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Devineé Lingo

Alyssa Perri

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The Effect of Diet on the Variation of Blood and Urine Heavy Metal Biomarkers Among National Health and Nutrition Examination Survey (NHANES) Subjects:

A Secondary Data Analysis

Devineé Lingo

Alyssa Perri

Edward Bitok

Gurinder Bains

Noha Daher

JeJe Noval

Department of Nutrition and Dietetics, Loma Linda University

Abstract

Background: Heavy metals are non-essential elements that provide no biological benefit to the human body. Heavy metals such as lead, mercury, arsenic, and cadmium are toxic to humans even in trace amounts. Humans are exposed to various heavy metals through ingestion of contaminated food and water supplies. Heavy metals can accumulate in body organs and displace essential minerals leading to detrimental health effects such as neurological, endocrine, and immune dysfunction, that can result in long-term damage.

Objective: The purpose of this graduate student research study was to examine the effect of diet on the variation of blood and urine lead, mercury, arsenic, and cadmium biomarkers among National Health and Nutrition Examination Survey (NHANES) participants.

Design: Secondary Data Analysis

Statistics: Using SPSS version 25.0, all food groups were stratified, and the data was fitted to a crude linear regression. Means of heavy metals in blood and urine were computed and percent variability of heavy metals accounted for by diet were expressed as R2 values. All analysis were considered significant at $p < 0.05$.

Results: Diet accounts for a small variation in blood and urine heavy metal levels (2.2-7.6%). Blood mercury and blood cadmium measured higher than the acceptable limits at 1.10 (±2.24) and 0.31 (±0.44) micrograms, respectively.

Conclusion: Factors including industrial exposure, air or water pollution, cigarette smoke, household exposure, medications, lead-based paint, improperly coated foods, and occupation may account for >90% variation in blood and urine heavy metal levels. Heavy metals have been shown to displace our bodies essential minerals such as zinc, magnesium, and calcium. It is extremely important that health professionals educate their patients about heavy metals. Further

statistical analysis should be conducted to account for the effect of confounding factors and to further elucidate the relationships between foods and heavy metals present in blood and urine biomarkers.

Introduction

Metals are fundamental elements of various anatomical and physiological mechanisms in the human body. Certain metals are considered essential nutrients and are required in a well-balanced diet.[1] Essential metals can be categorized into two categories: macrominerals and trace minerals.[1] The macrominerals include sodium, potassium, calcium, magnesium, phosphorus, chloride, and sulfur. The trace minerals consist of iodine, selenium, molybdenum, chromium, cobalt, copper, fluorine, manganese, iron, and zinc.[1] Conversely, there are nonessential metals, known as heavy metals, that have been found to provide no biological benefit to the human body.[2] These metals that are toxic in trace amounts include lead, mercury, arsenic, and cadmium.[2] Therefore, while certain metals are considered essential components of a well-balanced diet, others have no place in the diet and can be harmful, or toxic, if they remain in the body over time.

Background

Heavy metal toxicity occurs when these nonessential metals are ingested, bypass metabolism, fail to be excreted, and accumulate in body tissues and organs.[3] Since heavy metals are both geologically and anthropogenically derived, they often show up in the food supply via plant foods grown in contaminated soil and bioaccumulate in animals who consume those plants.[3,4] Humans are then exposed to various heavy metals through ingestion of contaminated food and water supplies. Other sources of exposure include polluted air, certain household products, and some beauty products when absorbed through the surface of the skin.[3] While entirely eliminating exposure is impossible, it is important to recognize the presence of heavy metals in everyday life and to become aware of the deleterious health effects that they may cause.

The acuity or chronicity of heavy metal toxicity depend upon the route and duration of exposure, which can lead to specific disorders. Heavy metal exposure imparts oxidative stress that arises from free radical formation.[4] These metals also exhibit their toxic effects by binding to the sulphydryl groups in proteins, thus disrupting normal cell function.[5] Cadmium, lead, arsenic, and mercury are known to accumulate in the kidneys, nervous system, liver, and bones. Under these mechanisms, toxic metals have the potential to displace essential minerals, compromise body organs, cause neurological dysfunction, and disrupt endocrine and immune system functioning, resulting in long-term damage.[5]

Heavy metals have previously been identified in the human diet by past research studies. Dietary sources of lead include whole milk and apples,[6] while contaminated drinking water and dietary supplements are also main sources of exposure.[3] Arsenic can be found in rice, grains, whole fruits, juices, and drinking water due to contamination from metal-containing pesticides.[6] Cadmium is ingested by way of cereal grains, tubers, pulses, and rice.[6] Mercury, presenting as ethyl, methyl, or organic mercury is present in marine foods such as fatty fish, as well as the livers of lean fish.[1]

Heavy metals from the environment biomagnify along the food chain. Biomagnification is the increased toxic concentration that takes place as bigger organisms or animals consume smaller organisms. This undesirable process is the key element that poses a potential health risk to consumers.[7] Species higher up on the food chain are at greater risk of biomagnification than their lower counterparts.[7] This explains the phenomena of larger fish, such as halibut and swordfish, containing significantly larger amounts of mercury than small fish such as tilapia.

With this principal in mind, the same biomagnification can potentially be expected within animal foods commonly consumed today.

Research surrounding heavy metals has historically focused on assessing the heavy metal content in various foods. The typical study designs include market basket analyses and single food or food group dietary assessments.[6] However, large-scale studies on heavy metal accumulation in the human body are limited. A novel dietary wide association study (DWAS) was conducted in 2014 by Davis et al. to assess the amounts of heavy metals in various foods.[6] The study found that diet accounted for more of the variation in mercury and arsenic than lead and cadmium.[6] While this research was the first of its kind, more dietary wide association studies need to be conducted to assess the relationship between heavy metal exposure and dietary intake.

Due to a lack of awareness of daily consumption of heavy metals, the problem this study has sought to address is the biochemical accumulation of toxic heavy metals in the body that can lead to deleterious health effects. Since there is limited literature exploring heavy metal load in correlation with dietary intake, this study examines the extent of heavy metal accumulation as a result of consuming foods contaminated by heavy metals. Therefore, the purpose of this graduate student research study was to examine the effect of diet on the variation of blood and urine lead, mercury, arsenic, and cadmium biomarkers among National Health and Nutrition Examination Survey (NHANES) participants.

Methods

The National Health and Nutrition Examination Survey (NHANES), administered by the National Center of Health Statistics (NCHS), was conducted to evaluate the health and nutritional status of US adults and children. Data was collected using a complex, cross-sectional, multistage probability design on civilians in various counties across the 50 states and D.C. each year.[8] Interviews, physical examinations, and laboratory data were all collected and uploaded to the CDC website. The data collected is representative of the noninstitutionalized US population and is a widely used source of National health statistics, and therefore is publicly available for download at http://www.cdc.gov/nchs/nhanes.htm. It has been de-identified for participant safety.[8] The NHANES broadly and largely assessed racial subgroups including Hispanic, Non-Hispanic black, Non-Hispanic Asian, and Non-Hispanic white in order to increase the precision and reliability of estimates regarding various health status indicators.[8]

The current study utilized the demographic data, dietary data, laboratory data, and questionnaire data from the 2013-2014 cycle of NHANES. The structured dietary pattern questionnaires were used to gauge how the participants usually ate. In addition, the first day in-person 24-hour recall was used to reflect the foods consumed the day heavy metal biomarkers were taken. The second 24-hour recall was conducted over the phone approximately one week after the first 24-hour recall and was, therefore, excluded. The heavy metal biomarkers were used to assess the level of lead, mercury, arsenic, and cadmium present in the participants' blood and urine.

Dietary Intake

Table 1. Characteristics of Study Participants NHANES 2013-2014, describes the participants included in this secondary data analysis study. Precisely 10,175 adults and children ages one year and older were included. There was an equal distribution of participants based on age, gender, education level, marital status, and household income.

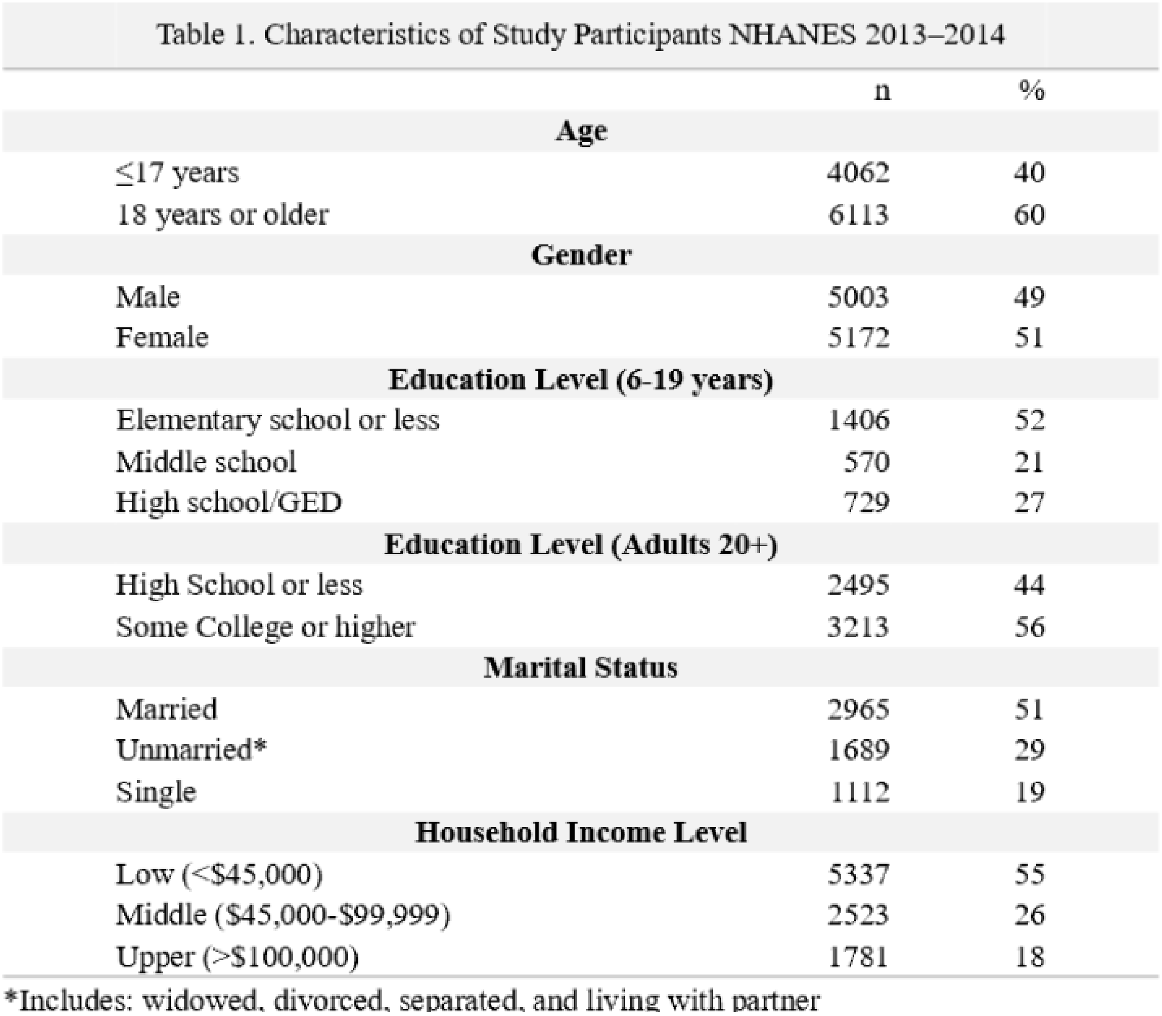

Table 1. Characteristics of Study Participants NHANES 2013–2014

	n	%
Age		
≤17 years	4062	40
18 years or older	6113	60
Gender		
Male	5003	49
Female	5172	51
Education Level (6-19 years)		
Elementary school or less	1406	52
Middle school	570	21
High school/GED	729	27
Education Level (Adults 20+)		
High School or less	2495	44
Some College or higher	3213	56
Marital Status		
Married	2965	51
Unmarried*	1689	29
Single	1112	19
Household Income Level		
Low (<$45,000)	5337	55
Middle ($45,000-$99,999)	2523	26
Upper (>$100,000)	1781	18

*Includes: widowed, divorced, separated, and living with partner

All NHANES participants were eligible for two 24-hour dietary recall interviews. Food consumption information was collected from participants using structured questionnaires administered by trained interviewers. The first dietary recall interview was collected in-person and the second was collected by telephone 3 to 10 days after the first. The primary focus of this study was dietary data from the first day, which included details about each food/beverage consumed by participants; whether the food was eaten in combination with other foods; meal name; amount of food/beverage consumed, in grams, and eight-digit USDA food code, among other measurements. The USDA food codes were used along with The What We Eat in America (WWEA) codes to classify foods into eight food groups: dairy; meat and poultry; seafood; egg; legumes; grains; fruits and vegetables; and fried foods. Foods that were eaten in combination were analyzed separately, which accounted for approximately 43% of foods consumed by our participants. These include beverages with additions; cereal with additions; bread/baked products with additions; salads; sandwiches; frozen meals; ice cream/frozen yogurt; dried beans or vegetables with additions; fruit with additions; and meat, fish, poultry.

Statistical Analysis

The mean values and the standard deviations of all the heavy metals were calculated by using Statistical Packages for Social Sciences (SPSS version 25.0. Armonk, NY: IBM Corp). As for the comparison of general characteristics of participants by ethnicity, their significance was verified through the ANOVA test. When the ANOVA test indicated significant differences among groups, those differences were further evaluated using the Tukey post-hoc test. Crude regression models were fitted for dietary food groups (predictor variables) and heavy metals (outcome variables). Foods that did not contribute significantly to the model were excluded in subsequent analysis. The proportion of variance (R^2) were derived to explain the extent to which differences in heavy metals in blood and urine can be explained by food groups. Standardized correlation coefficients (*Beta*) were used to determine the magnitude of the coefficients, i.e., to determine which foods had more effect on the model. All statistical analyses were considered significant at the level of $\alpha \leq 0.05$.

Results

Table 2. Means (SD) of Heavy Metals in Blood and Urine of the NHANES 2013-2014 Participants summarizes the average levels and standard deviation (SD) of each heavy metal in the study population. The table also displays minimum and maximum levels for each heavy metal as well as the acceptable reference ranges for each metal. Of the four heavy metals assessed, blood mercury and blood cadmium measured higher than the reference ranges at 1.10

(±2.24) and 0.31 (±0.44) micrograms, respectively. The maximum measurements that are higher than the reference ranges are indicative of heavy metal toxicity within the population.

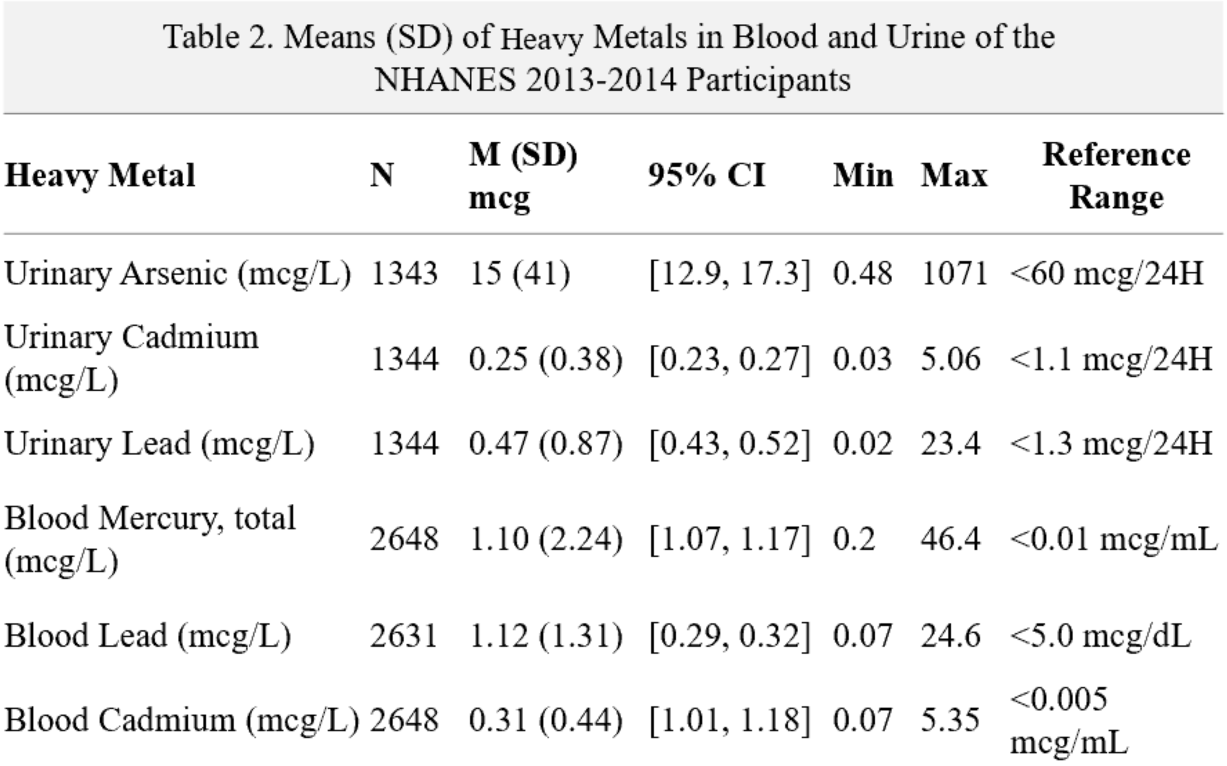

Table 2. Means (SD) of Heavy Metals in Blood and Urine of the NHANES 2013-2014 Participants

Heavy Metal	N	M (SD) mcg	95% CI	Min	Max	Reference Range
Urinary Arsenic (mcg/L)	1343	15 (41)	[12.9, 17.3]	0.48	1071	<60 mcg/24H
Urinary Cadmium (mcg/L)	1344	0.25 (0.38)	[0.23, 0.27]	0.03	5.06	<1.1 mcg/24H
Urinary Lead (mcg/L)	1344	0.47 (0.87)	[0.43, 0.52]	0.02	23.4	<1.3 mcg/24H
Blood Mercury, total (mcg/L)	2648	1.10 (2.24)	[1.07, 1.17]	0.2	46.4	<0.01 mcg/mL
Blood Lead (mcg/L)	2631	1.12 (1.31)	[0.29, 0.32]	0.07	24.6	<5.0 mcg/dL
Blood Cadmium (mcg/L)	2648	0.31 (0.44)	[1.01, 1.18]	0.07	5.35	<0.005 mcg/mL

Table 3. Heavy Metal Regression Analysis summarizes the major results of this study. The second column shows the percent variability in heavy metals that can be explained by the contributing food groups, listed in the middle column for each heavy metal.

Table 3. Heavy Metal Regression Analysis

Heavy Metal	**% Explained by Diet**	**Contributing Groups**	***β***	**p-value**
Lead - Blood	3.2%	Beverages	0.116	0.000
		Eggs	0.086	0.000
		Meat/poultry/fish	0.069	0.001
		Fried Foods	-0.046	0.031
ANOVA				0.000
Cadmium - Blood	7.6%	Beverages	0.203	0.000
		Sandwiches	0.091	0.000
		Dried Beans	0.059	0.006
		Fruit and Vegetables	-0.081	0.000
		Dairy	-0.126	0.000
ANOVA				0.000
Cadmium - Urine	2.2%	Beverages	0.117	0.000
		Legumes	0.092	0.002
ANOVA				0.000
Total Mercury - Blood	6.5%	Seafood	0.129	0.000
		Salad	0.102	0.000
		Soup	0.102	0.000
		Dried Beans	0.074	0.000
		Beverages	0.057	0.008
		Grains	0.053	0.014
		Tortilla Products	-0.049	0.019
		Dairy	-0.058	0.006
ANOVA				0.000
Total Arsenic - Urine	4.7%	Seafood	0.170	0.000
		Cereal	0.103	0.000
		Chips	0.094	0.001
ANOVA				0.000

Starting with blood lead, 3.2% of the variability can be explained by the food groups: beverages, eggs, meat poultry fish, and fried foods. There was a 7.6% variation in blood cadmium levels explained by the groups: beverages, sandwiches, dried beans, fruits and vegetables, and dairy. There was a 2.2% variation in urine cadmium levels that can be explained by beverages and legumes. There was a 6.5% variation in total blood mercury explained by: seafood, salad, soup, dried beans, beverages, grains, grains, tortilla products, and dairy. Finally, there was a 4.7% variation in urinary arsenic that can be explained by the groups: seafood, cereal, and chips. The groups listed in the "contributing groups" column are listed in order of contribution from highest to lowest.

The positive Beta values, such as for blood lead from beverages (0.116), implies that beverages are a risk factor for increased blood lead levels. Conversely, negative beta values such as blood cadmium from fruits and vegetables (-0.081) signifies a protective effect of fruits and vegetables against increased blood cadmium.

It is important to note that these results must be interpreted with caution, as they are not adjusted for confounding factors. As evident by the previously described results, most food groups are risk factors to the various heavy metals. Surprisingly, fried foods, dairy, and fruits and vegetables have a protective effect against heavy metals. The take home message here is: heavy metals are in the foods we eat daily.

Discussion

Heavy metals are naturally derived contaminates that are present in our food supply at varying levels. The differing levels of heavy metal contamination depend upon the amount that is present in the air, water, and soil used to grow crops, the crop's ability to absorb specific elements, as well as exposure from industrial processes.[9] Humans are particularly vulnerable to heavy metal accumulation in various body tissues, as a result of the increased concentration of heavy metals that occurs through the process of biomagnification. However, other sources of heavy metal exposure include household products, medications, cigarette smoke, lead-based paint, occupation, and environmental pollution. Therefore, the main outcome of this study revealed to what extent diet contributes to the variation of blood and urine lead, mercury, arsenic, and cadmium biomarkers among the 2013-2014 NHANES participants. Within this study, diet accounted for a small variation in blood and urine biomarkers for all four heavy metals. Similar to the current findings, the first and only dietary-wide association study (DWAS) conducted by Davis et. al found that lead accounted for an 1.6-2.9% variation, mercury an 4.5-10.5% variation, arsenic an 8.5-11.5% variation, and cadmium an 0.6-1.4% variation in blood and urine biomarkers among the 2005-2006 and 2007-2008 NHANES adults and children.[6] Nevertheless, while the present study found that diet accounted for more of the variation in cadmium and mercury, Davis et. al found more of the variation from arsenic and mercury.[6]

Of the food groups that contributed to the variation of heavy metal biomarkers, beverages were found to be the primary risk factor for increased lead and cadmium levels. Moreover, the positive beta values indicate that a unit increase in consumption of beverages was associated with a unit increase in blood and urinary lead and cadmium levels. According to the Food and Drug Administration (FDA), lead can inadvertently contaminate water used in food and beverage production during the manufacturing process due to plumbing that contains lead.[10] In addition, lead may be present in pottery or other food contact surfaces that can leach into beverages during preparation or storage.[10] Between the 1970s-1990s lead concentrations in the food supply dropped significantly; however, like all heavy metals, lead does not biodegrade from the environment over time.[10] Unfortunately, lead is often used in products that come from other countries. The major health effects of lead exposure include neurotoxic effects on intelligence, decreased memory, CVD diseases, reproductive toxicity, hemolytic anemia, lung cancer, and bladder cancer.[3] Comparable to lead, cadmium enters water supplies from mining, industry, burning coal, and household wastes.[11] In addition, cadmium is emitted as a byproduct from the smelting of zinc, lead, and copper ores. Cadmium is also used to manufacture plastics.[11] Therefore, it may be present in beverages due to contaminated water or plastics. Elevated cadmium exposure has been shown to result in neurodegenerative diseases, end stage renal disease, breast cancer, prostate cancer, demineralized bones, and diabetes.[3] In recent research, Izah et al. reported that heavy metal concentrations in commercially packaged beverages included both lead and cadmium at levels that exceed the maximum contaminant levels recommended by the World Health Organization (WHO).[13] Specifically, the WHO recommends that lead and cadmium levels should not exceed 0.01mg/L and 0.003 mg/L, respectively. However, research has shown detectable cadmium levels in various beverages ranging from 0.01-0.158 mg/L, while lead levels ranged from 0.002-1.21 mg/L.[14-18] Therefore, the presence of lead and cadmium in various beverages is a concern worth further investigation.

As expected, seafood was revealed to be the primary risk factor for increased levels of both mercury and arsenic. Previous studies have identified the main sources of mercury exposure are from fish and shellfish consumption, outgassing from dental amalgam, vaccines containing thiomersal, and occupational exposure, such as through agricultural products, industry, and gold mining.[19] While there are multiple forms of mercury in nature, all forms of mercury are poisonous.[19] Mercury toxicity can produce symptoms from nervous, renal, cardiovascular, respiratory systems, and skin, but any organ may be a target, such as the bone marrow.[3,19] This poses multiple health concerns, especially when toxic levels of mercury build up in body tissues.

A systematic review of 6,601 people revealed that the non-occupational pathways of exposure for mercury were: food (5,243), home near gold mining plus food (291), amalgam (454), environmental (82), medicine (54), bringing mercury home (29), suicide attempt (15), school (2), maternal exposure (2), aesthetical (1) and thermometer (1).[20] The main pathways of exposure among children and teenagers were: food (4,800), environmental (82), home near gold mining plus food (70) and bringing mercury home.[20] The researchers found that mercury's exposure biomarker was above the recommended threshold in about 81.87% of all cases.[20] In our current study, we were not able to control for such variables, like environmental and household exposures. These routes of exposure are important to consider as our study found that diet accounted only for a small variation in blood and urine heavy metal biomarkers. This means outside factors are responsible for the remaining portion of heavy metal exposure.

Similar to mercury, seafood is the main source of organic arsenic exposure from the diet.[21] Inorganic arsenic, however is largely found in drinking water, rice, and cereal grains worldwide.[21] Inorganic arsenic is a known human carcinogen that has been previously associated with developmental, cardiovascular, and metabolic disease.[3,21] A cross-sectional study using data from NHANES showed that participants in the highest quartiles of urine arsenic had higher 10-year predicted ASCVD risk than in the lowest quartiles.[21] This finding signifies the importance of identifying the sources of heavy metal contamination in order to reduce disease risk, such as cardiovascular disease.

Although certain food groups positively contributed to the variation of heavy metal blood and urine levels, there were food groups that displayed potential protective effects against heavy metal accumulation. One study looking at the concentrations of heavy metals in raw, organic cow's milk found that the breed of cow does effect heavy metal concentration.[22] The milk of Simmental cows, for example, had significantly lower concentration of Pb and Cd ($P < 0.001$) compared to Holstein-Frisian cows.[22] Relating back to our results, dairy was associated with a protective effect against heavy metals, specifically cadmium and mercury.[22] One potential mechanism is that casein micelles have been found to have a high ability to bind inorganic mercury, which aids with excretion.[23] In addition to the protective effects of dairy, we found that fruits and vegetables were associated with lower heavy metal biomarkers, as the negative beta value indicated that a unit increase in the intake of fruits and vegetables correlates with a unit decrease in blood cadmium levels. Research has shown that fruits and vegetables contain various plant compounds that support the body's natural detoxification pathways by helping to reduce the absorption and reabsorption of heavy metals.[24-25] Insoluble dietary fiber is a plant compound that has been revealed to interrupt the enterohepatic recirculation of heavy metals, as well as modulate intestinal flora.[24,26] Furthermore, cellulose, hemicellulose, lignin, and pectin in fiber containing foods hold the ability to bind heavy metal ions.[26] Natural plant polymers such as algal polysaccharides alginate and chlorella have also been shown to reduce heavy metals in the human body.[24] In addition, sulfur-containing peptides found in cruciferous vegetables (e.g., broccoli) and alliums (e.g., garlic) have a strong affinity for toxic heavy metals; therefore, they aid in heavy metal excretion. The consumption of the herb cilantro has also been shown to enhance heavy metal excretion and decrease the absorption of heavy metals into bone.[24] Antioxidants such as beta-carotene, vitamin C, and vitamin E found in plant foods help to fight free radical damage caused by heavy metals.[25] Therefore, fruit and vegetable intake imparts beneficial plant compounds that may reduce heavy metal accumulation.

Limitations to the study primarily surround the data analysis. Confounding factors were not accounted for, which led to the use of crude regression models. Future studies should consider controlling for confounding factors such as lead exposure through water pipes, serum cotinine levels to account for cadmium exposure through cigarette smoke, homes built prior to 1978 (the year lead paint was banned), cosmetic use, dental amalgam fillings, occupation, and urinary creatinine to account for urinary dilution, among other factors. Due to the nature of the statistical analysis conducted for this study, it is not possible to claim cause and effect. The results of this study can only be expressed as probable associations.

Conclusion

From our preliminary findings, diet accounts for a small variation (2-8%) in blood and urine heavy metal biomarkers among the 2013-2014 NHANES participants. Therefore, the majority of heavy metal exposure can result from multiple factors including, but not limited to, industrial exposure, air or water pollution, cigarette smoke, household exposure, medications, lead-based paint, improperly coated foods, and occupation. Collectively, these factors may account for >90% variation in blood and urine heavy metal levels. Foods may not account for a large variation in heavy metal blood and urine levels, but heavy metals have been shown to displace our bodies essential minerals such as zinc, magnesium, and calcium. Therefore, it is important for health professionals to educate their patients and clients on heavy metals, their sources, and the effects that they have on the human body. Further statistical analysis should be conducted to account for the effect of confounding factors and to further elucidate the relationships between foods and heavy metals present in blood and urine biomarkers. This study approach may be used as a method for identifying and monitoring heavy metal exposure through diet in the US population. The current practices of regulatory agencies, such as the FDA, that measure for heavy metals at the point of food distribution may be better supported by the analyzed in vivo data as seen in our study.

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