an adjuvant to increase Th1 and Th2 cytokines (32). In the composition of PG saponins present in PGW, it was confirmed that PGW has neuroinflammation inhibitory activity, as in the results of previous studies.

Neuroinflammation and apoptosis are related, and the inflammatory response can cause damage to nerve cells, leading to their apoptosis. Among the genes involved in regulating apoptosis, Bcl-2 and Bcl-XL have anti-apoptotic effects that promote cell survival (33). By contrast, Bax (Bcl-2-associated X protein), Bad, Bak, Bik, and Bcl-XS have proapoptotic effects. In addition, c-Jun N-terminal kinase (JNK) regulates apoptosis (34). JNK is activated by apoptotic signals and phosphorylates several intracellular factors, thereby regulating apoptosis. Neuronal injury caused by cytokines and inflammatory or heat shock-inducing factors triggers the JNK and p38 signaling pathways and induces ERK signaling, leading to oxidative

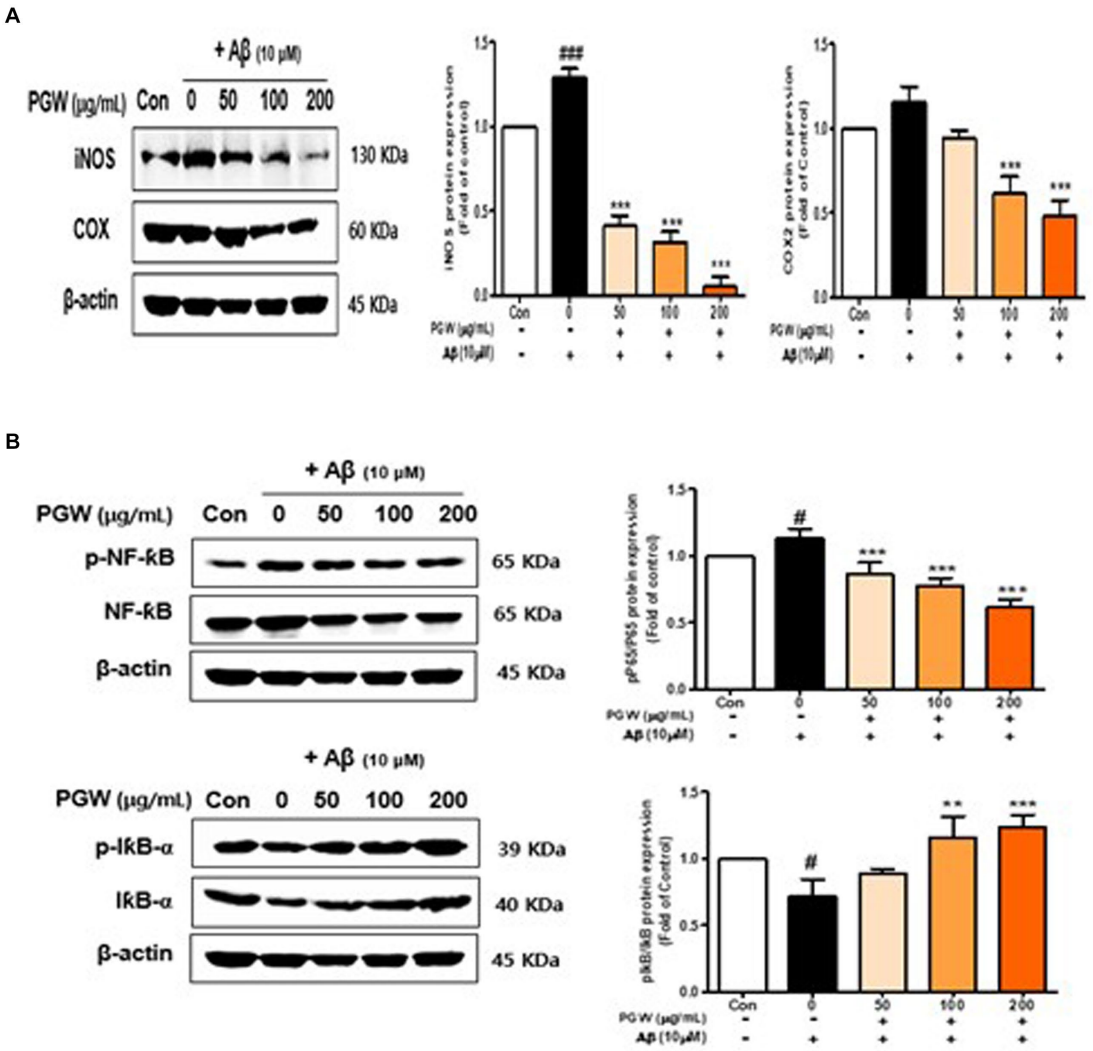

FIGURE 8
Effect of PGW on the NF-κB level in Aβ-induced cells. **(A)** iNOS and COX-2 levels. **(B)** p-NF-κB, NF-κB, I-κBα, and p-I-κBα levels. Cells were treated with PGW (50, 100, and 200 μg/mL) for 24 h, followed by Aβ (10 μM) for 24 h. Control groups were treated with the same volume of conditioned medium (0.2% DMSO). Western blotting was performed using β-actin as the loading control. Values are means ± SD ($n = 3$). Significance was determined by one-way ANOVA. *Post hoc* analysis was performed using Tukey's multiple comparison tests; #$p < 0.05$ compared with the control (white bar); **$p < 0.01$, ***$p < 0.001$ compared with Aβ treatment (black bar).

stress-mediated death of neurons (35). In this study, JNK was phosphorylated, the ratio of Bax/Bcl-2 was increased, and caspase-3 was activated in BV2 microglia treated with β-amyloid (10 μM) for 24 h (Figure 6).

In our previous study, saponin, an important component of PGE, not only alleviated oxidative stress and prevented neuronal death in Aβ-treated hippocampal HT22 cells, but also alleviated AD-related pathologies such as Aβ deposition, oxidative damage, and neuroinflammation. Additionally, bellflower root extract (PGE) inhibited Aβ accumulation in the brain of 5XFAD mice. In this study, we aimed to elucidate the mechanism by which PGW alleviates neuroinflammation in Aβ-treated BV2 microglia. Subsequent studies demonstrate that PGE administration significantly inhibits the deposition of Aβ in the brains of 5XFAD mice.

MAPKs (e.g., JNK, ERK, and p38) are important regulators of the production of proinflammatory cytokines such as iNOS and COX2, including NF-κB. Nam et al. (36) reported that the upregulation of proinflammatory cytokines induced by $A\beta_{25\text{-}35}$ was suppressed in HT22 neurons, and Ji et al. (10) showed that a bellflower saponin fraction significantly inhibited the $A\beta_{25\text{-}35}$-induced phosphorylation of ERK1/2 MAPK, in turn inhibiting inflammatory mediator production in neurons. In this study, p-JNK and p-P38 levels were increased, and that of p-ENK was decreased, by $A\beta_{25\text{-}35}$. By contrast, PGW ameliorated $A\beta_{25\text{-}35}$-induced neuroinflammation by inhibiting the p38 signaling pathway in BV2 microglial cells (Figure 7). Although the roles of MAPKs in cytokine synthesis vary depending on the cytokine, and synthesis can occur at different steps in the signaling cascade in the same cell, our data suggest that PGW has potential as an anti-inflammatory and neuroprotective agent.

We confirmed that $A\beta_{25\text{-}35}$-induced PGW significantly increased the production of IL-1β, IL-6, and TNF-α in $A\beta_{25\text{-}35}$-treated BV2 microglial cells. On the other hand, TNF-α did not show a significant

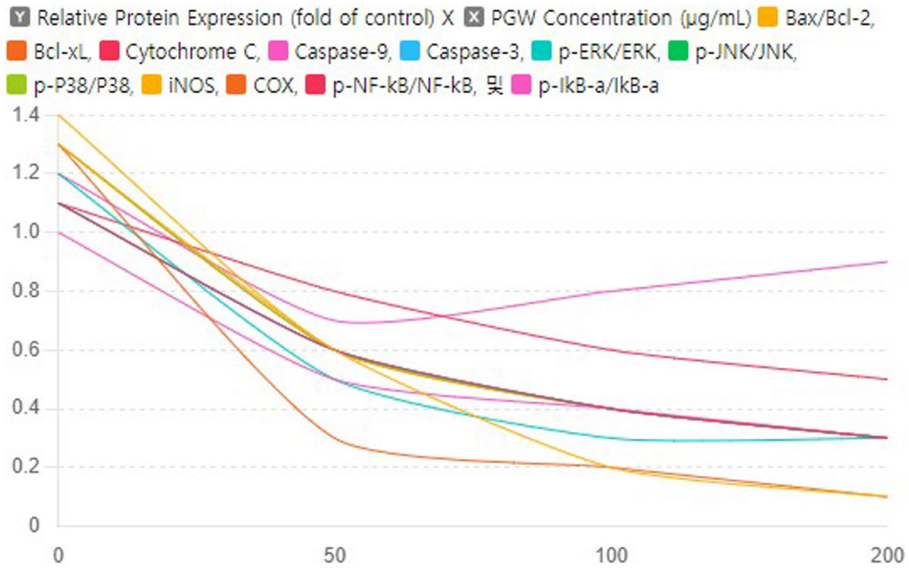

FIGURE 9
Impact of PGW on Protein Expression in Response to Aβ: Dose–Response Curves. Nonlinear regression analysis was performed to derive curves showing the relationship with PGW concentration for each protein expression level. Significance was determined by one-way ANOVA. *Post-hoc* analysis was performed using Tukey's multiple comparison tests; PGW treatment at 50, 100, and 200 μg/mL generally leads to very significant changes ($p < 0.001$) compared to the control.

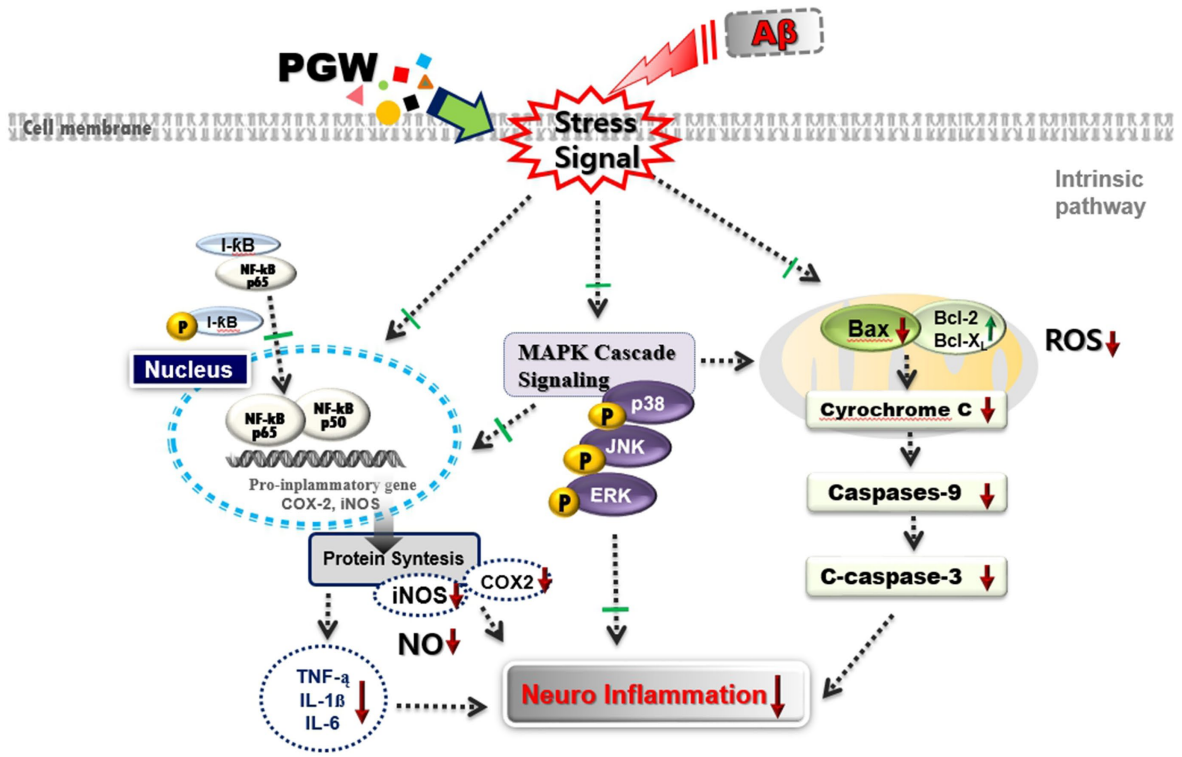

FIGURE 10
Schematic diagram of the mechanism of Anti-neuroflammatory effects of PGW in Aβ-stimulated BV2 microglia. PGW regulates Aβ toxicity-induced inflammatory response by inhibiting MAPK and NF-κB activation in BV2 microglia. ↑, increase or up-regulation; ↓, decrease or down-regulate; ⊥, block or inhibit.

decrease, but significant inhibitory activity was confirmed at 200 μg/mL (Figure 5). It is associated with the regulation of transcriptional activity. Jang et al. (27) reported that a water extract of *P. grandiflorum* root upregulated iNOS and TNF-α and transcriptionally activated NF-κB in LPS-stimulated RAW 264.7 cells. Ahn et al. showed that *P. grandiflorum* saponins exert anti-inflammatory effects by inhibiting

the LPS-induced expression of iNOS and COX-2 by blocking NF-κB activation in RAW 264.7 macrophages (37). In this study, PGW significantly suppressed the activation of NF-κB by Aβ and increased the expression of I-κBα (Figure 8B). PGW also inhibited NF-κB signaling by regulating the activation of NF-κB and IκBα. Additionally, the levels of iNOS and COX-2 were significantly decreased by PGW compared to Aβ alone (Figure 8A). Therefore, PGW attenuated Aβ-induced neuroinflammation by downregulating NF-κB signaling.

Based on the results of a study analyzing the effect of PGW on protein expression by concentration, the PG dose was investigated, and it was confirmed that a concentration of 100 μg/mL had the optimal effect (Figure 9). At this concentration, the expression of most proteins was maximally suppressed, and in particular, the expression of proteins that cause inflammation and apoptosis was significantly reduced. Therefore, in research and clinical applications, a PGW concentration of 100 μg/mL is most effective in maximally suppressing protein expression, providing important baseline data to increase the potential utilization of PGW as a neuroprotective and anti-inflammatory therapeutic agent.

In summary, PGW demonstrated anti-inflammatory activity by inhibiting the specific MAPK signaling pathways, including JNK and ERK, and suppressing the activation of NF-κB. This inhibition resulted in the downregulation of iNOS and COX-2 expression, leading to a reduction in the production of inflammatory mediators and pro-inflammatory cytokines. Based on these findings, PGW has been identified as a valuable material for effectively preventing and treating neurodegenerative inflammatory disorders through its anti-inflammatory regulatory efficacy.

5 Conclusion

As shown in the schematic diagram of Figure 10, PGW demonstrated anti-inflammatory activity by inhibiting the specific MAPK signaling pathways, including JNK and ERK, and suppressing the activation of NF-κB. This inhibition resulted in the downregulation of iNOS and COX-2 expression, leading to a reduction in the production of inflammatory mediators and pro-inflammatory cytokines. Based on these findings, PGW has been identified as a valuable material for effectively preventing and treating neurodegenerative inflammatory disorders through its anti-inflammatory regulatory efficacy.

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.

Ethics statement

Ethical approval was not required for the studies on animals in accordance with the local legislation and institutional requirements because only commercially available established cell lines were used.

Author contributions

Y-JJ: Writing – original draft, Writing – review & editing. MK: Formal analysis, Software, Writing – review & editing. G-SK: Conceptualization, Data curation, Writing – review & editing. HK: Conceptualization, Project administration, Writing – review & editing. GJ: Methodology, Writing – review & editing.

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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/fnut.2024.1427121/full#supplementary-material

References

1. Zhang L-L, Huang M-Y, Yang Y, Huang M-Q, Shi J-J, Zou L, et al. Bioactive platycodins from Platycodonis Radix: Phytochemistry, pharmacological activities, toxicology and pharmacokinetics. *Food Chem*. (2020) 327:127029. doi: 10.1016/j.foodchem.2020.127029

2. Noh E-M, Kim J-M, Lee HY, Song H-K, Joung SO, Yang HJ, et al. Immuno-enhancement effects of *Platycodon grandiflorum* extracts in splenocytes and a cyclophosphamide-induced immunosuppressed rat model. *BMC Complement Altern Med*. (2019) 19:1–12. doi: 10.1186/s12906-019-2724-0

3. Ke W, Wang P, Wang X, Zhou X, Hu X, Chen F. Dietary *Platycodon grandiflorus* attenuates hepatic insulin resistance and oxidative stress in high-fat-diet induced non-alcoholic fatty liver disease. *Nutrients*. (2020) 12:480. doi: 10.3390/nu12020480

4. Han L-K, Zheng Y-N, Okuda H, Xu B-J, Kimura Y. Saponins from Platycodi Radix ameliorate high fat diet–induced obesity in mice. *J Nutr*. (2002) 132:2241–5. doi: 10.1093/jn/132.8.2241

5. Zhao H, Harding S, Marinangeli C, Kim Y, Jones P. Hypocholesterolemic and anti-obesity effects of saponins from *Platycodon grandiflorum* in hamsters fed atherogenic diets. *J Food Sci*. (2008) 73:H195–200. doi: 10.1111/j.1750-3841.2008.00915.x

6. Qi C, Li L, Cheng G, Xiao B, Xing Y, Zhao X, et al. *Platycodon grandiflorus* polysaccharide with anti-apoptosis, anti-oxidant and anti-inflammatory activity against LPS/D-GalN induced acute liver injury in mice. *J Polym Environ*. (2021) 29:4088–97. doi: 10.1007/s10924-021-02179-2

7. Zhang Z, Wang S, Tan H, Yang P, Li Y, Xu L, et al. Advances in polysaccharides of natural source of the anti-Alzheimer's disease effect and mechanism. *Carbohydr Polym*. (2022) 296:119961. doi: 10.1016/j.carbpol.2022.119961

8. Ryu CS, Kim CH, Lee SY, Lee KS, Choung KJ, Song GY, et al. Evaluation of the total oxidant scavenging capacity of saponins isolated from *Platycodon grandiflorum*. *Food Chem*. (2012) 132:333–7. doi: 10.1016/j.foodchem.2011.10.086

9. Choi JH, Jin SW, Han EH, Park BH, Kim HG, Khanal T, et al. *Platycodon grandiflorum* root-derived saponins attenuate atopic dermatitis-like skin lesions via suppression of NF-κB and STAT1 and activation of Nrf2/ARE-mediated heme oxygenase-1. *Phytomedicine*. (2014) 21:1053–61. doi: 10.1016/j.phymed.2014.04.011

10. Ji Y-J, Kim S, Kim J-J, Jang GY, Moon M, Kim HD. Crude saponin from *platycodon grandiflorum* attenuates aβ-induced neurotoxicity via antioxidant, anti-inflammatory and anti-apoptotic signaling pathways. *Antioxidants*. (2021) 10:1968. doi: 10.3390/antiox10121968

11. Zhang J, Song N, Liu Y, Guo J. Platycodin D inhibits β-amyloid-induced inflammation and oxidative stress in BV-2 cells via suppressing TLR4/NF-κB signaling pathway and activating Nrf2/HO-1 signaling pathway. *Neurochem Res*. (2021) 46:638–47. doi: 10.1007/s11064-020-03198-6

12. Li Q, Wu Y, Chen J, Xuan A, Wang X. Microglia and immunotherapy in Alzheimer's disease. *Acta Neurol Scand*. (2022) 145:273–8. doi: 10.1111/ane.13551

13. Kim JH, Kim DH, Baek SH, Lee HJ, Kim MR, Kwon HJ, et al. Rengyolone inhibits inducible nitric oxide synthase expression and nitric oxide production by down-regulation of NF-κB and p38 MAP kinase activity in LPS-stimulated RAW 264.7 cells. *Biochem Pharmacol*. (2006) 71:1198–205. doi: 10.1016/j.bcp.2005.12.031

14. Kaminska B. MAPK signalling pathways as molecular targets for anti-inflammatory therapy—from molecular mechanisms to therapeutic benefits. *Biochimica et Biophysica Acta (BBA)-Proteins and Proteomics*. (2005) 1754:253–62. doi: 10.1016/j.bbapap.2005.08.017

15. Mandrekar-Colucci S, Landreth GE. Microglia and inflammation in Alzheimer's disease. *CNS & Neurolog Disord-Drug Targets (Formerly Current Drug Targets-CNS & Neurolog Dis)*. (2010) 9:156–67. doi: 10.2174/187152710791012071

16. Kim B, Lee KY, Park B. Isoorientin inhibits amyloid β25–35-induced neuronal inflammation in BV2 cells by blocking the NF-κB signaling pathway. *Molecules*. (2021) 26:7056. doi: 10.3390/molecules26227056

17. Cai Z, Hussain MD, Yan L-J. Microglia, neuroinflammation, and beta-amyloid protein in Alzheimer's disease. *Int J Neurosci*. (2014) 124:307–21. doi: 10.3109/00207454.2013.833510

18. Nyakudya E, Jeong JH, Lee NK, Jeong Y-S. Platycosides from the roots of Platycodon grandiflorum and their health benefits. *Prevent Nutrit Food Sci*. (2014) 19:59–68. doi: 10.3746/pnf.2014.19.2.059

19. Zhang L, Wang Y, Yang D, Zhang C, Zhang N, Li M, et al. *Platycodon grandiflorus*–an Ethnopharmacological, phytochemical and pharmacological review. *J Ethnopharmacol*. (2015) 164:147–61. doi: 10.1016/j.jep.2015.01.052

20. Graeber MB, Li W, Rodriguez ML. Role of microglia in CNS inflammation. *FEBS Lett*. (2011) 585:3798–805. doi: 10.1016/j.febslet.2011.08.033

21. Lee K-W, Jung SY, Choi S-M, Yang EJ. Effects of ginsenoside re on LPS-induced inflammatory mediators in BV2 microglial cells. *BMC Complement Altern Med*. (2012) 12:1–8. doi: 10.1186/1472-6882-12-196

22. Liu R-t, Zou L-b, Lü Q-j. Liquiritigenin inhibits Aβ25–35-induced neurotoxicity and secretion of Aβ1–40 in rat hippocampal neurons. *Acta Pharmacol Sin*. (2009) 30:899–906. doi: 10.1038/aps.2009.74

23. Tan BL, Norhaizan ME, Liew W-P-P, Sulaiman RH. Antioxidant and oxidative stress: a mutual interplay in age-related diseases. *Front Pharmacol*. (2018) 9:1162. doi: 10.3389/fphar.2018.01162

24. Calabrese V, Santoro A, Monti D, Crupi R, Di Paola R, Latteri S, et al. Aging and Parkinson's disease: Inflammaging, neuroinflammation and biological remodeling as key factors in pathogenesis. *Free Radic Biol Med*. (2018) 115:80–91. doi: 10.1016/j.freeradbiomed.2017.10.379

25. Kim M, Hwang IG, Kim SB, Choi AJ. Chemical characterization of balloon flower (*Platycodon grandiflorum*) sprout extracts and their regulation of inflammatory activity in lipopolysaccharide-stimulated RAW 264.7 murine macrophage cells. *Food Sci Nutr*. (2020) 8:246–56. doi: 10.1002/fsn3.1297

26. Serafini M, Peluso I. Functional foods for health: the interrelated antioxidant and anti-inflammatory role of fruits, vegetables, herbs, spices and cocoa in humans. *Curr Pharm Des*. (2016) 22:6701–15. doi: 10.2174/1381612823666161123094235

27. Jang K-J, Kim HK, Han MH, Oh YN, Yoon H-M, Chung YH, et al. Anti-inflammatory effects of saponins derived from the roots of *Platycodon grandiflorus* in lipopolysaccharide-stimulated BV2 microglial cells. *Int J Mol Med*. (2013) 31:1357–66. doi: 10.3892/ijmm.2013.1330

28. Lu H, Ju M, Chu S, Xu T, Huang Y, Chan Q, et al. Quantitative and chemical fingerprint analysis for the quality evaluation of Platycodi Radix collected from various regions in China by HPLC coupled with chemometrics. *Molecules*. (2018) 23:1823. doi: 10.3390/molecules23071823

29. Yoo DS, Choi YH, Cha MR, Lee BH, Kim S-J, Yon GH, et al. HPLC-ELSD analysis of 18 platycosides from balloon flower roots (Platycodi Radix) sourced from various regions in Korea and geographical clustering of the cultivation areas. *Food Chem*. (2011) 129:645–51. doi: 10.1016/j.foodchem.2011.04.106

30. Choi YH, Kim YS, Yeo SJ, Roh SH, Jeong YC, Kang JS, et al. Ameliorating effect of balloon flower saponin on the ethanol-induced memory impairment in mice. *Phytother Res*. (2008) 22:973–6. doi: 10.1002/ptr.2394

31. Choi JH, Yoo K-Y, Park OK, Lee CH, Won M-H, Hwang IK, et al. Platycodin D and 2 ″-o-acetyl-polygalacin D2 isolated from *Platycodon grandiflorum* protect ischemia/reperfusion injury in the gerbil hippocampus. *Brain Res*. (2009) 1279:197–208. doi: 10.1016/j.brainres.2009.05.005

32. Xie Y, Deng W, Sun H, Li D. Platycodin D2 is a potential less hemolytic saponin adjuvant eliciting Th1 and Th2 immune responses. *Int Immunopharmacol*. (2008) 8:1143–50. doi: 10.1016/j.intimp.2008.04.006

33. Czabotar PE, Garcia-Saez AJ. Mechanisms of BCL-2 family proteins in mitochondrial apoptosis. *Nat Rev Mol Cell Biol*. (2023) 24:732–48. doi: 10.1038/s41580-023-00629-4

34. Li M, Wang D, He J, Chen L, Li H. Bcl-XL: A multifunctional anti-apoptotic protein. *Pharmacol Res*. (2020) 151:104547. doi: 10.1016/j.phrs.2019.104547

35. Kumari S, Dhapola R, Reddy DH. Apoptosis in Alzheimer's disease: insight into the signaling pathways and therapeutic avenues. *Apoptosis*. (2023) 28:943–957. doi: 10.1007/s10495-023-01848-y

36. Nam Y, Shin SJ, Park YH, Kim M-J, Jeon SG, Lee H, et al. *Platycodon grandiflorum* root protects against Aβ-induced cognitive dysfunction and pathology in female models of Alzheimer's disease. *Antioxidants*. (2021) 10:207. doi: 10.3390/antiox10020207

37. Raso GM, Meli R, Di Carlo G, Pacilio M, Di Carlo R. Inhibition of inducible nitric oxide synthase and cyclooxygenase-2 expression by flavonoids in macrophage J774A. 1. *Life Sci*. (2001) 68:921–31. doi: 10.1016/S0024-3205(00)00999-1

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EDITED BY
Misha Vrolijk,
Maastricht University, Netherlands

REVIEWED BY
Mamunur Rashid,
University of Nebraska Medical Center, United States
Munkhtuya Tumurkhuu,
Wake Forest Baptist Medical Center, United States

*CORRESPONDENCE
Xinjun Yang
✉ yangxinj1989@sohu.com

†These authors have contributed equally to this work and share first authorship

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Association of dietary niacin intake with all-cause and cardiovascular mortality of adult patients with chronic kidney disease in the United States: results from NHANES 1999–2018

Chao Zhang[1†], Qi Cheng[2†], Xinjun Yang[1*], Wei Zhao[1], Kaifa Luo[1] and Yunlong Qin[1]

[1]Department of Nephrology, Bethune International Peace Hospital, Shijiazhuang, Hebei, China, [2]Outpatient Department, Bethune International Peace Hospital, Shijiazhuang, Hebei, China

Objective: The relationship between dietary niacin intake (DNI) and mortality rates among patients afflicted with chronic kidney disease (CKD) is a subject of debate. Utilizing data derived from the National Health and Nutrition Examination Survey (NHANES), this study adopts a retrospective cohort design with an aim to investigate the association in the American adult patients with CKD.

Methods: A cohort study was conducted in the National Health and Nutrition Examination Survey (NHANES) between 2009 and 2018 that enrolled 6,191 CKD patients aged 20 years and above. We collected data on mortality through 31 December 2018. DNI was measured using a 24-h recall method. The relationship between DNI levels and mortality from all causes and cardiovascular causes was analyzed using weighted Cox proportional hazards models. The Kaplan–Meier (K-M) survival curve was plotted to illustrate these associations.

Results: Following a median monitoring period of 85 months, it was observed that 2,419 individuals (33.08%) succumbed to all causes, whereas cardiovascular-related deaths were recorded for 746 participants (10.45%). When controlling for confounders, an inverse relationship was established between DNI and mortality rates. Specifically, a marginal increase of 1 mg/day in DNI corresponded to a reduced Hazard Ratios (HRs) of 0.993 (0.987, 0.999; $p = 0.027$) for all-cause mortality and 0.980 (0.969, 0.991; $p < 0.001$) for cardiovascular mortality. A further stratified analysis by quartiles of DNI, with the lowest quartile serving as the reference, revealed that the highest quartile was associated with HRs of 0.820 (0.697, 0.966) for all-cause mortality and 0.663 (0.465, 0.944) for cardiovascular mortality, both displaying a significant trend ($p < 0.001$). However, a subdivision of CKD patients by age showed that the protective effects of higher DNI were only confined to individuals aged 60 years and above but not to those under 60 years of age.

Conclusion: A negative correlation between DNI and mortality due to all causes and cardiovascular issues among CKD patients aged 60 and above was revealed based on the datasets; however, this association was not observed in younger individuals under 60. Consequently, enhancing DNI might serve as a beneficial therapeutic strategy specifically for the older CKD demographic.

KEYWORDS

niacin, chronic kidney disease, mortality, national health and nutrition examination survey, cohort study

1 Introduction

Chronic kidney disease (CKD) is characterized by persistent structural or functional anomalies of the kidneys, lasting over 3 months, inclusive of indicators of kidney damage or a reduction in renal filtration capacity (1). The prevalence of CKD ranges between 10 and 15% worldwide, with a year-on-year increment noted (2). This condition profoundly undermines the quality of life and elevates the risk of mortality from all causes as well as cardiovascular events. According to estimates by the World Health Organization (WHO), CKD is directly responsible for between 5 and 10 million deaths globally each year (3). In individuals with end-stage kidney disease (ESRD), cardiovascular complications remain the predominant cause of increased mortality, with the mortality risk from such diseases being over 20 times higher in CKD patients than in the general populace (4). CKD has become a serious public health issue, and therefore the search for measures to delay the progression of CKD and reduce mortality is one of the most concerned problems in the field of nephrology.

Niacin, also known as vitamin B3, is an essential nutrient obtained through dietary intake, where food sources include liver, meat, fish, and whole grains (5). Historically employed for pellagra management, niacin now demonstrates significant anti-inflammatory and antioxidant capabilities across a multitude of cell types and tissues, which plays a crucial role in addressing various pathologies such as diabetes mellitus (DM), obesity, coronary artery disease (CAD), and atherosclerosis (6). Research indicates potential benefits of niacin in CKD through its modulation of lipid metabolism abnormalities, reduction in phosphate levels, enhancement of endothelial function, and its anti-inflammatory and antioxidant attributes, thereby contributing to a decrease in all-cause and cardiovascular-related mortalities among CKD sufferers (7, 8). Nonetheless, findings concerning the effectiveness of niacin in CKD remain inconclusive (9, 10). We also note that these investigations predominantly focus on niacin supplements, with minimal emphasis on niacin obtained through dietary sources. Current research on the association between dietary niacin intake (DNI) and mortality rates in CKD patients is scant.

To investigate the association between DNI and mortality outcomes due to all causes as well as cardiovascular conditions, we conducted an extensive cohort analysis utilizing data from the National Health and Nutrition Examination Survey (NHANES) spanning from 1999 to 2018. This retrospective study was based on a large dataset of American adults.

2 Materials and methods

2.1 Data and sample sources

The NHANES is a series of studies conducted to evaluate the health and nutritional statuses across various demographics in the United States (11). Utilizing a stratified, multistage probabilistic sampling method, NHANES collects a comprehensive set of data, including demographic profiles, dietary information, physical examinations, and laboratory measurements, which are integral to research in epidemiology and health sciences. The National Center for Health Statistics (NCHS) ethical review board has granted approval for all study protocols related to NHANES, ensuring that informed consent was secured from every participant.

From 1999 to 2018, a total of 101,316 individuals have participated in 20 NHANES cycles (12). In the present study, we applied exclusion criteria to the participant pool as follows: individuals under 20 years of age were not included (totaling 46,235), as were those who were pregnant (1,541 excluded). Moreover, individuals demonstrating optimal renal function, defined by an estimated glomerular filtration rate (eGFR) of 60 mL/min/1.73 m^2 or higher and a urine albumin-to-creatinine ratio (UACR) below 30 mg/g, were also excluded (39,532 individuals). Additionally, exclusions were made for participants lacking mortality data (7 individuals) and those with incomplete data on DNI (1,156 individuals). Following these exclusions, the study ultimately encompassed 6,191 participants (Figure 1).

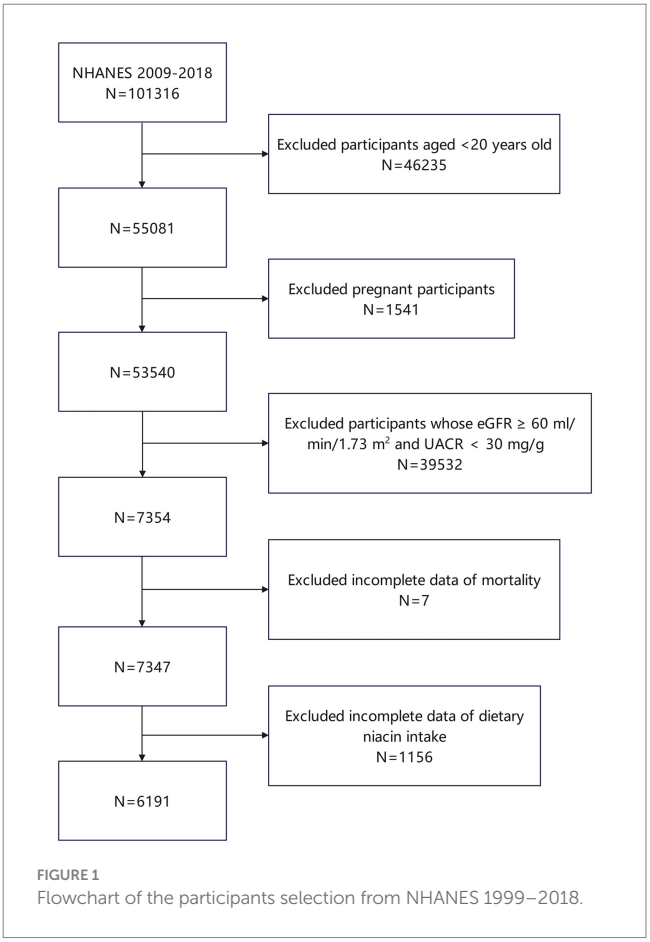

FIGURE 1
Flowchart of the participants selection from NHANES 1999–2018.

2.2 Independent variable

DNI (mg/day) was primarily assessed using two 24-h dietary recalls, which were conducted to capture participants' dietary intake, including both food and beverages, for the preceding 24-h period. The initial dietary recall was performed face-to-face in the Mobile Examination Center, ensuring accurate report under direct supervision. To enhance the accuracy of data, a second recall was conducted via telephone between 3 and 10 days after the initial session, allowing for the cross-verification of dietary intake. This recall approach helps to mitigate the variability of day-to-day food intake and reduces recall bias, thereby improving the reliability and validity of the dietary data. Trained data gatherers and nutritionists reviewed the data to ensure completeness and accuracy. Depending on the practicalities of data collection, in 1999–2002, dietary data from day 1 was used, and in 2003–2018, the average dietary data from 2 days was used. The exclusion criteria encompassed the absence of DNI among participants. The content of niacin in various foods and beverages was determined based on the U.S. Department of Agriculture's Food and Nutrient Database for Dietary Studies (13).

2.3 Dependent variable

The estimation of eGFR was performed utilizing the creatinine equation developed by the CKD Epidemiology Collaboration in 2021 (14). CKD was identified when the eGFR fell below 60 mL/min/1.73 m^2 or the UACR exceeded 30 mg/g (15). The study primarily investigated all-cause mortality, with cardiovascular mortality as a secondary consideration. The latter was specified as deaths attributed to cardiovascular disease, in accordance with the guidelines from the 10th revision of the International Statistical Classification of Diseases, Injuries, and Causes of Death (ICD-10) (15).

2.4 Covariates

Informed by prior research on niacin and CKD, the present study incorporated an array of covariates. Demographic variables such as sex, age, race, educational attainment, marital status, and the income-to-poverty ratio were considered. The presence of DM was established through a fasting blood glucose (FBG) level of 125 mg/dL or higher, self-reports of diabetes, or ongoing use of antihyperglycemic medications (16). Hypertension was identified when systolic blood pressure (SBP) averaged 140 mmHg or greater, or diastolic blood pressure (DBP) was 90 mmHg or higher, or if there was a physician's diagnosis, or current use of antihypertensive drugs (17). The analysis categorized smoking status into never smokers (less than 100 cigarettes consumed in a lifetime), former smokers (100 or more cigarettes previously, but none currently), and active smokers (100 or more cigarettes with occasional or daily smoking) (18). Body Mass Index (BMI) was segmented into under 25, between 25 and 29.9, and 30 kg/m^2 or above, denoting normal weight, overweight, and obesity, respectively (19). Self-reported health statuses ranged from poor to fair, to good, and then to very good or excellent. Covariates also included levels of alanine aminotransferase (ALT), aspartate aminotransferase (AST), and serum albumin (ALB). Data concerning cardiac diseases and cancer were extracted from self-administered questionnaires, with cardiac disease encompassing conditions such as heart failure, coronary heart disease, angina, or myocardial infarction.

2.5 Statistical analyses

According to the weight calculation method of NHANES, we used WTMEC4YR (1999–2002) and WTMEC2YR (2003–2018) to provide weights for all data. To assess baseline differences across various DNI levels, weighted t-tests and weighted chi-square tests were employed for continuous and categorical variables, respectively. Continuous variables were presented as mean values with standard deviations, while categorical data were shown as frequencies (percentages). To explore the association of DNI with both all-cause and cardiovascular mortality, Cox regression models were utilized, employing weights. Model 1 included no covariates to serve as a baseline comparison, allowing us to assess the unadjusted association between DNI and mortality. Model 2 was adjusted for a comprehensive set of demographic factors, including sex, age, race, educational attainment, marital status, and the income-to-poverty ratio. These variables were selected based on their established associations with both dietary intake and mortality outcomes in the literature. Model 3 included additional adjustments for health-related variables such as smoking status, BMI, self-reported health status, DM, hypertension, cardiac disease, cancer, and key biochemical indexes including ALT, AST, ALB. The selection of these covariates was based on their documented impact on mortality in CKD populations, as well as their potential to confound the relationship between DNI and mortality. A detailed list of covariables is shown in Supplementary Table S2. Considering the potential impact of age on DNI's effects, participants were categorized into two groups using a threshold age of 60 years for additional analyses. The findings were quantified as hazard ratios (HRs) with 95% confidence intervals (95%CI). Interaction terms were analyzed to determine variability in the relationships across different subgroups. All statistical analyses were performed using R software, version 4.3.2. Statistical significance was determined using a p-value threshold of 0.05. All analyses where the p-value was below this threshold were considered statistically significant.

3 Results

3.1 Baseline characteristics of participants

The study incorporated 6,191 subjects, correlating to a demographic scale of 20 million based on weighting calculations. Participants had an average age of 58.87 ± 17.94 years and consisted of 49.21% males and 50.79% females. DNI averaged 21.91 ± 11.19 mg/day. Throughout a median monitoring period of 85 months (interquartile range: 44, 140), all-cause mortality was recorded for 2,419 subjects (33.08%), and cardiovascular mortality was noted in 746 subjects (10.45%). Participants were divided into quartiles based on their DNI levels. The specific ranges for each quartile were as follows:

Quartile 1 (Q1): DNI < 14.4 mg/day.
Quartile 2 (Q2): 14.4 mg/day ≤ DNI < 19.7 mg/day.
Quartile 3 (Q3): 19.7 mg/day ≤ DNI < 26.9 mg/day.
Quartile 4 (Q4): DNI ≥ 26.9 mg/day.

These ranges were determined based on the distribution of DNI values among the participants and were used to explore the potential dose–response relationship between DNI and mortality outcomes. Baseline characteristics of the participants across these quartiles are detailed in Table 1.

Statistical analyses revealed significant disparities in several demographic and health variables across quartiles of DNI. These variables include sex, age, educational attainment, marital status, the ratio of income to poverty, presence of hypertension, self-reported health statuses, incidence of cardiac conditions, and mortality due to all causes and cardiovascular issues ($p < 0.05$). Conversely, no significant differences were observed among the quartiles in terms of race, smoking habits, BMI, DM, and cancer prevalence ($p \geq 0.05$). Individuals classified Q1 group (i.e., lower DNI) were predominantly female, of advanced age, with education extending to middle school or lower. This group also included a higher proportion of widowed, divorced, or separated individuals, a lower income-to-poverty ratio, and more frequent reports of fair to poor health. Moreover, these individuals exhibited a higher prevalence of hypertension and cardiac disorders, correlating with increased mortality rates from all causes and cardiovascular diseases compared to those in higher DNI quartiles.

3.2 Relationship between DNI and mortality

We conducted weighted Cox regression analyses to examine the relationship between DNI and mortality outcomes due to all causes and cardiovascular diseases. Model 1 involved no covariate adjustments. Model 2 included adjustments for demographic and socio-economic factors such as age, sex, race, marital status, education, and the income-to-poverty ratio. Model 3 encompassed adjustments for all previously mentioned covariates. Due to the direct correlation between cardiac disease and cardiovascular deaths, cardiac disease was excluded as a covariate in the cardiovascular mortality analysis within Model 3. Statistically significant relationships were found between DNI and mortality from all causes as well as cardiovascular conditions across all models ($p < 0.01$; Tables 2, 3). Specifically in Model 3, for each 1 mg/day increment in DNI, the HRs were 0.993 (0.987–0.999) for all-cause mortality and 0.980 (0.969–0.991) for cardiovascular mortality. Additionally, when examining the association of DNI across quartiles with mortality outcomes (with Q1 serving as the reference), the HRs for Q4 were 0.820 (0.697–0.966) for all-cause mortality and 0.663 (0.465–0.944) for cardiovascular mortality. The significant p for trend values ($p < 0.001$ for both all-cause and cardiovascular mortality) indicate a consistent and statistically significant decrease in mortality risk with increasing DNI levels across the quartiles. This suggested a dose–response relationship, where higher DNI intake was associated with progressively lower risks of mortality. These HR values suggested a modest but statistically significant protective effect of higher DNI against mortality. Clinically, this implied that even a small increase in dietary niacin intake could contribute to a reduction in the risk of death, particularly from cardiovascular causes. For example, the HR of 0.980 for cardiovascular mortality indicated that each additional 1 mg/day of DNI was associated with a 2% reduction in the risk of cardiovascular death. Although this reduction may appear small on an individual level, it could translate into substantial public health benefits when considered across a larger population, especially in high-risk groups such as CKD patients. The consistent protective trend observed across different models and quartiles underscored the potential of DNI as a modifiable dietary factor in improving long-term outcomes in CKD patients. K-M curves, stratified by quartiles of DNI, were displayed in Figure 2. The curve representing the Q1 showed the lowest survival probability for all-cause mortality, with a statistically significant difference (log-rank test, $p < 0.001$). A similar trend was noted for cardiovascular mortality. These results underscored the inverse relationship between DNI levels and mortality rates in patients with CKD.

However, in the analysis of CKD patients at different ages, we found that the associations between DNI and mortality were distinctly different above and under 60 years of age (Tables 2, 3). In the age group ≥60 years, weighted COX regression analysis demonstrated a consistent inverse relationship between DNI and mortality due to all causes and cardiovascular events across Models 1 to 3 ($p < 0.01$). Specifically, in Model 3, the risk of mortality from all causes was reduced by 21.1%, and cardiovascular mortality was reduced by 30.1% in the Q4 compared to the Q1, with a significant trend ($p < 0.05$). Conversely, for participants below 60, the analyses across Models 1 to 3 revealed no significant association between DNI or its quartile divisions and mortality rates from either all causes or cardiovascular issues ($p \geq 0.05$). The K-M curves for these two groups similarly reflected this difference (Figure 3).

3.3 Subgroup analysis

Subsequent subgroup analysis targeted individuals aged 60 years or older, with the data depicted in forest plots (Figure 4). These subgroups underwent stratification based on various demographic and health-related factors, including sex, race, educational level, marital status, income-to-poverty ratio, smoking habits, BMI, self-reported health statuses, DM, cancer, and cardiac disease (only in all-cause mortality). The investigations revealed a consistently negative correlation between DNI and mortality rates—both all-cause and cardiovascular—across all stratifications among patients with CKD who were 60 years of age or older ($p > 0.05$ for all interactions).

4 Discussion

Niacin has been recognized as the earliest therapeutic agent for dyslipidemia management and is effective in mitigating the risk of atherosclerotic cardiovascular diseases (20). Currently, the data on niacin's impact on CKD present mixed outcomes. Some researches indicate the potential of niacin to mitigate several adverse factors affecting renal function, such as hyperlipidemia, oxidative stress, inflammation, and endothelial dysfunction (21). These benefits contribute to the deceleration of the decline in glomerular filtration rate, reduction in cardiovascular risks, and overall enhancement of CKD outcomes. Additionally, niacin may decrease serum phosphorus by inhibiting the gastrointestinal absorption of dietary phosphorus, thus ameliorating mineral and bone disorders in ESRD patients (22). Contrarily, other investigations have found niacin to be ineffective in improving CKD. A multicenter prospective study in the United States revealed that, although niacin combined with simvastatin improved triglycerides (TG) and high-density lipoprotein (HDL) levels in CKD patients, it did not enhance cardiovascular or renal outcomes and was

TABLE 1 Baseline characteristics of weighted sample by the quartiles of DNI.

	Overall	Q1 < 14.4 mg/day	Q2 14.4–19.7 mg/day	Q3 19.7–26.9 mg/day	Q4 ≥ 26.9 mg/day	*p*-value
	N = 6,191	*N* = 1,544	*N* = 1,540	*N* = 1,558	*N* = 1,549	
Sex (%)						< 0.001
Female	50.79	70.56	61.23	46.64	30.06	
Male	49.21	29.44	38.77	53.36	69.94	
Age (years)	58.87 ± 17.94	61.09 ± 18.58	61.70 ± 17.25	58.56 ± 17.78	54.93 ± 17.46	< 0.001
Race (%)						0.069
Mexican American	7.32	7.81	6.86	7.05	7.59	
Non-Hispanic Black	13.58	16.96	12.51	12.92	12.49	
Non-Hispanic White	67.78	62.73	70.27	68.6	68.74	
Other Hispanic	5.2	6.46	4.53	5.24	4.77	
Other Race	6.13	6.05	5.83	6.19	6.4	
Education (%)						< 0.001
College or more	49.99	39.36	47.31	53.81	57.12	
High school	25.56	25.01	28.21	23.68	25.38	
Middle school or lower	24.44	35.62	24.48	22.51	17.5	
Marital status (%)						< 0.001
Never married	11.13	9.51	9.06	11.38	13.99	
Married/with partner	59.91	51.88	58.43	62.47	65.09	
Widowed/divorced/separated	28.96	38.61	32.51	26.16	20.92	
Income-to-poverty ratio (%)						< 0.001
<1.3	26.24	36.44	26.21	22.47	21.7	
≥1.3	73.76	63.56	73.79	77.53	78.3	
Smoke status (%)						0.125
never smokers	48.2	50.6	49.5	47.62	45.72	
ever smokers	33.41	29.48	34.27	34.22	34.97	
current smokers	18.4	19.93	16.24	18.16	19.32	

(Continued)

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TABLE 1 (Continued)

	Overall	Q1 < 14.4 mg/day	Q2 14.4–19.7 mg/day	Q3 19.7–26.9 mg/day	Q4 ≥ 26.9 mg/day	*p*-value
	N = 6,191	*N* = 1,544	*N* = 1,540	*N* = 1,558	*N* = 1,549	
BMI (%)						0.058
<25	25.6	26.7	26.52	26.31	23.32	
25–30	32.13	35.88	30.52	30.63	32.05	
≥30	42.27	37.43	42.96	43.07	44.63	
Health Status (%)						0.004
Poor to Fair	30.05	36.31	29.77	29.22	26.61	
Good	39.09	37.13	39.51	40.51	38.8	
Very good to Excellent	30.86	26.56	30.72	30.27	34.59	
Hypertension (%)						0.035
Yes	63.36	64.74	66.58	63.29	59.53	
No	36.64	35.26	33.42	36.71	40.47	
DM (%)						0.181
Yes	33.93	32.78	34.25	36.69	32.03	
No	66.07	67.22	65.75	63.31	67.97	
Cardiac disease (%)						0.024
Yes	8.73	10.47	9.1	8.69	7.1	
No	91.27	89.53	90.9	91.31	92.9	
Cancer (%)						0.281
Yes	16.56	17.15	17.95	16.76	14.69	
No	83.44	82.85	82.05	83.24	85.31	
All-cause mortality (%)	33.08	41.89	36.62	30.46	25.52	< 0.001
Cardiovascular mortality (%)	10.45	13.62	11.72	9.89	7.39	< 0.001

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TABLE 2 The association between DNI and all-cause mortality in CKD patients, weighted.

	OR (95% CI)		
All participants	Model 1	Model 2	Model 3
DNI (mg/day)	0.982 (0.976,0.987)	0.990 (0.985,0.996)	0.993 (0.987,0.999)
p value	< 0.001	0.001	0.027
DNI quartiles			
Q1, < 14.4 mg/day	Ref	Ref	Ref
Q2, 14.4–19.7 mg/day	0.952 (0.810,1.120)	0.936 (0.803,1.092)	0.983 (0.845,1.143)
Q3, 19.7–26.9 mg/day	0.769 (0.654,0.904)	0.843 (0.714,0.995)	0.839 (0.712,0.990)
Q4, ≥ 26.9 mg/day	0.613 (0.522,0.719)	0.764 (0.652,0.895)	0.820 (0.697,0.966)
p for trend	< 0.001	< 0.001	0.005
Participants≥60years old			
DNI (mg/day)	0.986 (0.981,0.991)	0.985 (0.979,0.992)	0.990 (0.983,0.996)
p value	< 0.001	< 0.001	0.002
DNI quartiles			
Q1, < 13.8 mg/day	Ref	Ref	Ref
Q2, 13.8–18.7 mg/day	0.880 (0.761,1.019)	0.906 (0.765,1.072)	0.919 (0.779,1.085)
Q3, 18.7–25.2 mg/day	0.783 (0.670,0.915)	0.764 (0.628,0.928)	0.804 (0.674,0.958)
Q4, ≥ 25.2 mg/day	0.715 (0.620,0.824)	0.717 (0.603,0.851)	0.789 (0.656,0.950)
p for trend	< 0.001	< 0.001	0.005
Participants<60years old			
DNI (mg/day)	1.003 (0.992,1.015)	1.002 (0.987,1.017)	1.001 (0.986, 1.016)
p value	0.580	0.800	0.886
DNI quartiles			
Q1, < 16.11 mg/day	Ref	Ref	Ref
Q2, 16.11–21.95 mg/day	1.194 (0.755,1.89)	1.123 (0.588,2.112)	0.981 (0.606,1.589)
Q3, 21.95–30.48 mg/day	0.953 (0.632,1.44)	0.797 (0.575,1.808)	0.801 (0.485,1.322)
Q4, ≥ 30.48 mg/day	1.144 (0.764,1.71)	0.975 (0.608,1.647)	0.917 (0.583,1.440)
p for trend	0.748	0.651	0.588

TABLE 3 The association between DNI and cardiovascular mortality in CKD patients, weighted.

	OR (95% CI)		
All participants	Model 1	Model 2	Model 3
DNI (mg/day)	0.973 (0.964,0.982)	0.981 (0.971,0.990)	0.980 (0.969,0.991)
p value	< 0.001	< 0.001	< 0.001
DNI quartiles			
Q1, < 14.4 mg/day	Ref	Ref	Ref
Q2, 14.4–19.7 mg/day	0.935 (0.738,1.186)	0.921 (0.714,1.189)	0.966 (0.717,1.301)
Q3, 19.7–26.9 mg/day	0.765 (0.590,0.993)	0.861 (0.660,1.124)	0.821 (0.635,1.062)
Q4, ≥ 26.9 mg/day	0.544 (0.413,0.718)	0.673 (0.499,0.907)	0.663 (0.465,0.944)
p for trend	< 0.001	0.006	0.007
Participants≥60years old			
DNI (mg/day)	0.979 (0.968,0.990)	0.978 (0.967,0.990)	0.977 (0.964,0.991)
p value	< 0.001	< 0.001	0.001
DNI quartiles			
Q1, < 13.8 mg/day	Ref	Ref	Ref
Q2, 13.8–18.7 mg/day	0.900 (0.716,1.131)	0.891 (0.684,1.160)	0.984 (0.719,1.347)
Q3, 18.7–25.2 mg/day	0.812 (0.646,1.019)	0.816 (0.613,1.085)	0.866 (0.633,1.185)
Q4, ≥ 25.2 mg/day	0.673 (0.509,0.891)	0.666 (0.490,0.904)	0.697 (0.492,0.987)
p for trend	0.002	0.006	0.021
Participants<60years old			
DNI (mg/day)	0.993 (0.973,1.013)	0.985 (0.960,1.010)	0.984 (0.958,1.011)
p value	0.500	0.226	0.234
DNI quartiles			
Q1, < 16.11 mg/day	Ref	Ref	Ref
Q2, 16.11–21.95 mg/day	1.333 (0.590,3.011)	1.512 (0.642,3.561)	1.243 (0.566,2.727)
Q3, 21.95–30.48 mg/day	1.224 (0.628,2.386)	1.125 (0.513,2.465)	1.155 (0.436,3.062)
Q4, ≥ 30.48 mg/day	0.960 (0.382,2.412)	0.798 (0.270,2.359)	0.833 (0.240,2.898)
p for trend	0.873	0.507	0.734

linked to increased all-cause mortality (10). Tilman, a researcher from France, has argued the lack of sufficient evidence supporting the survival benefit of niacin in regulating serum phosphate in CKD patients (9). Furthermore, current researches on niacin and CKD mainly focus on niacin supplements, and no large-scale studies specifically addressing the relationship between DNI and CKD have been retrieved. In light of the above, utilizing the NHANES database, we conducted a comprehensive retrospective cohort investigation to delve into the association between DNI and mortality rates among CKD patients.

The present study encompassed 6,191 subjects, each showing an average DNI of 21.91 ± 11.19 mg/day. Recorded rates of all-cause and cardiovascular mortality stood at 33.08 and 10.45%, respectively. Employing weighted Cox regression analyses, we observed a significant inverse association between higher DNI levels and reduced mortality

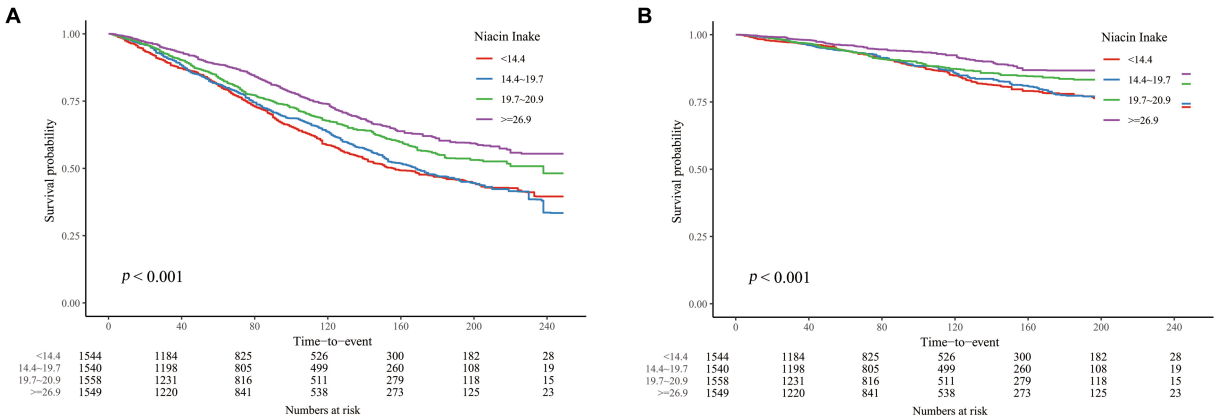

FIGURE 2
K-M curves describing the relationship between DNI and mortality in CKD patients. **(A)** all-cause mortality **(B)** cardiovascular mortality.

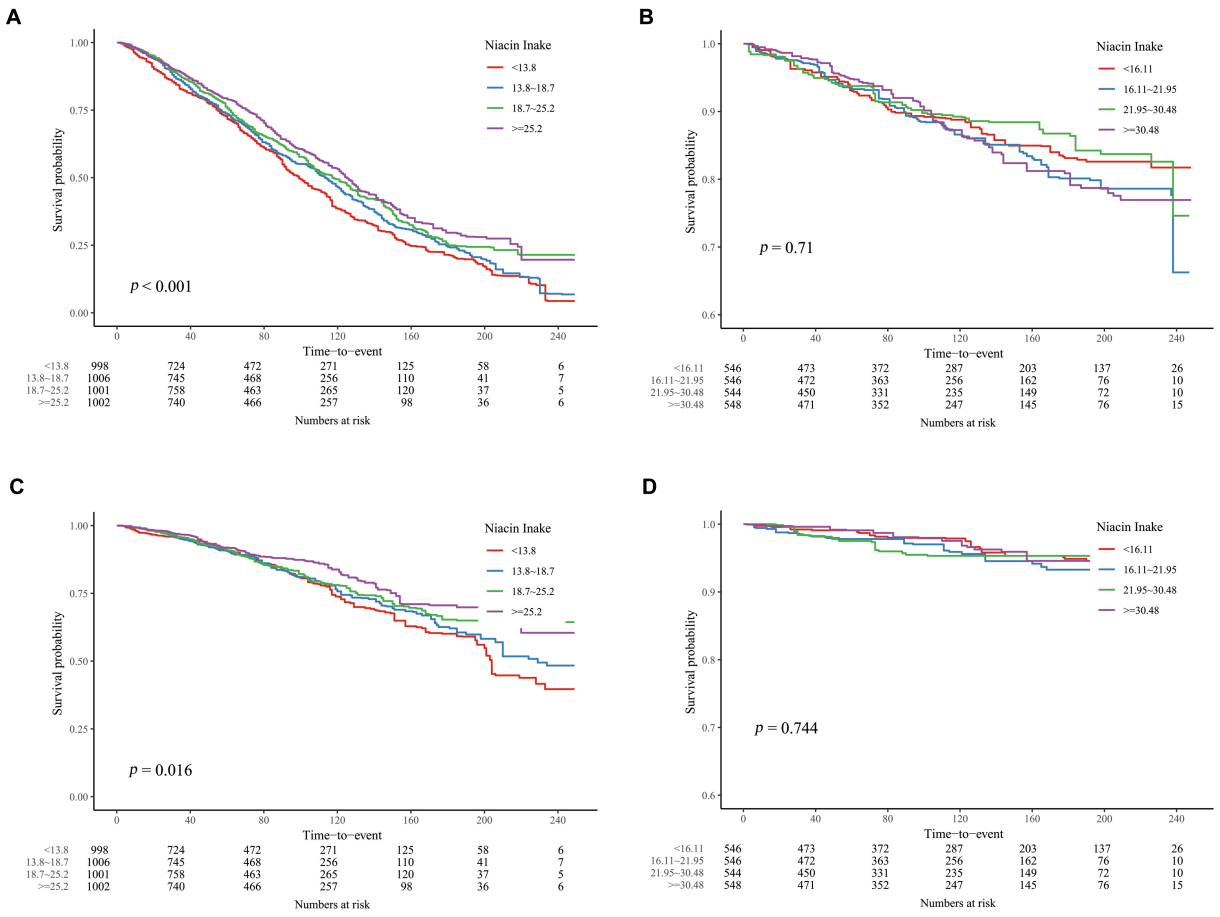

FIGURE 3
K-M curves describing the relationship between DNI and mortality in CKD patients of different age stages. **(A)** all-cause mortality, ≥ 60 years old; **(B)** all-cause mortality, < 60 years old; **(C)** cardiovascular mortality, ≥ 60 years old; **(D)** all-cause mortality, < 60 years old.

due to all causes and cardiovascular events in patients suffering from CKD, after adjusting for pertinent confounders. However, subgroup analysis by age revealed that this inverse relationship was evident exclusively in individuals aged 60 or above, whereas it was not detectable in those under 60. Thus, the results of this study supported a beneficial effect of dietary niacin on the prognosis of CKD, but this effect was only present in patients aged 60 and above. The age-specific protective effect of higher DNI observed in individuals aged 60 and

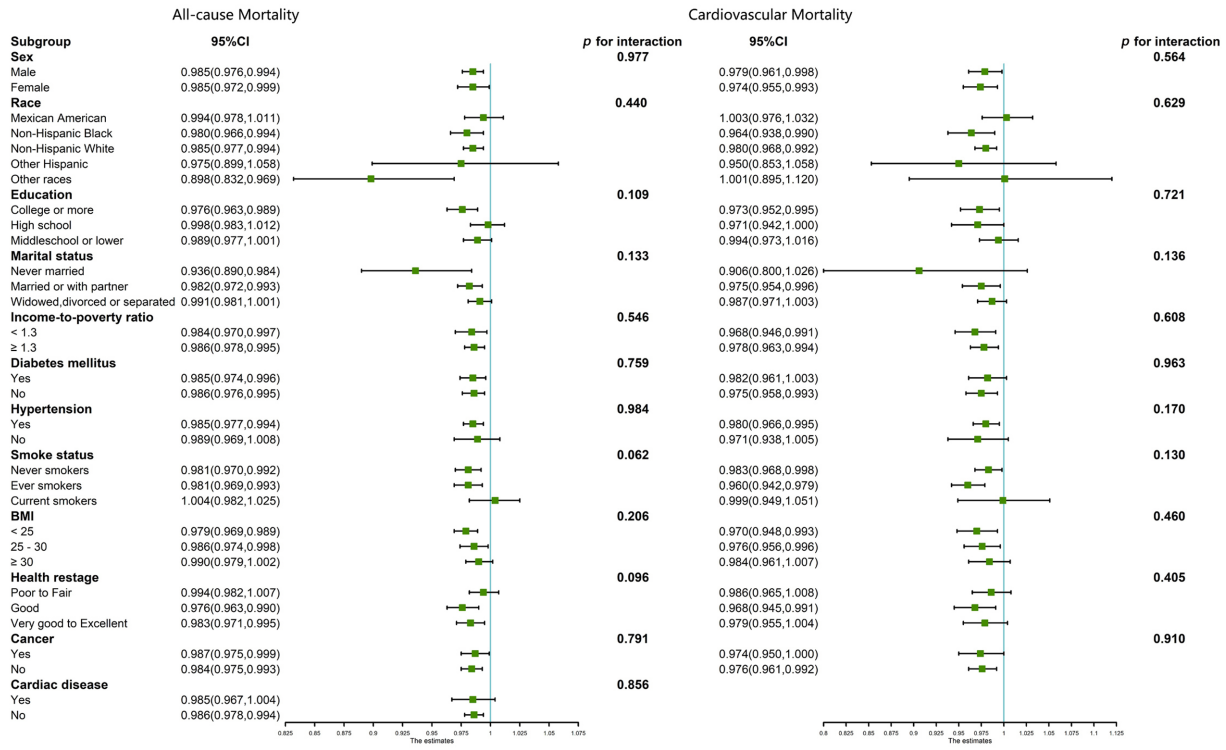

FIGURE 4
Subgroup analysis for the association between DNI and all-cause and cardiovascular mortality in CKD patients ≥60 years of age, weighted.

above could be attributed to several biological and lifestyle factors. First, as people age, metabolism also changes, including alterations in lipid metabolism, where niacin is known to influence positively. Second, older individuals may also experience a decline in renal function and an increase in oxidative stress and inflammation, conditions that niacin's antioxidant and anti-inflammatory properties could help mitigate. Third, the prevalence of cardiovascular diseases increases with age, and the role of niacin in improving lipid profiles and reducing atherosclerotic risk could be more pronounced in this demographic. Fourth, older individuals often have different dietary patterns and nutrient absorption rates compared to younger individuals, potentially making them more responsive to the effects of niacin. These factors combined suggest that the elderly may particularly benefit from higher DNI as a protective measure against mortality, which aligns with our findings. It is also important to recognize that younger patients with CKD generally face a lower risk of mortality, a factor that could mask the potential effects of DNI. In this study, the small sample size of CKD patients under 60 years may have limited the statistical power needed to identify a significant association. This underscores the necessity for larger sample sizes and further research to investigate nutritional interventions that could be particularly relevant to younger CKD patients.

Based on previous studies, we collected four factors related to niacin and CKD from NHANES: TG, HDL, serum phosphorus, and systemic immunoinflammatory index (SII), and looked at their distributions in different DNI groups. High TG, low HDL and high blood phosphorus are thought to have a detrimental effect on the progression and prognosis of CKD (23–25). SII is a new index of systemic inflammation, and some studies have confirmed that it is positively associated with mortality in CKD patients (26). Among CKD patients aged 60 or older, grouped by quartiles of DNI, the distributions of the above four indicators were shown in Supplementary Table S1. TG, HDL and serum phosphorus were statistically different between the four groups, while SII was not. However, serum phosphorus, although decreasing with increasing DNI, did not show large gaps between the means of the four groups (Q1 3.71 mg/dL vs. Q4 3.63 mg/dL), which did not seem to be sufficient to have a significant effect on CKD mortality from clinical experience. TG and HDL, although significantly different between the four groups, tended to change in the opposite direction than expected: there was a tendency for TG to increase (Q1 165.88 mg/dL vs. Q4 176.57 mg/dL) and HDL to decrease (Q1 1.40 mmol/L vs. Q4 1.29 mmol/L) as DNI increased, which in turn may have had a detrimental effect on CKD. Consequently, the findings of our investigation implied that the influences of DNI on mortality among CKD patients might not be mediated through enhancements in TG, HDL, serum phosphorus, or inflammation levels. Further research is called for to elucidate the underlying mechanisms.

According to the US Institute of Medicine, the established daily niacin intake recommendation is 16 milligrams per day for adult males and 14 milligrams per day for adult females, with the tolerable upper intake level set at 35 milligrams per day (27). In our study, the mean intake of dietary niacin of the group ≥60 years old was 20.5 mg/day, which was above average but still far from the upper limit. Also, the upper limit for niacin (35 mg/day) is based on excessive intake of niacin in the form of supplements and additives, and does not apply to niacin in foods (28). Adverse effects due to excessive intake of niacin from foods have not been reported. Therefore, it may be feasible to appropriately elevate DNI to lower the mortality of CKD patients ≥60 years old.

Although the 24-h dietary recall method was widely used for dietary assessment due to its practicality and ability to capture detailed intake data, it was not without limitations. A primary limitation was the reliance on participants' memory, which could introduce recall bias. Participants may forget or misreport their food intake, leading to inaccuracies in the reported DNI. Additionally, the 24-h recall captured dietary intake for only one or two specific days, which might not represent usual intake, particularly in individuals with highly variable diets. This limitation could result in measurement errors, potentially attenuating the observed associations between DNI and mortality. To mitigate this, we only included participants whose dietary recall status was reliable and used the mean of two dietary recalls where data allowed, but some degree of measurement error might still exist. These limitations suggested that while our findings indicated a significant association between DNI and mortality, caution should be exercised in interpreting the results, and further studies using more comprehensive dietary assessment methods may be needed to confirm these findings. Another limitation of this study was that we did not conduct a gender-specific analysis to explore potential differences in the relationship between DNI and mortality across genders. Since our primary focus was on age-related differences, future research is needed to investigate whether gender plays a significant role in modifying the effects of DNI on mortality outcomes.

5 Conclusion

Research indicates that in CKD patients aged 60 years or older residing in the United States, there is a negative correlation between DNI and mortality rates due to all causes and cardiovascular issues. Conversely, this correlation is absent in CKD patients under the age of 60. It is suggested that enhancing DNI could serve as a beneficial intervention for older CKD patients.

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://www.cdc.gov/nchs/nhanes/index.htm.

Ethics statement

The studies involving humans were approved by National Center for Health Statistics Ethics Review Board. The studies were conducted in accordance with the local legislation and institutional requirements. The participants provided their written informed consent to participate in this study.

Author contributions

CZ: Conceptualization, Data curation, Investigation, Methodology, Resources, Software, Validation, Writing – original draft, Writing – review & editing. QC: Conceptualization, Data curation, Investigation, Methodology, Resources, Validation, Writing – original draft, Writing – review & editing. XY: Conceptualization, Methodology, Supervision, Validation, Writing – original draft, Writing – review & editing. WZ: Methodology, Validation, Writing – original draft, Writing – review & editing. KL: Methodology, Validation, Writing – original draft, Writing – review & editing. YQ: Methodology, Validation, Writing – original draft, Writing – review & editing.

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Conflict of interest

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

Publisher's note

Supplementary material

The Supplementary material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fnut.2024.1436836/full#supplementary-material

References

1. Ammirati AL. Chronic kidney disease. *Rev Assoc Med Bras*. (2020) 1992:s03–9. doi: 10.1590/1806-9282.66.S1.3

2. Borg R, Carlson N, Søndergaard J, Persson F. The growing challenge of chronic kidney disease: an overview of current knowledge. *Int J Nephrol*. (2023) 2023:9609266. doi: 10.1155/2023/9609266

3. Evans M, Lewis RD, Morgan AR, Whyte MB, Hanif W, Bain SC, et al. A narrative review of chronic kidney disease in clinical practice: current challenges and future perspectives. *Adv Ther*. (2022) 39:33–43. doi: 10.1007/s12325-021-01927-z

4. Adedinsewo DA, Porter IE, White RO, Hickson LJ. Racial and ethnic disparities in cardiovascular disease risk among patients with chronic kidney disease. *Curr Cardiovasc Risk Rep*. (2022) 16:145–57. doi: 10.1007/s12170-022-00701-2

5. Wuerch E, Urgoiti GR, Yong VW. The promise of niacin in neurology. *Neurotherapeutics*. (2023) 20:1037–54. doi: 10.1007/s13311-023-01376-2

6. Gasperi V, Sibilano M, Savini I, Catani MV. Niacin in the central nervous system: an update of biological aspects and clinical applications. *Int J Mol Sci*. (2019) 20:974. doi: 10.3390/ijms20040974

7. Malhotra R, Katz R, Hoofnagle A, Bostom A, Rifkin DE, Mcbride R, et al. The effect of extended release niacin on markers of mineral metabolism in ckd. *Clin J Am Soc Nephrol*. (2018) 13:36–44. doi: 10.2215/CJN.05440517

8. Streja E, Kovesdy CP, Streja DA, Moradi H, Kalantar-Zadeh K, Kashyap ML. Niacin and progression of ckd. *Am J Kidney Dis*. (2015) 65:785–98. doi: 10.1053/j.ajkd.2014.11.033

9. Drueke TB, Massy ZA. Lowering expectations with niacin treatment for ckd-mbd. *Clin J Am Soc Nephrol*. (2018) 13:6–8. doi: 10.2215/CJN.12021017

10. Kalil RS, Wang JH, De Boer IH, Mathew RO, Ix JH, Asif A, et al. Effect of extended-release niacin on cardiovascular events and kidney function in chronic kidney disease: a post hoc analysis of the aim-high trial. *Kidney Int*. (2015) 87:1250–7. doi: 10.1038/ki.2014.383

11. Taylor CL, Madans JH, Chapman NN, Woteki CE, Briefel RR, Dwyer JT, et al. Critical data at the crossroads: the national health and nutrition examination survey faces growing challenges. *Am J Clin Nutr*. (2023) 117:847–58. doi: 10.1016/j.ajcnut.2023.03.007

12. Akinbami L, Chen T, Davy O, Ogden C, Fink S, Clark J, et al. National health and nutrition examination survey, 2017-march 2020 prepandemic file: sample design, estimation, and analytic guidelines. *Vital and Health statistics Ser*. (2022) 1:1–36. doi: 10.15620/cdc:115434

13. Rhodes DG, Morton S, Myrowitz R, Moshfegh AJ. Food and nutrient database for dietary studies 2019–2020: an application database for national dietary surveillance. *J Food Compos Anal*. (2023) 123:105547. doi: 10.1016/j.jfca.2023.105547

14. Inker LA, Eneanya ND, Coresh J, Tighiouart H, Wang D, Sang Y, et al. New creatinineand cystatin c–based equations to estimate gfr without race. *N Engl J Med*. (2021) 385:1737–49. doi: 10.1056/NEJMoa2102953

15. Li F, Sun A, Wu F, Zhang D, Zhao Z. Trends in using of antihypertensive medication among us ckd adults, nhanes 2001–2018. *Front Cardiovascular Med*. (2023) 10:990997. doi: 10.3389/fcvm.2023.990997

16. Mathur P, Leburu S, Kulothungan V. Prevalence, awareness, treatment and control of diabetes in India from the countrywide national ncd monitoring survey. *Front Public Health*. (2022) 10:748157. doi: 10.3389/fpubh.2022.748157

17. Santos D, Dhamoon MS. Trends in antihypertensive medication use among individuals with a history of stroke and hypertension, 2005 to 2016. *JAMA Neurol*. (2020) 77:1382–9. doi: 10.1001/jamaneurol.2020.2499

18. Fan H, Xiong Y, Huang Y, Wang L, Xu C, Li W, et al. Moderate selenium alleviates the pulmonary function impairment induced by cadmium and lead in adults: a population-based study. *Sci Total Environ*. (2023) 903:166234. doi: 10.1016/j.scitotenv.2023.166234

19. Li M, Gong W, Wang S, Li Z. Trends in body mass index, overweight and obesity among adults in the Usa, the nhanes from 2003 to 2018: a repeat cross-sectional survey. *BMJ Open*. (2022) 12:e065425. doi: 10.1136/bmjopen-2022-065425

20. Memoni A, Akram M, Bhatti U, Khan AS, Rani K, Riaz H. Vitamin b3 (niacin) in diet act as protective negotiator for development of myocardial infarction. *Pakistan J Med Health Sci*. (2021) 15:1127–9. doi: 10.53350/pjmhs211551127

21. Zeman M, Vecka M, Perlık F, Stankova B, Hromadka R, Tvrzicka E, et al. Pleiotropic effects of niacin: current possibilities for its clinical use. *Acta Pharma*. (2016) 66:449–69. doi: 10.1515/acph-2016-0043

22. Ahmed HM, Yossif E, Abd-Elkader AS, Abdel Aziz EM. The efficacy and safety of niacin on hyperphosphatemia in esrd patients undergoing hemodialysis: randomized controlled trial. *Egyptian J Internal Med*. (2022) 34:33. doi: 10.1186/s43162-021-00080-x

23. Bowe B, Xie Y, Xian H, Balasubramanian S, Al-Aly Z. Low levels of high-density lipoprotein cholesterol increase the risk of incident kidney disease and its progression. *Kidney Int*. (2016) 89:886–96. doi: 10.1016/j.kint.2015.12.034

24. Da J, Xie X, Wolf M, Disthabanchong S, Wang J, Zha Y, et al. Serum phosphorus and progression of ckd and mortality: a meta-analysis of cohort studies. *Am J Kidney Dis*. (2015) 66:258–65. doi: 10.1053/j.ajkd.2015.01.009

25. Soohoo M, Moradi H, Obi Y, Kovesdy CP, Kalantar-Zadeh K, Streja E. Serum triglycerides and mortality risk across stages of chronic kidney disease in 2 million us veterans. *J Clin Lipidol*. (2019) 13:744–753.e15. doi: 10.1016/j.jacl.2019.08.001

26. Lai W, Xie Y, Zhao X, Xu X, Yu S, Lu H, et al. Elevated systemic immune inflammation level increases the risk of total and cause-specific mortality among patients with chronic kidney disease: a large multi-center longitudinal study. *Inflamm Res*. (2023) 72:149–58. doi: 10.1007/s00011-022-01659-y

27. Institute of Medicine Staff and Food and Nutrition Board Staff. Dietary reference intakes for thiamin, riboflavin, niacin, vitamin B6, folate, vitamin B12, pantothenic acid, biotin, and choline. Washington, D.C.: National Academies Press (2000) 123–45.

28. Meyer-Ficca M, Kirkland J. Niacin. *Advan Nutrition (Bethesda, Md)*. (2016) 7:556–8. doi: 10.3945/an.115.011239

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EDITED BY
Siddika Yalcin,
Hacettepe University, Türkiye

REVIEWED BY
Ruijie Xie,
German Cancer Research Center (DKFZ), Germany
Marzieh Saei Ghare Naz,
Shahid Beheshti University of Medical Sciences, Iran

*CORRESPONDENCE
Wencai Tian
✉ 202200121@sr.gxmu.edu.cn

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Association between dietary vitamin B6 intake and endometriosis risk: evidence from the national health and nutrition examination survey

Ling Yin[1], Feng Liang[2], Baoli Xie[3], Yanlin Su[1], Li Cheng[1], Xin Wei[1] and Wencai Tian[1]*

[1]Department of Obstetrics and Gynecology, The Affiliated Changsha Central Hospital, Hengyang Medical School, University of South China, Changsha, China, [2]Gynecology Department, The Reproductive Hospital of Guangxi Zhuang Autonomous Region, Nanning, China, [3]Gynecology Department, The First People's Hospital of Nanning, Nanning, China

Background: Endometriosis is a multifaceted disorder with genetic, immune, inflammatory, and multifactorial origins. Vitamin B6 serves as a pivotal coenzyme in various metabolic pathways involving lipids, hemes, nucleic acids, proteins, and carbohydrates. Dysregulation or deficiency of vitamin B6 can perturb human physiology. However, the relationship between dietary vitamin B6 and endometriosis remains elusive. This study aims to explore how dietary intake of vitamin B6 is associated with the risk of endometriosis.

Methods: Using cross-sectional data from the National Health and Nutrition Examination Survey, we analyzed information from American women aged 20–54 years between 1999 and 2006. After adjusting for relevant covariates, multivariable logistic regression analysis was employed to evaluate correlations.

Results: A total of 4,453 women were included in the study. The multiple linear regression model revealed a positive association between dietary vitamin B6 intake and the risk of endometriosis, even after controlling for confounding variables. Compared to individuals with lower vitamin B6 consumption (Q1: <0.94 mg/day), the adjusted odds ratio (OR) values for dietary vitamin B6 intake and endometriosis in Q2 (0.95–1.39 mg/day), Q3 (1.40–1.99 mg/day), and Q4 (>1.90 mg/day) were 1.22 (95% CI: 0.88–1.69, $p = 0.23$), 1.22 (95% CI: 0.86–1.73, $p = 0.279$), and 1.51 (95% CI, 1.01–2. 24, $p = 0.04$), respectively.

Conclusion: Our findings suggest a positive correlation between endometriosis and dietary vitamin B6 intake. Further investigations are imperative to establish a causal relationship between dietary vitamin B6 intake and endometriosis.

KEYWORDS

endometriosis, vitamin B6, NHANES, cross-sectional study, dietary intake

Introduction

Endometriosis is an inflammatory condition characterized by the ectopic growth of endometrial-like tissue outside the uterus, often affecting pelvic organs (1). It affects around 176 million women globally, leading to symptoms such as pelvic pain and infertility in 5–10% of cases (2). Those affected typically incur double the healthcare costs compared to unaffected individuals (3), making it a significant public health concern (4). Despite its impact, there is limited understanding of modifiable risk factors associated with its development.

Vitamin B6, a water-soluble vitamin found naturally in various foods, is involved in numerous metabolic processes as a coenzyme (5). It exists in three forms: pyridoxamine, pyridoxal, and pyridoxine, with pyridoxal-phosphate (PLP) being the active form. Vitamin B6 also plays a role in the immune and endocrine systems and has antioxidant properties (6, 7). Studies have suggested that vitamin B6 deficiency may increase the risk of various cancers, while adequate levels might reduce the risk, although findings are mixed (8–13). There is also evidence linking high doses of vitamin B6 with bone issues and other health concerns (14–16). Despite these associations, its potential protective effects against conditions like depression, cardiovascular disease, and cognitive decline remain unconfirmed (17–19).

The biological behaviors of endometriosis and malignancies, particularly in pathways regulating inflammation and cell proliferation, indicate a potential role for vitamin B6 in modulating these processes (20). Limited research has explored the relationship between dietary vitamin B6 and endometriosis. One study reported lower vitamin B6 levels in patients with advanced endometriosis (21). This study seeks to investigate the association between dietary vitamin B6 intake and endometriosis in a large sample of American women aged 20 to 54, aiming to offer new insights into potential preventive strategies.

Methods

Data source

The National Health and Nutrition Examination Survey (NHANES) and the National Center for Health Statistics (NCHS) played essential roles in collecting data for this investigation. We acquired data from three consecutive 2-year NHANES cycles conducted between 1999 and 2006, utilizing a nationally representative stratified sample through interviews and physical examinations. The NCHS Ethics Review Committee granted ethical approval, and all subjects provided signed informed consent.

Study design and population

The original dataset included 5,557 female participants. After excluding individuals with missing endometriosis-related information and those outside the age range of 20 to 54 years, our final study population comprised 4,453 women. Among them, 337 were diagnosed with endometriosis, while 4,116 were not. Exclusions were made for 991 pregnant women and 113 individuals lacking information on dietary vitamin B6 intake. Figure 1 provides a detailed illustration of the inclusion and exclusion process.

Participants and definition

We determined endometriosis based on participants' responses to a specific question in the reproductive health questionnaire: "Has a doctor or other health professional ever diagnosed you with endometriosis?" We categorized those who answered affirmatively as patients. Professional interviewers assessed dietary vitamin B6 consumption during the NHANES dietary survey, a component of the "What We Eat in America" survey, conducted at the Mobile Examination Center (MEC) using a 24-h recall method. The NHANES computer-assisted dietary interview (CADI) system recorded participants' food and beverage intake from the 24 h preceding the interview.

According to the study procedure, we randomly assigned participants to data collection sessions that occurred in the morning, afternoon, or evening. We determined dietary vitamin B6 and nutrient consumption using the US Department of Agriculture Survey Nutrients Database and the University of Texas Food Intake Analysis System. We excluded pharmaceuticals and dietary supplements from the nutritional calculations. We conducted two 24-h dietary recall interviews, followed by a phone interview 3 to 10 days later. We selected the first interview, conducted in person at the MEC, for analysis, as the 24-h recall method is the most commonly used in large-scale surveys (22).

Measurements

In our study, we considered a wide range of covariates sourced from the literature (23–25), including Age, marital status, race/ethnicity, education level, family income, smoking status, physical activity, BMI, alcohol consumption, use of birth control pills, high blood pressure, diabetes, coronary heart disease, chronic bronchitis, caloric consumption, total fat intake, total cholesterol intake, and usage of nutritional supplements. We categorized race and ethnicity into Non-Hispanic White, non-Hispanic Black, Mexican American, and other races. We classified marital status as either living with a partner or living alone. We stratified educational attainment into three levels: fewer than 9, 9 to 12 years, and more than 12 years. We assessed family income using the Poverty Income Ratio (PIR) and categorized it into low, medium, and high income, based on ranges from 1.3 to 3.5, according to the US government's Agriculture report (26). We dichotomized smoking status into smokers and never smokers (those who have smoked fewer than 100 cigarettes in total). Alcohol drinking status was determined by the survey question, "In any 1 year, have you had at least 12 drinks of any type of alcoholic beverage?" Participants who answered "yes" were defined as alcohol drinkers. We divided physical activity levels into three categories: unable to perform physical activity, moderate (defined as at least 10 min of movement within the previous 30 days resulting in light perspiration or a mild to moderate increase in respiration or heart rate), and vigorous (at least 10 min of activity within the last 30 days resulting in profuse sweating or an increased heart rate). The determination of previous disease (high blood pressure, diabetes, coronary heart disease and chronic bronchitis) was based on the inquiry in the questionnaire of whether the doctor had been informed of the condition in the past.

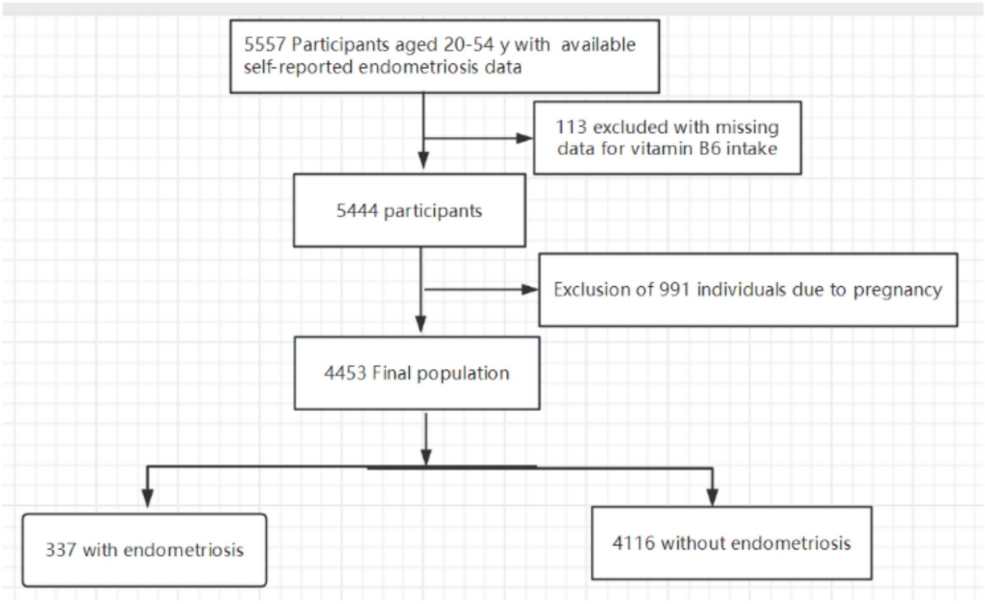

FIGURE 1
The study's flow diagram.

Prior to the MEC interview, participants completed a food recall questionnaire in order to get 24-h nutritional data, including macronutrient profiles and calorie intake. Additionally, details regarding medications, including dietary supplements consumed within the preceding month, were documented.

Statistical analyses

In this study, we conducted a secondary analysis of publicly available datasets and used descriptive statistics to characterize continuous variables (mean/SD or median/IQR) and proportions (%) for categorical variables. We assessed group differences using Kruskal-Wallis tests and one-way analyses of variance. We employed logistic regression models to explore the relationship between dietary vitamin B6 intake and endometriosis across three models. Model 1 adjusted for sociodemographic (age, race/ethnicity, education level, family income and marital status); Model 2 was adjusted for Model 1 plus BMI, smoking status, vigorous activity, moderate activity, alcohol consumption, birth control pills taken, high blood pressure, diabetes, coronary heart disease and chronic bronchitis; and Model 3 was adjusted for Model 2 plus calorie consumption, total fat consumption, total cholesterol consumption and dietary supplements taken. These models aimed to comprehensively address potential confounding factors and to enhance our understanding of the relationship between dietary vitamin B6 intake and endometriosis.

Furthermore, we investigated potential modifiers of the association between dietary vitamin B6 intake and endometriosis by incorporating variables such as family income (low vs. medium or high), marital status (living with a partner vs. living alone), smoking status, and dietary supplements taken. We assessed heterogeneity among subgroups using multivariate logistic regression and examined interactions between subgroups and dietary vitamin B6 intake through likelihood ratio testing. To ensure the robustness of our findings, we conducted sensitivity analyses by excluding participants with extreme energy intake, defined as consuming less than 500 or more than 5,000 kcal per day. This meticulous approach aimed to assess the consistency and reliability of our results under different conditions, thereby enhancing the validity of the study outcomes.

We determined the sample size based on available data, without conducting *a priori* statistical power assessments. We performed statistical analyses using R 3.3.2 and Free Statistics Software 1.5 (27). We conducted a comprehensive descriptive study on all individuals. For hypothesis testing, we utilized a two-tailed analysis, considering a significance level of 0.05 for determining statistical significance. This widely accepted threshold ensures standard confidence levels for interpreting results and drawing meaningful conclusions from the analyses.

Results

Baseline characteristics

Table 1 presents the essential characteristics of the 4,453 participants in the study. Among the sample, 337 individuals (7. 57%) received a diagnosis of endometriosis, and 2,820 (63.92%) were classified as overweight. A total of 1741 participants (39.10%) reported being smokers, while 2099 (47.14%) acknowledged the use of dietary supplements. Regarding age distribution, 2,378 participants (53.40%) were below 40 years old, 1,424 (31.98%) were between 40 and 50 years old, and 651 (14.62%) were above 50 years old. Notably, dietary vitamin B6 intake appeared higher in individuals who used dietary supplements, engaged in moderate exercise, were aged under 40, had a BMI below 25, had an education level exceeding 12 years, did not smoke, and lived with a partner or had a higher income. These baseline characteristics provide an overview of the diversity

TABLE 1 Population characteristics by categories of dietary vitamin B6 intake.

			Dietary vitamin B6 intake(mg/d)			
Variables	Total	Q1(<0.938)	Q2(0.939–1.385)	Q3(1.386–1.985)	Q4(>1.985)	*p*-value
No.	4,453	1,106	1,118	1,115	1,114	
Age (year), n (%)						0.355
< 40	2,378 (53.40)	581 (52.53)	593 (53.04)	578 (51.8)	626 (56.19)	
40–50	1,424 (31.98)	365 (33.00)	352 (31.48)	377 (33.81)	330 (29.62)	
> 50	651 (14.62)	160 (14.47)	173 (15.47)	160 (14.35)	158 (14.18)	
BMI (Kg/m^2), n (%)						0.041
< 25	1,592 (36.08)	360 (32.97)	391 (35.32)	408 (36.89)	1,592 (36.08)	
25–30	1,185 (26.86)	320 (29.30)	313 (28.27)	279 (25.23)	1,185 (26.86)	
> 30	1,635 (37.06)	412 (37.73)	403 (36.40)	419 (37.88)	1,635 (37.06)	
Family Income, n (%)						< 0.001
Low	1,177 (28.28)	346 (33.49)	279 (26.72)	276 (26.54)	276 (26.41)	
Medium	1,521 (36.54)	390 (37.75)	377 (36.11)	385 (37.02)	369 (35.31)	
High	1,464 (35.18)	297 (28.75)	388 (37.16)	379 (36.44)	400 (38.28)	
Race/Ethnicity, n (%)						0.012
Mexican American	1,006 (22.59)	214 (19.35)	263 (23.52)	267 (23.95)	262 (23.52)	
Other Race	400 (8.98)	101 (9.13)	101 (9.03)	109 (9.78)	89 (7.99)	
Non-Hispanic White	2051 (46.06)	501 (45.30)	509 (45.53)	503 (45.11)	538 (48.29)	
Non-Hispanic Black	996 (22.37)	290 (26.22)	245 (21.91)	236 (21.17)	225 (20.20)	
Education Level (year), n (%)						< 0.001
< 9	383 (8.61)	87 (7.88)	97 (8.68)	109 (9.78)	90 (8.09)	
9–12	1,695 (38.10)	488 (44.20)	419 (37.51)	407 (36.50)	381 (34.23)	
> 12	2,371 (53.29)	529 (47.92)	601 (53.80)	599 (53.72)	642 (57.68)	
Family Income, n (%)						< 0.001
Low	1,177 (28.28)	346 (33.49)	279 (26.72)	276 (26.54)	276 (26.41)	
Medium	1,521 (36.54)	390 (37.75)	377 (36.11)	385 (37.02)	369 (35.31)	
High	1,464 (35.18)	297 (28.75)	388 (37.16)	379 (36.44)	400 (38.28)	
Marital Status, n (%)						0.007
Living with a partner	2,613 (60.19)	593 (55.63)	672 (61.37)	680 (62.33)	668 (61.34)	
Living alone	1728 (39.81)	473 (44.37)	423 (38.63)	411 (37.67)	421 (38.66)	
Smoking status, n (%)						< 0.001
Yes	1741 (39.12)	489 (44.21)	431 (38.55)	425 (38.19)	396 (35.58)	
No	2,709 (60.88)	617 (55.79)	687 (61.45)	688 (61.81)	717 (64.42)	
Vigorous activity, n (%)						< 0.001
Yes	1,509 (33.90)	319 (28.84)	364 (32.59)	376 (33.72)	450 (40.43)	
No	2,855 (64.14)	757 (68.44)	734 (65.71)	720 (64.57)	644 (57.86)	
Unable to do activity	87 (1.95)	30 (2.71)	19 (1.70)	19 (1.70)	19 (1.70)	
Moderate activity, n (%)						< 0.001
Yes	2,320 (52.12)	518 (46.84)	569 (50.89)	577 (51.80)	656 (58.94)	
No	2065 (46.39)	566 (51.18)	532 (47.58)	523 (46.95)	444 (39.89)	
Unable to do activity	66 (1.48)	22 (1.99)	17 (1.53)	14 (1.26)	13 (1.17)	
Dietary supplements taken, n (%)						< 0.001
Yes	2099 (47.12)	461 (41.68)	526 (47.05)	522 (46.86)	590 (53.11)	
No	2,350 (52.82)	645 (58.32)	592 (52.95)	592 (53.14)	521 (46.89)	

(Continued)

TABLE 1 (Continued)

Variables	Total	Dietary vitamin B6 intake(mg/d)				*p*-value
		Q1(<0.938)	Q2(0.939–1.385)	Q3(1.386–1.985)	Q4(>1.985)	
Diabetes, n (%)						0.608
Yes	236 (5.33)	52 (4.72)	60 (5.42)	62 (5.64)	62 (5.58)	
No	4,176 (93.81)	1,041 (94.12)	1,046 (93.57)	1,043 (93.52)	1,046 (93.89)	
Birth control pills taken, n (%)						0.881
Yes	3,362 (75.52)	825 (74.63)	850 (76.03)	847 (76.01)	840 (75.48)	
No	1,087 (24.43)	279 (25.23)	267 (23.89)	267 (23.93)	274 (24.64)	
Coronary heart disease, n (%)						0.855
Yes	37 (0.81)	10 (0.90)	11 (1.00)	10 (0.92)	6 (0.54)	
No	4,413 (99.12)	1,095 (99.02)	1,107 (99.04)	1,104 (99)0.03	1,107 (99.4.04)	
Chronic bronchitis, n (%)						0.177
Yes	317 (7.12)	90 (8.13)	86 (7.74)	72 (6.45)	69 (6.23)	
No	4,128 (92.67)	1,012 (91.45)	1,032 (92.33)	1,041 (93.24)	1,043 (93.56)	
High blood pressure, n (%)						0.934
Yes	867 (19.45)	213 (19.23)	223 (19.93)	218 (19.62)	213 (19.14)	
No	3,558 (79.92)	885 (80.04)	889 (79.53)	890 (79.82)	894 (80.34)	
Calorie consumption(kcal/d), Mean (SD)	1911.90(816.11)	1332.51(558.03)	1734.22(575.24)	2056.63(651.52)	2520.71(916.92)	< 0.001
Total cholesterol consumption(mg/d), Median (IQR)	194.03 (114.02, 329.03)	117.0 4 (71.02, 202.14)	181.03 (113.04, 292.82)	224.04(148.04, 366.45)	272.02 (170.14, 436.83)	< 0.001
Total fat consumption(g/d), Median (IQR)	66.04 (45.40, 91.38)	47.33 (30.43, 65.89)	62.44 (45.45, 82.44)	74.67 (53.43, 99.73)	86.92 (61.64, 117.89)	< 0.001

within the study population and establish the foundation for subsequent analyses exploring the association between these factors and endometriosis.

Relationship between dietary vitamin B6 intake and endometriosis

Table 2 illustrates the relationships between dietary vitamin B6 intake and endometriosis. Univariate analysis revealed significant associations, indicating correlations between dietary vitamin B6 intake, age, race, family income, education level, use of birth control pills, high blood pressure, chronic bronchitis, and the use of dietary supplements with the presence of endometriosis. These findings underscore a complex interplay between dietary factors, demographic variables, and lifestyle choices influencing the occurrence of endometriosis. Subsequent multivariate analyses will further dissect these associations to elucidate the independent contributions of each factor to the risk of endometriosis.

Compared to individuals with lower vitamin B6 consumption (Q1 < 0.94 mg/day), the adjusted odds ratio (OR) values for dietary vitamin B6 intake and endometriosis in Q2 (0.95–1.39 mg/day), Q3 (1.40–1.99 mg/day), and Q4 (>1.99 mg/day) were 1.22 (95% CI: 0.88–1.69, $p = 0.24$), 1.22 (95% CI: 0.86–1.73, $p = 0.279$), and 1.51 (95% CI: 1.01–2. 24, $p = 0.04$), respectively (Table 3).

Stratified analyses based on additional variables

In a thorough examination of various subgroups, stratified analyses were conducted to assess potential effect modifications on the relationship between dietary vitamin B6 intake and endometriosis (refer to Figure 2). Notably, no significant interactions were identified in any subgroups, whether stratified by age, BMI, marital status, family income, smoking status, education level, or dietary supplements taken. These results suggest that the observed association between dietary vitamin B6 intake and endometriosis remains consistent across diverse demographic and lifestyle factors, reinforcing the robustness of the findings.

Sensitivity analysis

Furthermore, after excluding individuals with extreme energy intake, the dataset comprised 4,375 individuals, and the association between dietary vitamin B6 intake and endometriosis remained robust. Compared to individuals with lower vitamin B6 consumption (<0.96 mg/day), and after adjustments for age, race, education level, smoking status, dietary supplements taken,

TABLE 2 Association of covariates and endometriosis risk.

Variable	OR_95CI	P_value	Variable	OR_95CI	P_value
Age(year)			Moderate activity		
<40	1 (reference)		Yes	1 (reference)	
40–50	0.53 (0.42 ~ 0.68)	<0.001	No	1.21 (0.96 ~ 1.51)	0.108
>50	0.54 (0.39 ~ 0.74)	<0.001	Unable to do activity	0.49 (0.25 ~ 0.98)	0.043
BMI(Kg/m^2)			Dietary supplements taken		
<25	1 (reference)		Yes	1 (reference)	
25–30	0.94 (0.71 ~ 1.24)	0.671	No	1.59 (1.27 ~ 1.99)	<0.001
>30	1.07 (0.82 ~ 1.39)	0.629	Diabetes		
Family Income			Yes	1 (reference)	
Low	1 (reference)		No	0.76 (0.44 ~ 1.32)	0.33
Medium	0.79 (0.57 ~ 1.08)	0.134	Birth control pills taken		
High	0.51 (0.38 ~ 0.69)	<0.001	Yes	1 (reference)	
Race/Ethnicity			No	2.77 (1.95 ~ 3.93)	<0.001
Mexican American	1 (reference)		Coronary heart disease		
Other Race	0.65 (0.35 ~ 1.19)	0.162	Yes	1 (reference)	
Non-Hispanic White	0.23 (0.15 ~ 0.34)	<0.001	No	1.92 (0.74 ~ 4.96)	0.178
Non-Hispanic Black	0.42 (0.27 ~ 0.67)	<0.001	Chronic bronchitis		
Education Level(year)			Yes	1 (reference)	
<9	1 (reference)		No	2.67 (1.93 ~ 3.67)	<0.001
9–12	0.19 (0.08 ~ 0.43)	<0.001	High blood pressure		
>12	0.17 (0.08 ~ 0.39)	<0.001	Yes	1 (reference)	
Marital Status			No	1.61 (1.25 ~ 2.07)	<0.001
Living with a partner	1 (reference)		Calorie consumption(kcal/d)	1 (1 ~ 1)	0.296
Living alone	1.23 (0.97 ~ 1.55)	0.082	Total fat consumption(g/d)	1 (1 ~ 1)	0.651
Vigorous activity			Total cholesterol consumption(mg/d)	1 (1 ~ 1)	0.072
Yes	1 (reference)		Dietary vitamin B6 intake(mg/d)	1.16 (1.02 ~ 1.32)	0.026
No	0.97 (0.77 ~ 1.24)	0.835			
Unable to do activity	0.55 (0.28 ~ 1.06)	0.075			

TABLE 3 Association between dietary vitamin B6 intake and endometriosis.

			OR(95%CI)				
Variable	No.	Model 1	*p*-value	Model 2	*p*-value	Model 3	*p*-value
Dietary vitamin B6 intake(mg/d)	4,453	1.19 (1.04 ~ 1.37)	0.011	1.17 (1.02 ~ 1.34)	0.023	1.18 (1.01 ~ 1.39)	0.042
Q1(<0.94)	1,106	1(Ref)		1(Ref)		1(Ref)	
Q2(0.95–1.39)	1,118	1.25 (0.91 ~ 1.71)	0.173	1.22 (0.88 ~ 1.68)	0.229	1.22 (0.88 ~ 1.69)	0.242
Q3(1.40–1.99)	1,115	1.26 (0.92 ~ 1.73)	0.151	1.23 (0.89 ~ 1.69)	0.214	1.22 (0.86 ~ 1.73)	0.266
Q4(>1.99)	1,114	1.58 (1.13 ~ 2.2)	0.007	1.49 (1.06 ~ 2.09)	0.021	1.51 (1.01 ~ 2.24)	0.044
Trend test	4,453	1.15 (1.03 ~ 1.27)	0.01	1.13 (1.01 ~ 1.25)	0.027	1.13 (1 ~ 1.28)	0.06

Q, quartiles; OR, odds ratio; CI, confidence interval; Ref: reference. Model 1 adjusted for sociodemographic (age, race/ethnicity, education level, family income and marital status). Model 2 was adjusted for Model 1 plus BMI, smoking status, vigorous activity, moderate activity, alcohol consumption, use of birth control pills, high blood pressure, diabetes, coronary heart disease and chronic bronchitis. Model 3 was adjusted for Model 2 plus calorie consumption, total fat consumption, total cholesterol consumption and dietary supplements taken.

vigorous activity, moderate activity, marital status, family income, and BMI, the adjusted odds ratio (OR) values for dietary vitamin B6 intake and endometriosis in Q2 (0.96–1.39 mg/day), Q3 (1.40–2.05 mg/day), and Q4 (>2.05 mg/day) were 1.26 (95% CI: 0.91–1.73, $p = 0.16$), 1.27 (95% CI: 0.92–1.75, $p = 0.15$), and 1.61 (95% CI: 1.14–2.26, $p = 0.01$), respectively (Supplementary Table S1).

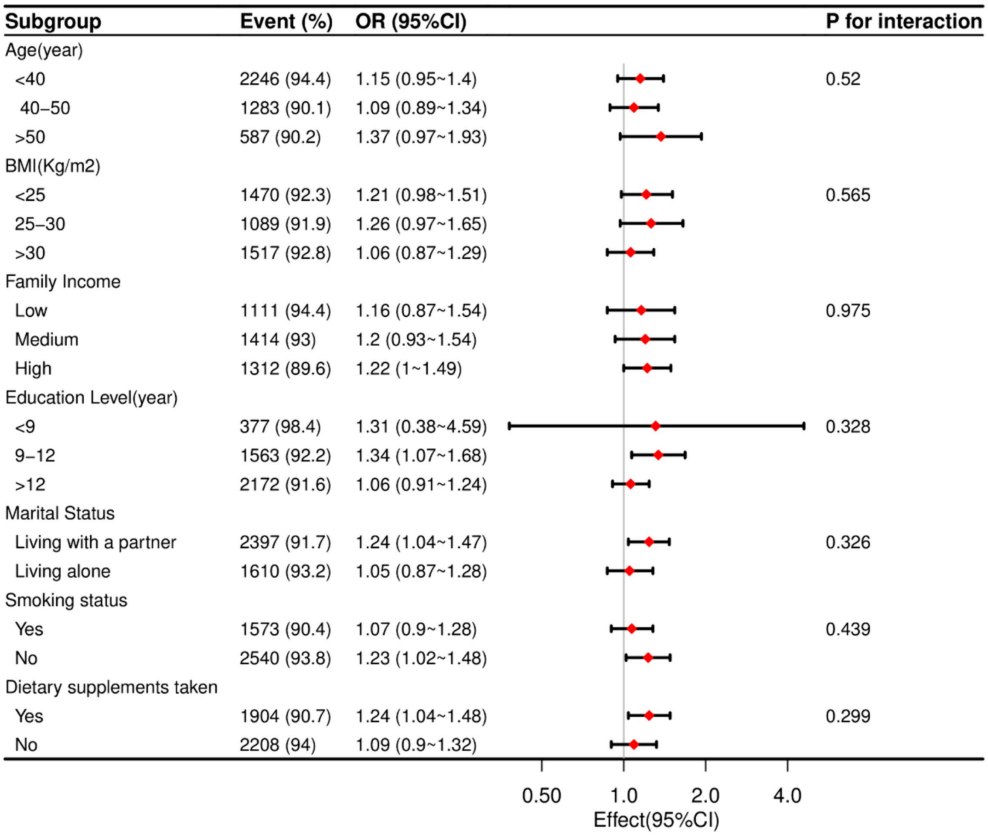

FIGURE 2
The relationship between dietary vitamin B6 intake and endometriosis according to basic features. Except for the stratification component itself, each stratification factor was adjusted for all other variables (age, marital status, race/ethnicity, education level, family income, BMI, vigorous activity, moderate activity, dietary supplements taken and smoking status).

Discussion

In this comprehensive cross-sectional study involving American adults, a noteworthy and previously unexplored positive correlation between endometriosis and dietary vitamin B6 intake was identified. Upon stratified analysis, these associations were found to be more significant among patients from high-income families, with 9–12 years of education, living with a partner, non-smokers, and those eligible for dietary supplements. Endometriosis has been frequently linked to inflammation (28), and evidence from studies suggested that women who have the disease exhibited greater levels of both systemic and localized inflammation. This led to the hypothesis that antioxidants, including vitamin B6, may contribute to the modulation of endometriosis, given its roles as an antioxidant regulator. This finding suggested a possible association between dietary factors and endometriosis, indicating a need for further research to explore this relationship in detail.

Given the observed correlation, it is essential to delve deeper into the specific biochemical mechanisms by which Vitamin B6 may influence endometriosis, particularly through its antioxidant and anti-inflammatory effects. Vitamin B6, present in forms such as pyridoxine, pyridoxal, and pyridoxamine, along with their phosphorylated derivatives, is known for its potent antioxidant properties, particularly in scavenging reactive oxygen species (ROS) (7, 29). This function is crucial in managing conditions like endometriosis, characterized by chronic inflammation and oxidative stress. The active form, pyridoxal 5′-phosphate (PLP), is vital in the transsulfuration pathway, where it helps convert homocysteine into cysteine, a precursor for the synthesis of glutathione (30). Glutathione, a key antioxidant, neutralizes ROS, thus protecting cells from oxidative damage and maintaining a balanced redox state (31, 32).

In endometriosis, elevated ROS levels lead to cellular damage, such as lipid peroxidation, DNA damage, and protein oxidation, which exacerbate inflammation in endometrial tissues (31). Vitamin B6 contributes to reducing oxidative stress and inflammation by promoting glutathione production, thereby mitigating these damaging effects (32, 33). This antioxidant defense and regulation of inflammation are crucial in controlling the progression and symptoms of endometriosis.

Additionally, research indicates that sufficient levels of Vitamin B6 can lower pro-inflammatory cytokines like IL-6 and TNF-α, which are significant mediators in inflammatory responses and are linked to worsening chronic inflammatory conditions (34). This anti-inflammatory property of Vitamin B6 suggests its potential therapeutic use in not only endometriosis but also other inflammatory diseases, such as rheumatoid arthritis and cardiovascular disorders (35).

Moreover, the enhancement of glutathione activity by Vitamin B6 underscores its role in maintaining cellular redox balance, which is

essential for preventing oxidative damage and managing inflammation in chronic diseases (36). Studies have shown that Vitamin B6 supplementation can improve markers of oxidative stress and reduce inflammation, demonstrating its protective effects against cellular damage (37). In summary, Vitamin B6 is integral in supporting glutathione synthesis and modulating inflammatory responses, offering potential benefits in managing chronic inflammatory diseases like endometriosis. Further research is necessary to fully understand these mechanisms and develop effective therapeutic applications.

Our study possessed several strengths. Firstly, it was the first research explicitly examining the association between dietary vitamin B6 consumption and endometriosis. We identified a positive correlation between dietary vitamin B6 intake and endometriosis, and these results remained robust after conducting multiple regression and sensitivity analyses. Our study had several limitations, though. Firstly, because of the cross-sectional design, we were unable to prove directionality or causation. The existence of unmeasured variables may have added confounding effects even when possible confounders were carefully adjusted for in the logistic regression model. Interestingly, our model included several dietary parameters in an attempt to reduce confounding effects. Secondly, it was still challenging to measure the complete body's vitamin B6 status precisely. We had inherent constraints in measuring vitamin B6 consumption because we relied on dietary questionnaires and 24-h memory. Because self-reported dietary data were prone to recall bias, they could not provide accurate assessments of a person's overall vitamin B6 level. The accuracy of these assessments could be improved in the future by adopting more advanced approaches for assessing vitamin B6 levels. Thirdly, the study's unique emphasis on citizens of the US meant that conclusions should be extrapolated with caution to other populations. Our results may not have been as broadly applicable as they could be due to the distinctive lifestyle and demographic characteristics of the US. Therefore, to confirm and broaden the applicability of our findings, carefully planned multicenter controlled studies including a variety of populations are essential. In conclusion, although our investigation illuminated the complex correlation between vitamin B6 intake in the diet and endometriosis, the limitations we found emphasized the necessity for additional study to fully comprehend this link and its wider consequences.

Conclusion

In this study, we identified a positive association between dietary vitamin B6 intake and the risk of endometriosis among American women aged 20 to 54 years. The findings suggest that higher dietary intake of vitamin B6 may be linked to an increased risk of developing endometriosis, particularly in women with specific sociodemographic characteristics. This study adds to the growing body of evidence highlighting the potential role of diet in the pathogenesis of endometriosis. However, given the observational nature of this study, further research is warranted to explore the underlying mechanisms and to establish causal relationships. Future studies should also consider a broader range of populations and utilize longitudinal designs to validate these findings and examine the long-term effects of dietary vitamin B6 on endometriosis risk.

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.

Ethics statement

Ethical approval was not required for the study involving humans in accordance with the local legislation and institutional requirements. Written informed consent to participate in this study was not required from the participants or the participants' legal guardians/next of kin in accordance with the national legislation and the institutional requirements.

Author contributions

LY: Conceptualization, Methodology, Writing – original draft. FL: Data curation, Formal analysis, Methodology, Software, Writing – original draft. BX: Data curation, Formal analysis, Resources, Software, Writing – review & editing. YS: Data curation, Formal analysis, Methodology, Software, Writing – review & editing. LC: Data curation, Formal analysis, Methodology, Software, Writing – review & editing. XW: Data curation, Formal analysis, Methodology, Validation, Visualization, Writing – review & editing. WT: Conceptualization, Writing – original draft, Writing – review & editing.

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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/fnut.2024.1407099/full#supplementary-material

References

1. Zondervan KT, Becker CM, Koga K, Missmer SA, Taylor RN, Vigano P. Endometriosis. *Nat Rev Dis Primers*. (2018) 4:9. doi: 10.1038/s41572-018-0008-5

2. Giampaolino P, Della Corte L, Foreste V, Barra F, Ferrero S, Bifulco G. Dioxin and endometriosis: a new possible relation based on epigenetic theory. *Gynecol Endocrinol*. (2020) 36:279–84. doi: 10.1080/09513590.2019.1698024

3. Soliman AM, Surrey ES, Bonafede M, Nelson JK, Vora JB, Agarwal SK. Health care utilization and costs associated with endometriosis among women with Medicaid insurance. *J Manag Care Spec Pharm*. (2019) 25:566–72. doi: 10.18553/jmcp.2019.25.5.566

4. Agarwal SK, Chapron C, Giudice LC, Laufer MR, Leyland N, Missmer SA, et al. Clinical diagnosis of endometriosis: a call to action. *Am J Obstet Gynecol*. (2019) 220:354.e1–e12. doi: 10.1016/j.ajog.2018.12.039

5. Vrolijk MF, Hageman GJ, van de Koppel S, van Hunsel F, Bast A. Inter-individual differences in pharmacokinetics of vitamin B6: a possible explanation of different sensitivity to its neuropathic effects. *Pharma Nutrit*. (2020) 12:100188. doi: 10.1016/j.phanu.2020.100188

6. Hellmann H, Mooney S. Vitamin B6: a molecule for human health? *Molecules*. (2010) 15:442–59. doi: 10.3390/molecules15010442

7. Zhang P, Suda T, Suidasari S, Kumrungsee T, Yanaka N, Kato N. Novel preventive mechanisms of vitamin B6 against inflammation, inflammasome, and chronic diseases In: VB Patel, editor. Molecular Nutrition. US: Academic Press (2020). 283–99.

8. Gylling B, Myte R, Schneede J, Hallmans G, Haggstrom J, Johansson I, et al. Vitamin B-6 and colorectal cancer risk: a prospective population-based study using 3 distinct plasma markers of vitamin B-6 status. *Am J Clin Nutr*. (2017) 105:897–904. doi: 10.3945/ajcn.116.139337

9. Kayashima T, Tanaka K, Okazaki Y, Matsubara K, Yanaka N, Kato N. Consumption of vitamin B6 reduces colonic damage and protein expression of HSP70 and HO-1, the anti-tumor targets, in rats exposed to 1,2-dimethylhydrazine. *Oncol Lett*. (2011) 2:1243–6. doi: 10.3892/ol.2011.370

10. Windebank AJ, Low PA, Blexrud MD, Schmelzer JD, Schaumburg HH. Pyridoxine neuropathy in rats: specific degeneration of sensory axons. *Neurology*. (1985) 35:1617–7.

11. Meyer HE, Willett WC, Fung TT, Holvik K, Feskanich D. Association of High Intakes of vitamins B6 and B12 from food and supplements with risk of hip fracture among postmenopausal women in the Nurses' health study. *JAMA Netw Open*. (2019) 2:e193591. doi: 10.1001/jamanetworkopen.2019.3591

12. Li Z, Zhang S, Wan L, Song X, Yuan D, Zhang S, et al. Vitamin B6 as a novel risk biomarker of fractured ankles. *Medicine*. (2021) 100:e27442. doi: 10.1097/MD.0000000000027442

13. Badawy AA. Pellagra and alcoholism: a biochemical perspective. *Alcohol Alcohol*. (2014) 49:238–50. doi: 10.1093/alcalc/agu010

14. Inubushi T, Takasawa T, Tuboi Y, Watanabe N, Aki K, Katunuma N. Changes of glucose metabolism and skin-collagen neogenesis in vitamin B6 deficiency. *Biofactors*. (2005) 23:59–67. doi: 10.1002/biof.5520230201

15. Vilter RW, Mueller JF, Glazer HS, Jarrold T, Abraham J, Thompson C, et al. The effect of vitamin B6 deficiency induced by desoxypyridoxine in human beings. *J Lab Clin Med*. (1953) 42:335–57.

16. Hsu CC, Cheng CH, Hsu CL, Lee WJ, Huang SC, Huang YC. Role of vitamin B6 status on antioxidant defenses, glutathione, and related enzyme activities in mice with homocysteine-induced oxidative stress. *Food Nutr Res*. (2015) 59:25702. doi: 10.3402/fnr.v59.25702

17. Aisen PS, Schneider LS, Sano M, Diaz-Arrastia R, van Dyck CH, Weiner MF, et al. High-dose B vitamin supplementation and cognitive decline in Alzheimer disease: a randomized controlled trial. *JAMA*. (2008) 300:1774–83. doi: 10.1001/jama.300.15.1774

18. Okereke OI, Cook NR, Albert CM, Van Denburgh M, Buring JE, Manson JE. Effect of long-term supplementation with folic acid and B vitamins on risk of depression in older women. *Br J Psychiatry*. (2015) 206:324–31. doi: 10.1192/bjp.bp.114.148361

19. Zhang DM, Ye JX, Mu JS, Cui XP. Efficacy of vitamin B supplementation on cognition in elderly patients with cognitive-related diseases. *J Geriatr Psychiatry Neurol*. (2017) 30:50–9. doi: 10.1177/0891988716673466

20. Laganà AA-O, Salmeri FM, Ban Frangež H, Ghezzi F, Vrtačnik-Bokal E, Granese R. Evaluation of M1 and M2 macrophages in ovarian endometriomas from women affected by endometriosis at different stages of the disease. *Gynecol Endocrinol*. (2020) 36:441–4. doi: 10.1080/09513590.2019.1683821

21. Cirillo M, Coccia ME, Petraglia F, Fatini C. Role of endometriosis in defining cardiovascular risk: a gender medicine approach for women's health. *Hum Fertil (Camb)*. (2022) 25:745–53. doi: 10.1080/14647273.2021.1919764

22. Foster E, Lee C, Imamura F, Hollidge SE, Westgate KL, Venables MC, et al. Validity and reliability of an online self-report 24-h dietary recall method (Intake24): a doubly labelled water study and repeated-measures analysis. *J Nutr Sci*. (2019) 8:e29. doi: 10.1017/jns.2019.20

23. Liu HA-O, Wang L, Chen C, Dong ZA-O, Yu S. Association between dietary niacin intake and migraine among American adults: National Health and nutrition examination survey. *Nutrients*. (2022) 14:3052. doi: 10.3390/nu14153052

24. Xiao Q, Cai B, Yin A, Huo H, Lan K, Zhou G, et al. L-shaped association of serum 25-hydroxyvitamin D concentrations with cardiovascular and all-cause mortality in individuals with osteoarthritis: results from the NHANES database prospective cohort study. *BMC Med*. (2022) 20:308. doi: 10.1186/s12916-022-02510-1

25. Zhang YY, Qiu HB, Tian JW. Association between vitamin D and hyperuricemia among adults in the United States. *Front Nutrit*. (2020) 7:592777. doi: 10.3389/fnut.2020.592777

26. Agriculture ArRSUDo. What We Eat in America: Data Tables. (2024). Available from: https://www.ars.usda.gov/northeast-area/beltsville-md-bhnrc/beltsville-human-nutrition-research-center/food-surveys-research-group/docs/wweia-data-tables/ (Accessed March 20, 2024).

27. Yang Q, Zheng J, Chen W, Chen X, Wen D, Chen W, et al. Association between preadmission metformin use and outcomes in intensive care unit patients with sepsis and type 2 diabetes: a cohort study. *Front Med (Lausanne)*. (2021) 8:640785. doi: 10.3389/fmed.2021.640785

28. Symons LK, Miller JE, Kay VR, Marks RM, Liblik K, Koti M, et al. The Immunopathophysiology of endometriosis. *Trends Mol Med*. (2018) 24:748–62. doi: 10.1016/j.molmed.2018.07.004

29. Vrolijk MF, Opperhuizen A, Jansen E, Hageman GJ, Bast A, Haenen G. The vitamin B6 paradox: supplementation with high concentrations of pyridoxine leads to decreased vitamin B6 function. *Toxicol In Vitro*. (2017) 44:206–12. doi: 10.1016/j.tiv.2017.07.009

30. Matxain JM, Padro D, Ristila M, Strid Å, Eriksson LA. Evidence of high• OH radical quenching efficiency by vitamin B6. *J Phys Chem B*. (2009) 113:9629–32. doi: 10.1021/jp903023c

31. Abramiuk M, Mertowska P, Frankowska K, Świechowska-Starek P, Satora M, Polak G, et al. How can selected dietary ingredients influence the development and progression of endometriosis? *Nutrients*. (2024) 16:154. doi: 10.3390/nu16010154

32. Arab A, Karimi E, Vingrys K, Kelishadi MR, Mehrabani S, Askari G. Food groups and nutrients consumption and risk of endometriosis: a systematic review and meta-analysis of observational studies. *Nutr J*. (2022) 21:58. doi: 10.1186/s12937-022-00812-x

33. Roshanzadeh G, Jahanian Sadatmahalleh SA-O, Moini A, Mottaghi A, Rostami F. The relationship between dietary micronutrients and endometriosis: a case-control study. *Int J Reproductive BioMed*. (2023) 21:333. doi: 10.18502/ijrm.v21i4.13272

34. Back E, Biesalski HK. Vitamin B6 In: S Offermanns and W Rosenthal, editors. Encyclopedia of molecular pharmacology. Berlin, Heidelberg: springer (2008). 1290–1.

35. Ueland PM, McCann A, Midttun O, Ulvik A. Inflammation, vitamin B6 and related pathways. *Mol Asp Med*. (2017) 53:10–27. doi: 10.1016/j.mam.2016.08.001

36. Rosenberg J, Ischebeck T, Commichau FM. Vitamin B6 metabolism in microbes and approaches for fermentative production. *Biotechnol Adv*. (2016) 35:31–40. doi: 10.1016/j.biotechadv.2016.11.004

37. Parra M, Stahl S, Hellmann H. Vitamin B_6 and its role in cell metabolism and physiology. *Cells*. (2018) 7:84. doi: 10.3390/cells7070084

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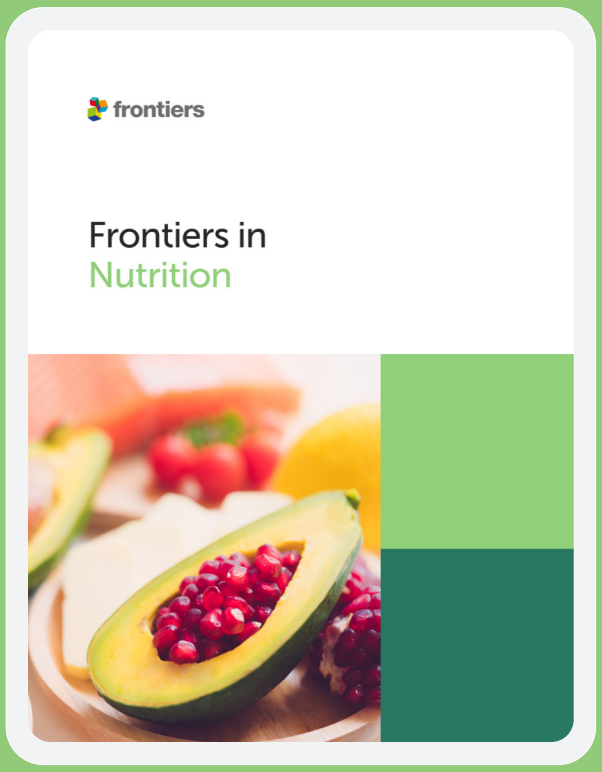

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