Urinary Metals and Type 2 Diabetes Mellitus

by

Xin Wang

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Doctoral Committee:

Associate Professor Sung Kyun Park, Chair
Professor Stuart Batterman
Professor Siobán D. Harlow
Assistant Professor Carrie A. Karvonen-Gutierrez
Professor Bhramar Mukherjee

Xin Wang

xwangsph@umich.edu

ORCID iD: 0000-0002-0851-6605

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Clinical Centers: University of Michigan, Ann Arbor – Siobán Harlow, PI 2011 – present, MaryFran Sowers, PI 1994-2011; Massachusetts General Hospital, Boston, MA – Joel Finkelstein, PI 1999 – present; Robert Neer, PI 1994 – 1999; Rush University, Rush University Medical Center, Chicago, IL – Howard Kravitz, PI 2009 – present; Lynda Powell, PI 1994 – 2009; University of California, Davis/Kaiser – Ellen Gold, PI; University of California, Los Angeles – Gail Greendale, PI; Albert Einstein College of Medicine, Bronx, NY – Carol Derby, PI 2011 – present, Rachel Wildman, PI 2010 – 2011; Nanette Santoro, PI 2004 – 2010;

University of Medicine and Dentistry – New Jersey Medical School, Newark – Gerson Weiss, PI 1994 – 2004; and the University of Pittsburgh, Pittsburgh, PA – Karen Matthews, PI.

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Central Laboratory: University of Michigan, Ann Arbor – Daniel McConnell (Central Ligand Assay Satellite Services).

SWAN Repository: University of Michigan, Ann Arbor – Siobán Harlow 2013 - Present; Dan McConnell 2011 - 2013; MaryFran Sowers 2000 – 2011.

Coordinating Center: University of Pittsburgh, Pittsburgh, PA – Maria Mori Brooks, PI 2012 - present; Kim Sutton-Tyrrell, PI 2001 – 2012; New England Research Institutes, Watertown, MA - Sonja McKinlay, PI 1995 – 2001.

Steering Committee: Susan Johnson, Current Chair

Chris Gallagher, Former Chair

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Table of Contents

List of Tables

List of Figures

Abstract

Type 2 diabetes mellitus is a major global health concern. Most epidemiologic studies of diabetes risk factors to date have focused on genetics, unhealthy diets, and physical inactivity. The potential contributions of environmental chemicals, specifically metals, to the epidemic of diabetes have received less attention. The general population is exposed to a myriad of metals through food, drinking water and ambient air. Biological evidence suggests that exposure to metals may play a role in the development of diabetes. Selected metals, especially arsenic, have been examined in relation to diabetes risk in human populations but little is known about the role of other metals in diabetes risk. This dissertation examined exposures to multiple metals and their impacts on diabetes risk in midlife women who may experience unfavorable changes in glucose metabolism over menopausal transition using data from the Study of Women's Health Across the Nation (SWAN).

In aim 1, we examined the distributions of 21 urinary metal concentrations, identified subgroups of women with different exposure patterns to metal mixtures, and evaluated associations of demographic, socioeconomic, lifestyle, dietary characteristics with each urinary metal, as well as with exposure patterns to metal mixtures, in 1335 SWAN participants. We found that Asian women, both Chinese and Japanese, had higher urinary concentrations of arsenic, cadmium, copper, mercury, molybdenum, lead and thallium, compared with other race/ethnic groups, independent of all other factors. Two distinct overall exposure patterns to metal mixtures- "high" vs. "low" -- were identified using the k-means clustering method.

Women in the “high” overall exposure pattern were more likely to be Asians, current smokers, and to report high intake of seafood and rice.

In aim 2, we evaluated associations of individual metals measured in urine and overall exposure patterns as metal mixtures with incident diabetes over 16 years of follow-up in 1237 SWAN participants. After multivariable adjustment, the hazard ratios (HR) (95%CI) of diabetes associated with each doubling increase in urinary metal concentrations were 1.24 (1.14, 1.35) for arsenic and 1.23 (1.08, 1.40) for lead, in Cox proportional hazards models. A doubling in urinary excretion of zinc was associated with higher diabetes risks (HR=1.31, 95%CI 1.11, 1.55). The multivariable adjusted HR of diabetes associated with the “high” exposure pattern to metal mixtures compared with the “low”, which were generated by k-means clustering, was 1.46 (1.11, 1.91).

In aim 3, we further examined associations between metal mixtures and longitudinal changes in insulin resistance and β-cell dysfunction, important etiopathogenic underlying mechanisms of diabetes, among 1262 SWAN participants. Using adaptive elastic-net models, urinary copper, lead, and zinc were associated with higher homeostatic model assessments for insulin resistance (HOMA-IR) at baseline, whereas molybdenum was associated with lower baseline HOMA-IR. Urinary zinc was also associated with a faster rate of increase in HOMA-IR. Urinary arsenic and zinc were associated with lower baseline HOMA β-cell function (HOMA-β), whereas cobalt was associated with higher baseline HOMA-β. Arsenic was also associated with a faster rate of decline in HOMA-β.

Overall, this dissertation suggests that metal exposures differ by race/ethnicity, as well as by sociodemographic, lifestyle, and dietary factors, and that metal exposures may influence diabetes risk, possibly through effects on insulin resistance and β-cell dysfunction, even decades

before its onset. Future studies need to confirm these findings to strengthen the foundation of knowledge on environmental chemicals as risk factors of diabetes.

Chapter I. Introduction

The Public Health Problem: Type 2 Diabetes Mellitus

Type 2 diabetes mellitus is a metabolic disease characterized by dysregulation of carbohydrate, lipid and protein metabolism, and results from impaired insulin secretion, insulin resistance or a combination of both (DeFronzo et al. 2015). Of the three major types of diabetes, type 2 diabetes mellitus (hereafter called diabetes) is far more common (accounting for more than 90% of all cases) than either type 1 diabetes mellitus or gestational diabetes (IDF 2020). The burden of diabetes is increasing globally. In 2019, 463 million people worldwide (6.1%) were estimated to have diabetes (IDF 2020). If current trends continue, this number is expected to rise to 700 million by 2045 (IDF 2020). Diabetes itself also constitutes a health risk as diabetes patients are at high risk for both microvascular complications (including retinopathy, nephropathy and neuropathy) and macrovascular complications (such as cardiovascular comorbidities) (DeFronzo et al. 2015). Due to the skyrocketing rates and the huge health impact, extensive research has been conducted to identify the potential risk factors and provide strategies for disease prevention, amelioration, and management.

Diabetes is caused by a combination of genetic and environmental factors (DeFronzo et al. 2015). Considering that genetic factors remain largely unchanged over the past several decades, the vertiginous rise in diabetes is more likely triggered by major changes in environmental factors, including increased caloric consumption combined with a decrease in physical activity levels (Chen et al. 2009), and a parallel upward swing in obesity prevalence

(Field et al. 2001; Ford et al. 1997; Renehan and Howell 2005; Sheehan and Ulchaker; WHO 2017). Beyond those conventional risk factors, increasing and widespread exposures to environmental pollutants from industrial, agricultural and consumer applications, such as metals, may also act as a key contributor to the growing epidemics of diabetes (Franks and McCarthy 2016).

Environmental Exposure to Metals

Metals occur naturally in the environment and are widely used in industrial, agricultural, and manufacturing processes. Individuals are commonly exposed to metals found in soil, water, air, dust, food, and consumer products (Alloway 2013; Bosch et al. 2016; Mohod and Dhote 2013). Essential metals, which have a known physiological function and are necessary at certain levels in order to avoid deficiency-related health outcomes, include, for example, cobalt, copper, manganese, molybdenum, vanadium and zinc. In contrast, non-essential metals that have no known physiological functions include, for example, arsenic, barium, cadmium, cesium, mercury, lead, antimony, tin, and thallium. Depending on the level of exposure, both essential and non-essential metals may cause tissue damage and adverse health impacts (Zoroddu et al. 2019). A remarkable reduction in environmental sources has been achieved in the US over the last several decades for metals with known high toxicities such as arsenic, cadmium, lead, and mercury (Calafat 2012); however low-to-moderate chronic exposure has been associated with numerous health outcomes, including cardiovascular diseases, kidney diseases, metabolic diseases, neurocognitive outcomes, some cancers, and mortality (Lanphear et al. 2018; Mohammed Abdul et al. 2015; Navas-Acien et al. 2007; Satarug et al. 2009; Zahir et al. 2005).

Exposure to metals is unequally distributed, and this unequal distribution is often related to sociodemographic factors. For example, lead body burden varies across socioeconomic and racial/ethnic groups (Hu et al. 1996; Theppeang et al. 2008). Exposure to arsenic, lead, cadmium, mercury, cesium, thallium and antimony has been observed to be a function of the poverty-income-ratio among the US adults (Tyrrell et al. 2013). Reported differences in metal exposures are not likely to be fully explained by socioeconomic and racial/ethnic factors themselves; rather the residual differences could be explained by individual-level behaviors, lifestyles and other circumstances. For example, in a cohort of middle-aged-to-elderly men in the Boston area, higher bone lead concentrations were observed in men with fewer years of education. The authors suggested that the disparity might be explained by differences in home exposure to lead in dust and drinking water, home proximity to vehicular traffic, and/or occupational lead exposures (Hu et al. 1996).

Health behaviors such as smoking and diet may contribute to the body burden of metals. Cadmium, chromium, and lead found in tobacco smoke can accumulate in tissues and fluids through smoking (Al-Bader et al. 1999; Ashraf 2012; Galażyn-Sidorczuk et al. 2008; Pääkkö et al. 1989). Tobacco smoking has also been suggested to be the single most important source of cadmium exposure in the general population (Al-Bader et al. 1999). Regular seafood intake, particularly fish and shellfish, may contribute to overexposure to methyl mercury, organic arsenic (arsenobetaine and arsenocholine), cadmium and lead (Bae et al. 2013; Burger and Gochfeld 2005; Castro-González and Méndez-Armenta 2008; Falcó et al. 2006; Storelli 2008). Rice intake has gained recent attention as a potential source of toxic inorganic arsenic exposure in the general population (Azizur Rahman et al. 2008; Davis et al. 2012a; Gilbert-Diamond et al. 2011; Melkonian et al. 2013). More recently, a Western overall dietary pattern characterized by

high intake of processed meat, red meat, refined grains, butter, high-fat dairy products, eggs and French fries, was found to be associated with a greater lead body burden among middle-aged-to-elderly men (Wang et al. 2017). One explanation for this finding could be related to high fat intake, which has been found to increase lead concentrations in multiple biological matrices in animal models, possibly through a reduced calcium absorption rate and a faster rate of turnover in trabecular bones, which might further contribute to enhanced bone resorption and lead circulation (Wang et al. 2017).

Exposures to different metals is frequently correlated due to common environmental sources or similarities in metabolic pathways (Pang et al. 2016). Identification of overall exposure patterns is critical for evaluating the associations between metal mixtures and health outcomes. These issues have been recognized by the National Institute of Environmental Health Sciences (NIEHS), which has set understanding the field of exposures to mixtures of environmental chemicals as one of the goals of the 2018-2023 Strategic Plan (NIEHS 2018).

Metal Exposures and Type 2 Diabetes Mellitus

An increasing number of studies have examined the role of metal exposures in thedevelopment of type 2 diabetes mellitus. Arsenic has been most extensively examined and has been associated with diabetes in a number of human studies (Thayer et al. 2012). Other metals including cadmium, mercury, lead, nickel, and zinc have been examined in relation to diabetes but the evidence is limited and inconsistent. Evaluation of most other metals has not taken place and the potential underlying mechanisms are less well understood.

1. Arsenic

Arsenic is a ubiquitous industrial and naturally occurring environmental toxicant. The principal route of exposure to arsenic for the general population is likely to be the oral route, primarily in food and drinking water (ATSDR 2007a). After absorption mainly through the gastrointestinal tract, arsenic is widely distributed in organs such as skin, lungs, liver and kidneys by the blood throughout the body (ATSDR 2007a). Arsenic can also accumulate in the pancreas and may subsequently impair insulin secretion as well as viability of the β cells (Lu et al. 2011). Arsenic in the human body is excreted mainly through the renal system via urine.

Suggestive diabetogenic effects of arsenic are presented in **Figure I.1**. Arsenic may induce insulin resistance by altering gene expression of factors in inflammatory pathways, including NFκB, p38 mitogen-activated protein kinase (MAPK), tumor necrosis factor-α (TNFα), interleukin-6 (IL-6), peroxisome proliferator activated receptor γ (PPARγ), phosphatidydylinositol-3-kinase (PI3K) and PI3K-dependent phosphorylation of protein kinase B (PKB/Akt), and affecting the insulin-stimulated glucose uptake (ISGU) in adipocytes or skeletal muscle cells (Le Roith and Zick 2001; Mohammed Abdul et al. 2015; Somwar et al. 2002; Sriwijitkamol et al. 2006; Walton et al. 2004). Arsenic has the ability to induce oxidative stress and increase the activity of reactive oxygen species (ROS) (Jomova et al. 2011). Experimental studies have suggested that in the pancreas arsenic may increase amyloid formation, leading to apoptotic death/damage of pancreatic β cells through the generation of oxidative stress (Lu et al. 2011; Mukherjee et al. 2006; Yen et al. 2007). Additionally, arsenate (AsO_3^{3-}, As(V)) has been found to replace phosphate in energy transfer phosphorylation reactions, resulting in the formation of ADP-arsenate instead of ATP (Gresser 1981). Arsenite (AsO^{2-}, As(III)) has high affinity for sulfhydryl groups of proteins which has shown to inhibit pyruvate dehydrogenase and further impair the production of ATP by blocking the processing of

citric acid cycle (Petrick et al. 2001). In this way, arsenic was suggested to substitute phosphate and interact sulfhydryl groups, which could impair the glucose transport, interrupt the production of energy, and interfere with the ATP-dependent insulin secretion of β cells (Tseng 2004).

In human studies, long-term exposure to inorganic arsenic has been reported to be associated with diabetes. In a meta-analysis based on data from 17 studies, a pooled relative risk of diabetes for the highest versus lowest category of inorganic arsenic in drinking water was 1.75 (95% CI: 1.20, 2.54) (Wang et al. 2014). Based on epidemiologic evidence, high arsenic (≥50 μg/L in drinking water) was considered to have "limited to sufficient" evidence of causing diabetes, with increased prevalence of diabetes significantly associated with chronic exposure to inorganic arsenic in drinking water in areas such as Taiwan and Bangladesh where arsenic contamination has been a historical problem (Kuo et al. 2013; Nabi et al. 2005; Rahman et al. 1998, 1999; Thayer et al. 2012; Tsai et al. 1999; Tseng et al. 2000a, 2000b; Wang et al. 2003). Evidence of exposure to low to moderate arsenic (<50 μg/L in drinking water) and diabetes is more divergent. Urinary arsenic was associated with higher diabetes prevalence in US adults (Navas-Acien et al. 2008) and was associated with elevated diabetes incidence in a large cohort of American Indian adults (Grau-Perez et al. 2017). On the contrary, no evidence of a relationship between urinary arsenic and diabetes prevalence was found in a large cross-sectional studies in China (Li et al. 2013). More epidemiologic studies, particularly using prospective cohort designs, are needed to confirm this association at low-moderate levels of arsenic exposure (<50 μg/L in drinking water) and levels common in the US and other countries (< 10 μg/L).

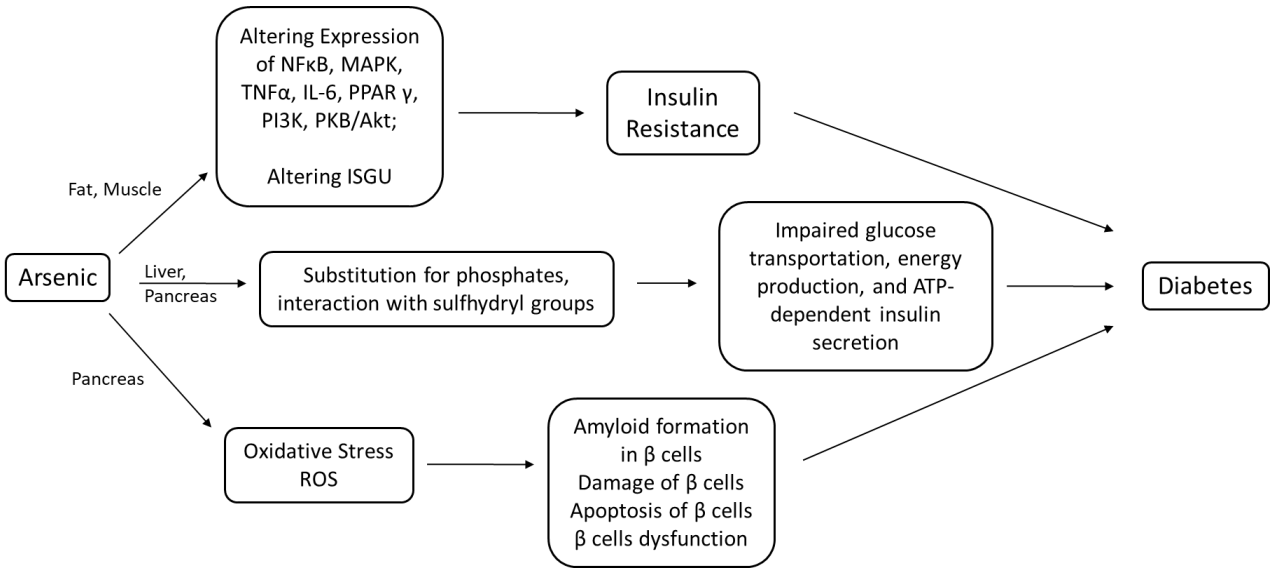

Figure I. 1. Underlying mechanisms of arsenic exposure in development of type 2 diabetes mellitus.

2. Cadmium

Cadmium is a recognized toxicant and carcinogen. Non-occupational exposure to cadmium occurs mainly through tobacco smoking with each cigarette containing an average of 1.1 μg cadmium (Al-Bader et al. 1999). For nonsmokers, secondhand smoke and food are the major sources of cadmium exposure (Satarug et al. 2009). Cadmium accumulation is primarily found in the kidney cortex after long-term exposure (Park et al. 2017). Cadmium may also accumulate in the liver, lung and reproductive tissues, bones, and pancreas (Lei et al. 2007; Satarug et al. 2009; Thompson and Bannigan 2008; Uetani et al. 2006). Findings from human tissue samples indicate that within the pancreas, cadmium may preferentially accumulate in islets (El Muayed et al. 2012).

Suggestive diabetogenic effects of cadmium exposure are shown in **Figure I.2**. In both kidney and liver tissues, cadmium is hypothesized to disrupt gluconeogenesis by increasing the activity of gluconeogenic enzymes including glucose-6-phosphatase (G6Pase), fructose-1,6-

diphosphatase (FDPase), and phosphoenolpyruvate carboxykinase (PEPCK) (Chapatwala et al. 1982; Rajanna et al. 1984), leading to the disruption of gluconeogenesis. However, the exact mechanism by which cadmium alters activity of those enzymes is still unclear. Cadmium may impact insulin resistance; it has been observed that glucose transport activity is reduced through a decreasing expression of the glucose transporter (GLUT4) in adipocytes (Han et al. 2003) and of the sodium-glucose cotransporter 1 (SGLT1) in renal cortical cells treated with cadmium (Blumenthal et al. 1998). In the pancreas, cadmium can reduce insulin secretion by altering the ADP/ATP ratio (Ježek et al. 2012; Takebayashi et al. 2003), blocking calcium channels (Gavazzo et al. 2005), and breaking cell-cell adhesion in pancreas islets (Bosco et al. 2007; Edwards and Prozialeck 2009; Rogers et al. 2007; Wells et al. 2007); these activities impact the insulin release of β cells. Cadmium is also a well-known inducer of oxidative stress in a variety of tissues and cell types (Tinkov et al. 2017). The accumulated cadmium in pancreas islets could possibly cause an induced degeneration, necrosis, and weak degranulation in β cells (Demir et al. 2006; Kurata et al. 2003; Messner et al. 2012; Wang et al. 2016) via induction of oxidative stress.

Human studies of cadmium exposure and diabetes risk have yielded inconsistent results. Based on a total of 9 cross-sectional studies, a recent meta-analysis reported a pooled odds ratio of 1.02 (95% CI: 1.00, 1.05) of diabetes for the highest versus lowest concentrations of urinary cadmium (Li et al. 2017). Among these findings, the strongest association was observed in the National Health and Nutrition Examination Survey (NHANES) III. People in the highest tertile of cadmium exposure were 1.45 (95% CI: 1.07, 1.97) times more likely to have diabetes compared to those in the lowest tertile (Schwartz et al. 2003). In a more recent study of US adults aged 40 and older from NHANES 2005-2010 datasets, urinary cadmium was associated

with a non-linear increase in the odds ratio for prediabetes (Wallia et al. 2014). In contrast, neither blood nor urinary cadmium was associated with incident diabetes, impaired glucose tolerance, blood glucose levels, insulin production, insulin resistance, or haemoglobin A1c (HbA1c) in a prospective cohort of elderly women in Sweden (Barregard et al. 2013). Another longitudinal cohort study of middle-aged-to-elderly Swedes also showed no association between cadmium and risk of diabetes; however an association of cadmium with a higher HbA1c in former and current smokers was reported (Borné et al. 2014).

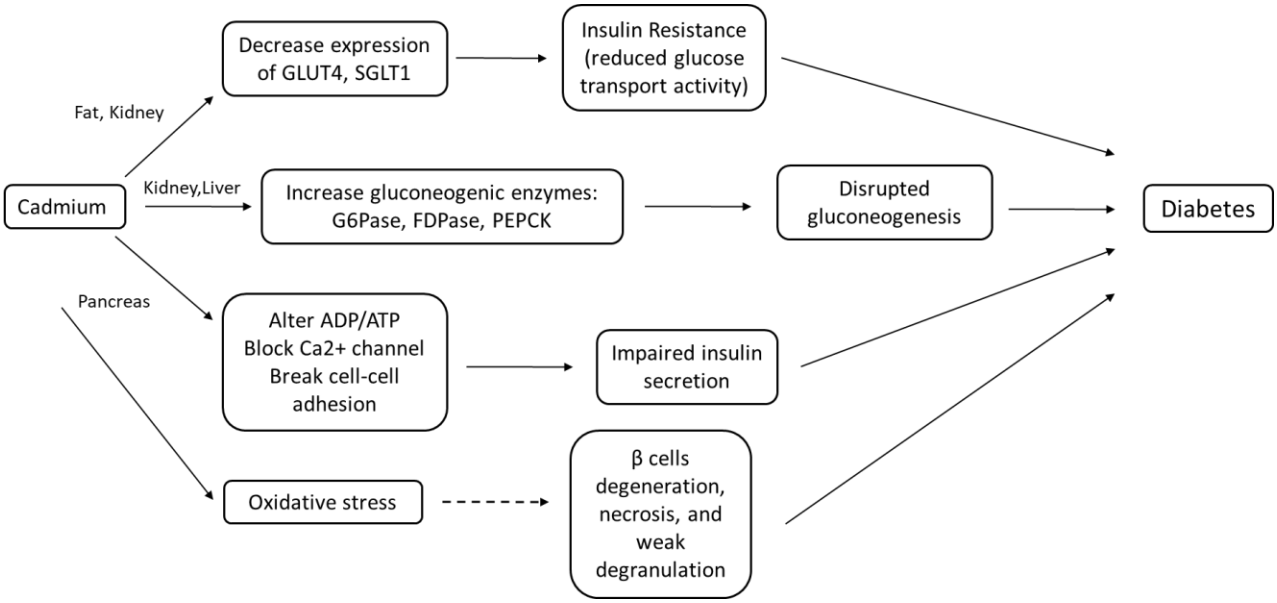

Figure I. 2. Underlying mechanisms of cadmium exposure in development of type 2 diabetes mellitus.

3. Mercury

Mercury is an ubiquitous and persistent toxicant that has elemental (metallic), inorganic, and organic forms (Roy et al. 2017). Exposure to metallic mercury is generally low in the general population, but may be significant in occupational settings (Roy et al. 2017). Nonoccupational exposure to inorganic mercury occurs majorly through occlusal surfaces of teeth that are filled

with mercury-containing amalgams (Mutter 2011) and skin lightening products with inorganic mercury compounds (Park and Zheng 2012). Once water has been contaminated by the direct release of elemental and inorganic mercury, both forms can be transformed by microorganisms to methylmercury (organic form) which can bioaccumulate in aquatic and terrestrial food chains (ATSDR 1999). General populations can be exposed to methylmercury mainly through the consumption of highly-contaminated large or long-lived fish species (Sundseth et al. 2017). In humans, blood mercury mainly reflects exposure to the dietary intake of organic mercury while urinary mercury mainly reflects inorganic mercury (ATSDR 1999).

Possible mechanisms underlying mercury diabetogenic toxicity are summarized in **Figure I.3**. Inorganic mercury has been suggested to impair insulin secretion by inducing β cell depolarization and altering intracellular calcium homeostasis (Liu and Lin-Shiau 2002). Mercury has also been known to increase ROS formation and cause oxidative stress that impairs the function of pancreatic β cells (Chen et al. 2006a). Both inorganic and organic mercury have been demonstrated to cause pancreatic β cell death via the oxidative stress-induced apoptotic and necrotic pathways, as well as β cell dysfunction through a PI3K-activated or oxidative stress-triggered Akt pathway (Chen et al. 2006b, 2006a, 2006d, 2006c, 2010). Inorganic mercury was also suggested to be linked with insulin resistance through a significant inhibition of both peroxisome PPARα and PPARγ mRNA expression in adipocytes(Kawakami et al. 2012).

Epidemiological studies that have investigated the association between mercury and diabetes, have yielding inconsistent results. A large longitudinal study of US young adults found that people with high mercury exposure in young adulthood had elevated risk of diabetes and decreased β cell function later in life (He et al. 2013). On the contrary, mercury exposure was not associated with diabetes risks in both the Health Professionals Follow-up Study and the Nurses'

Health Study, the two other large longitudinal studies of US adults (Mozaffarian et al. 2013). Associations between mercury exposure and metabolic syndrome and diabetes were evaluated in cross-sectional studies of the Korea National Health and Nutrition Examination Survey (KNHANES) with mixed results. Among the six studies based on the different cycles of the KNHANES, two observed a significant association between total mercury and metabolic syndrome (Chung et al. 2015; Park and Seo 2016), while the other four reported no evidence of an association (Kim et al. 2015; Lee and Kim 2013, 2016; Moon 2014),. In the most updated systematic review regarding the role of mercury exposure on development of metabolic syndrome and diabetes, the associations based on 25 epidemiologic studies were weak to moderate, ranging from no association at all to an odds ratio of 7.35 (95% CI: 1.73, 31.1) when total mercury was measured in hair (Roy et al. 2017).

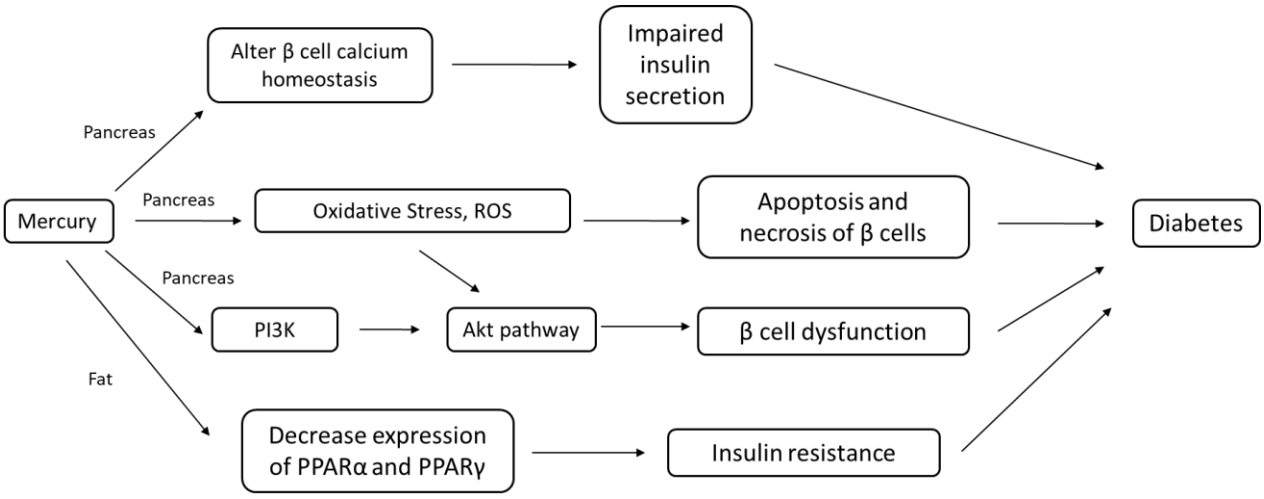

Figure I. 3. Underlying mechanisms of mercury exposure in development of type 2 diabetes mellitus.

4. Lead

Lead toxicity is acknowledged as a prevalent and persistent public health problem. Environmental exposure to lead occurs through various routes, including air, dust, paint, water, and food (Nordberg et al. 2014). Existing evidence on the influence of lead exposure on diabetes risk is limited and inconsistent: higher lead concentrations in different biological matrices were observed in diabetic patients compared to referents in case-control studies (Afridi et al. 2013; Nagaraj et al. 2009). On the contrary, no associations were found in two cross-sectional studies in both the US and South Korea (Menke et al. 2015; Moon 2013). One recent study in China found that higher blood lead concentration was associated with an increased risk of non-alcoholic fatty liver disease, which commonly coexists with type 2 diabetes and has been suggested as a predictor of diabetes risk (Zhai et al. 2017). Lead is a well-known toxicant that can induce oxidative stress through ROS generation, where the ROS pathway has been suggested in the pathogenesis of diseases including diabetes (Leff et al. 2018). Lead is also thought to induce the insulin resistance through disruption of a variety of intracellular signaling pathways by interfering with calcium homeostatsis and calcium cellular uptake, and modulating activity of protein kinase C (Leff et al. 2018).

5. Nickel

Nickel is a hard metal that occurs naturally in the environment and is commonly used for electroplating, alloy production and the production of nickel-cadmium batteries (ATSDR 2005; Cempel et al.). General populations are exposed to nickel through various routes such as air, water, food and tobacco smoking (ATSDR 2005; Caruso et al. 2013). Nickel accumulates primarily in the kidneys and also in other organs including the lungs, liver and heart (Das et al.

2008; Dieter et al. 1988). Most of the absorbed nickel is excreted in the urine, regardless of the route of exposure (ATSDR 2005).

Nickel has identified as a new chemical potentially linked with diabetes risk although the underlying mechanism is not fully elucidated (**Figure I.4**). Existing evidence suggests that nickel can increase hepatic glycolysis and pancreatic glucagon release, decrease peripheral utilization of glucose, induce gluconeogenesis, and impair islet function possibly through induction of oxidative stress as nickel can increase lipid peroxides and reduce antioxidant enzymes activities of superoxide dismutase, catalase and glutathione peroxidase, as well as hepatic glutathione concentrations (Das et al. 2001; KADOTA and KURITA 1955). Nickel-induced glucose deregulation was reduced with treatment of antioxidants (Das et al. 2001). In the pancreas, nickel has been suggested to raise the expression of inducible nitric oxide synthase (iNOS) protein, followed by an increase in cyclic guanosine monophosphate (cGMP) in adrenals, brain and pancreas, which might lead to hyperglycaemia by stimulating endocrine secretions (Gupta et al. 2000).

Evidence from human studies evaluating the association between nickel and diabetes is limited. In a large cross-sectional study of Chinese adults, urinary nickel concentration was associated with higher prevalence of diabetes, higher fasting glucose, higher HbA1c, higher insulin levels, and increased insulin resistance (Liu et al. 2015). A case-control study from Turkey showed that urinary nickel concentrations were higher in people with diabetes and impaired fasting glucose than people without diabetes (Serdar et al. 2009). However, in another case-control study conducted in Italy, blood nickel concentrations in diabetes patients were lower than those in non-diabetic controls (Forte et al. 2013).

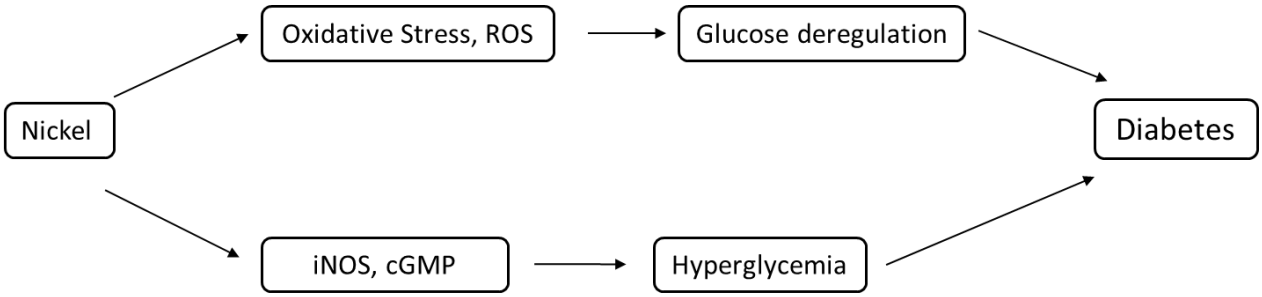

Figure I. 4. Underlying mechanisms of nickel exposure in development of type 2 diabetes mellitus.

6. Zinc

Unlike the aforementioned toxic metals (arsenic, cadmium, mercury, lead and nickel), zinc is an essential nutrient for humans that is necessary for biochemical pathways such as transcription, translation and cell divisions (Jansen et al. 2009). More than 300 enzymes need zinc for their catalytic activities (Jansen et al. 2009). Humans rely on a daily intake of dietary zinc to maintain health and prevent disease. In the US, the recommended dietary allowance (RDA) for zinc is 11 mg/day in men and 8 mg/day in women (Maret and Sandstead 2006). Zinc leaves the body in urine and feces (Roohani et al. 2013). Urinary concentrations of zinc reflect excretion of zinc in the urine (Roohani et al. 2013).

Zinc intake has been associated with a lower risk of type 2 diabetes in women (Sun et al. 2009; Vashum et al. 2013). In pancreatic β cells, zinc is necessary for insulin synthesis, storage and secretion, and accounts for the conformational integrity of insulin in its hexameric crystalline form (Jansen et al. 2009). Zinc transporter (ZnT8) is a key protein for the regulation of insulin secretion in that the alteration in its gene expression has been linked with diabetes (Wijesekara et al. 2010). Excessive urinary excretion of zinc was found to lead to a loss of zinc in β-cells, resulting in a reduced insulin secretion (Jansen et al. 2009). Certain zinc complexes showed

insulin-mimetic effects including reducing hyperglycemia and increasing lipogenesis in animal models (Jansen et al. 2009). Zinc has also been shown to improve glucose transportation in peripheral tissues by improving binding of insulin to its receptor through enhancing tyrosine kinase phosphorylation (Jansen et al. 2009). Additionally, zinc is a structural part of antioxidant enzymes such as superoxide dismutase that could protect insulin and β-cells from being attacked by free radicals (Jansen et al. 2009). On the other hand, hyperglycemia is suggested to interfere the active transportation of zinc back to renal cells, leading to a loss of this mineral in the urine (Chausmer 1998).

7. Other metals

Other metals may affect diabetes risks but evidence from human studies is limited or the underlying mechanisms are poorly understood. Some metals may be essential metals in which deficiencies contribute to diabetes development, while others could be non-essential with exposures linked with higher diabetes risk (Khan and Awan 2014).

Antimony is a silvery white metal that is used in manufacturing of electronics, metal alloys, and as a fire-retardant in paints, ceramics, fireworks, enamels, and glass (ATSDR 2017a). The general population is exposed to low doses of antimony primarily through ingestion of food and drinking water and possibly via inhalation of particulate matters containing antimony in ambient air (ATSDR 2017a). Urinary antimony was positively associated with prevalence of diabetes in both US and Chinese adults (Feng et al. 2015; Menke et al. 2015). Antimony may affect diabetes risks through disruption of estrogen (Choe et al. 2003).

Molybdenum is an essential element for humans and animals that is required for certain enzymes such as sulfite oxidase (Mendel and Bittner 2006). General populations are exposed to

molybdenum almost entirely through food, such as beans, cereal grains, leafy vegetables, legumes, liver, and milk, which are important sources of molybdenum in the daily diet (ATSDR 2017b). The limited evidence of molybdenum on diabetes risk is contradictory. A potential beneficial effect of molybdenum on insulin sensitivity is supported by a study of mice in which molybdenum treatment improved glucose tolerance, replenished glycogen stores, and corrected lipogenic enzyme gene expression (Tanju Özcelikay et al. 1996), likely through its insulin-like actions (Fillat et al. 1992). On the contrary, another mechanistic study suggested that molybdenum could induce β-cell dysfunction and apoptosis via c-jun N-terminal kinases (JNK) and AMP-activated protein kinase (AMPK) activation downstream-regulated mitochondrial-dependent and endoplasmic reticulum (ER) stress-triggered apoptosis pathways (Yang et al. 2016). In humans, urinary molybdenum concentration was associated with higher prevalence of diabetes in both US and Chinese adults (Feng et al. 2015; Menke et al. 2015).

Copper is also an essential element that is needed for multiple biological functions such as production of hemoglobin and maintaining the strength of the skin, blood vessels, and epithelial throughout the body (ATSDR 2004c). However, long-term exposure to excess copper through occupational hazard and environmental contamination has also been shown to induce oxidative damages (Gaetke and Chow 2003). Experimental research suggested the potential role of copper in the pathogenesis of diabetes through induction of hydrogen peroxide generation, leading to damage and death of pancreatic β cells (Masad et al. 2007). In a study of diabetic mice, the treatment of a copper chelating agent was found to reduce insulin resistance and ameliorate glucose intolerance (Tanaka et al. 2009).

Magnesium is an essential macronutrient for human health (Lopez-Ridaura et al. 2004), but excessive high concentration of manganese may have toxic effects, particularly to the central

nervous systems which plays an important role in glucose homeostasis (Guilarte 2010). In a study comprising participants from two large prospective cohorts in the US, a magnesium deficiency led to an increased diabetes risks and a decrease in insulin mediated glucose uptake (Lopez-Ridaura et al. 2004). A double blind randomized clinical trial suggested that magnesium supplementation could reduce insulin resistance in non-diabetic persons (Mooren et al. 2011).

Chromium (trivalent form) is an essential nutrient required for normal energy metabolism (Guidotti et al. 2008). Chromium reduces insulin resistance possibly via stimulating the insulin signaling pathway and metabolism by up-regulating the mRNA level of insulin receptor, GLUT4, glycogen synthase (GS), and uncoupling protein-3 (UCP3) in muscle cells (Qiao et al. 2009). Chromium inhibits oxidative stress and TNFα secretion which could suppress sensitivity and action of insulin (Jain and Kannan 2001).

Vanadium is also an essential nutrient, however, a functional role for vanadium in humans has not been established (ATSDR 2012b). Food is the primary route of exposure for the general population (ATSDR 2012b). An intervention study showed that the oral vanadium treatment improved insulin activity and lowered blood glucose levels (Soveid et al. 2013). Vanadium could exert its potential hypoglycemic effect through activation of signal pathways in GLUT translocation to plasma membrane by increasing the phosphorylation levels of various insulin pathway intermediaries, as well as disruption of gluconeogenesis by its inhibitory effect on the expression of the neoglucogenic enzymes PEPCK and G6Pase (Niu et al. 2016).

For other metals, in a cross-sectional study investigating urine metals with diabetes in NHANES 1999-2010, uranium and tungsten were positively associated with higher prevalence of diabetes; barium was positively while cesium was negatively associated with homeostatic model assessments for insulin resistance (HOMA-IR) (Menke et al. 2015). In a large cross-

sectional study of Chinese adults, urinary barium was associated with higher odds of impaired fasting glucose (IFG) while urinary tungsten was associated with higher fasting glucose levels and diabetes prevalence (Feng et al. 2015). Thallium, cobalt and tin were not associated with diabetes, HOMA-IR, IFG or fasting glucose in either of these two studies (Feng et al. 2015; Menke et al. 2015). Barium, thallium and cesium may affect diabetes risk through their link with obesity: higher urinary concentrations of barium and thallium while lower concentrations of cesium were associated with higher body mass index (BMI)/waist circumference in US adults (Padilla et al. 2010). The exact physiological roles of uranium, tungsten, cobalt and tin in diabetes are still unknown. No other studies investigating platinum, beryllium exposure and diabetes were located.

Evidence of metal exposures on diabetes is summarized in **Table I.1**.

Table I. 1. Summary of previous evidence of metal exposures on diabetes

Metals	Evidence
Arsenic	Insulin resistance (Le Roith and Zick 2001; Mohammed Abdul et al. 2015; Somwar et al. 2002; Sriwijitkamol et al. 2006; Walton et al. 2004): alteration in expression of NFκB, MAPK, PI3K, PKB/Akt in adipocytes and muscles Amyloid formation, apoptosis and necrosis of β cells (Lu et al. 2011; Mukherjee et al. 2006; Yen et al. 2007): oxidative stress Impair glucose transport, metabolism and energy production (Tseng 2004): replace phosphate or interact with sulfhydryl groups Epidemiologic evidences: higher risk/prevalence of diabetes
Cadmium	Disrupt gluconeogenesis (Chapatwala et al. 1982; Rajanna et al. 1984): increase activity of G6pase, FDPase, PEPCK Insulin resistance(Han et al. 2003): decrease expression of GLUT4 in adipocytes Impair insulin secretion: altering ADP/ATP ratio (Ježek et al. 2012; Takebayashi et al. 2003), blocking calcium channel (Gavazzo et al. 2005), as well as breaking cell-cell adhesion in pancreas islets (Bosco et al. 2007; Edwards and Prozialeck 2009; Rogers et al. 2007; Wells et al. 2007) Degeneration, necrosis, and weak degranulation of β cells (Demir et al. 2006; Kurata et al. 2003; Messner et al. 2012; Wang et al. 2016): oxidative stress Epidemiologic evidences (Borné et al. 2014; Li et al. 2017): higher prevalence of diabetes but inconsistent
Mercury	Impair insulin secretion (Liu and Lin-Shiau 2002): induce β cell depolarization and alter intracellular calcium homeostasis

	Apoptosis and necrosis of β cells/β cell dysfunction (Chen et al. 2006b, 2006a, 2006d, 2006c, 2010): PI3K-activated or oxidative stress-triggered Akt pathway Insulin resistance (Kawakami et al. 2012): inhibition of PPARα and PPARγ mRNA expression in adipocytes Epidemiologic evidences(Roy et al. 2017), (He et al. 2013)-(Moon 2014): inconsistent
Lead	Oxidative stress could be possible mechanism in the pathogenesis of diabetes (Patra et al. 2011) Epidemiologic evidences (Afridi et al. 2013)-(Moon 2013): limited and inconsistent
Nickel	Increase glycolysis and glucagon release/Insulin resistance/Impair islet function (Das et al. 2001; KADOTA and KURITA 1955; Tikare et al. 2008): oxidative stress Disrupt endocrine secretion (Gupta et al. 2000): raise expression of iNOS and cGMP Epidemiologic evidences (Kuo and Navas-Acien 2015)-(Forte et al. 2013): limited and inconsistent
Zinc	Necessary for insulin synthesis, storage and secretion (Jansen et al. 2009) ZnT8 is a key protein for the regulation of insulin secretion (Wijesekara et al. 2010) Antioxidant (Jansen et al. 2009): reduce oxidative stress-induced damage Epidemiologic evidences (Jayawardena et al. 2012): zinc deficiency with hyperglycemia; zinc supplement benefit glucose control
Antimony	Antimony may affect diabetes risks through disruption of estrogen (Choe et al. 2003) Epidemiologic evidences (Feng et al. 2015; Menke et al. 2015): higher prevalence of diabetes in two cross-sectional studies
Molybdenum	Beneficial effect on insulin sensitivity by improving glucose tolerance, replenished glycogen stores, and corrected lipogenic enzyme gene expression (Tanju Özcelikay et al. 1996) Apoptosis and necrosis of β cells/β cell dysfunction (Yang et al. 2016): possibly mitochondrial-dependent and ER stress-triggered apoptosis pathway Epidemiologic evidences (Feng et al. 2015; Menke et al. 2015): higher prevalence of diabetes in two cross-sectional studies
Copper	Required for activity of SOD; however long-term exposure induce oxidative stress (Gaetke and Chow 2003) Epidemiologic evidences: higher copper concentrations in diabetes patients than in healthy individuals (Qiu et al. 2017)
Magnesium	Epidemiologic evidences: magnesium deficiency increase diabetes risks in two prospective cohort studies(Lopez-Ridaura et al. 2004); supplement reduce insulin resistance in an RCT (Mooren et al. 2011)
Chromium	Reduce insulin resistance: possibly up-regulate the mRNA level of GLUT4, GS, UCP3; inhibit oxidative stress and TNFα secretion
Vanadium	Hypoglycemic effect: possibly activate GLUT translocation, inhibit expression of PEPCK and G6Pase (Niu et al. 2016) Epidemiologic evidences: improve insulin activity and lower blood glucose levels in a cross-sectional study (Fortoul et al. 2014)

Barium, Cesium, Cobalt, Tin, Tungsten, Thallium, Uranium	Evidence only available in two cross-sectional studies (Feng et al. 2015; Menke et al. 2015): barium, uranium and tungsten positively associated with diabetes prevalence, HOMA-IR, IFG or fasting glucose; cesium negatively associated with HOMA-IR; no association found for thallium, cobalt and tin
Beryllium, Platinum	No study can be identified

Gaps in Scientific Knowledge

While the evidence discussed above supports the role of metal exposures as potential risk factors for diabetes, several important challenges are still remaining.

First, almost all previous epidemiologic studies examining associations between metals and diabetes have been cross-sectional. Given the cross-sectional nature which precludes the ability to determine temporality of metal exposures and diabetes events, reverse causality cannot be ruled out as an possible explanation for observed associations since chronic conditions such as diabetes may affect metal excretions in urine (Chaumont et al. 2012).

Second, most previous studies have focused on only a limited number of metals, particularly those "priority toxic metals" including arsenic, cadmium, lead, and mercury. It is important not only to validate diabetic impacts of known toxicants in a well-designed cohort but also to explore the potential effects of other metals which have been rarely examined previously in human populations.

Third, although plausible biological mechanisms have been proposed, data on underlying mechanisms in human studies is lacking. Molecular epidemiologic approaches using a continuum of events between metal exposures and incident diabetes provide opportunities for elucidating underlying mechanisms in human populations. For example, etiopathogenic mechanisms underlying type 2 diabetes mellitus involves insulin resistance and β-cell dysfunction (DeFronzo 2004; Kahn; 1990). Longitudinal studies allow us to assess the impact of

metal exposures on the changes in intermediate quantitative traits such as homeostatic model assessments over time, providing better pictures of potential mechanisms.

Fourth, most previous studies have been limited to single metals, i.e., the unit of analysis is based on a single metal. This could be partly due to statistical challenges such as complex correlations among metals, confounding by correlated co-pollutants, and lack of well-established statistical methods to evaluate the combined effects of exposure to metal mixtures (Braun et al. 2016; Wang et al. 2018). Quantifying the impact of exposure to metal mixtures is needed for better understanding the role of metal exposures in pathogenesis of diabetes. However, almost all previous studies have not examined the associations of metal mixtures with diabetes and its related intermediate quantitative traits.

Specific Aims of Dissertation

The overall goal of this dissertation was to better understand the role of metals in the development of type 2 diabetes mellitus. The objectives of the present study were (1) to evaluate the distributions of urinary concentrations of a comprehensive list of metals and identify key determinants of each individual metal as well as metal mixtures; (2) to assess the associations of each individual metal, as well as metal mixtures, with incidence of diabetes; and (3) to examine whether exposures to metal mixtures affect insulin resistance and β-cell dysfunction, which help elucidate potential biological mechanisms linking metal exposures related to diabetes. To achieve this goal, I took advantage of the rich longitudinal features of the Study of Women's Health Across the Nation (SWAN), a multi-site, multi-ethnic cohort of women, evaluated annually over 16 years since 1996, when women were 42-52 years of age. Three specific aims were as follows:

Specific aim 1: To report on measurements of 21 metals (arsenic, barium, beryllium, cadmium, cobalt, chromium, cesium, copper, mercury, manganese, molybdenum, nickel, lead, antimony, tin, thallium, uranium, vanadium, tungsten, and zinc) in urine samples collected from the participants of the SWAN. The objectives were (1) to examine the distributions of urinary concentrations of metals, (2) to identify subgroups exposed to different patterns of metals using the k-means clustering method, a nonparametric clustering method seeking a minimum error sum of squares, which could suggest specific exposure patterns of metals, and (3) to evaluate associations of demographic, socioeconomic, lifestyle, dietary and geographical characteristics with each urinary metal, as well as with exposure patterns of multiple metals, in this diverse population of midlife women.

Specific aim 2: To examine the associations of urinary metal concentrations with the incidence of diabetes over 16 years of follow-up in the SWAN. Based on findings of the aim 1, I further evaluated the associations between metal mixtures captured by k-means clusters and incidence of diabetes.

Specific aim 3: To evaluate associations of urinary metal concentrations with longitudinal changes in insulin resistance (HOMA-IR) and β-cell function (HOMA-β) over 16 years of follow-up in the SWAN, which might be mechanisms by which metals may affect diabetes risks.

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Chapter II. Urinary Metals and Metal Mixtures in Midlife Women: the Study of Women's Health Across the Nation (SWAN)

Xin Wang, Bhramar Mukherjee, Stuart Batterman, Siobán D. Harlow, Sung Kyun Park

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Abstract

Background: Little is known about the extent of exposure to metals and metal mixtures among midlife women.

Objectives: We assessed exposure to multiple metals in the Study of Women's Health Across the Nation (SWAN), a multi-site, multi-racial/ethnic cohort of women at midlife.

Methods: We measured urinary concentrations of 21 metals (arsenic, barium, beryllium, cadmium, cobalt, chromium, cesium, copper, mercury, manganese, molybdenum, nickel, lead, platinum, antimony, tin, thallium, uranium, vanadium, tungsten and zinc) using high-resolution inductively coupled plasma-mass spectrometry among 1,335 white, black, Chinese and Japanese women aged 45-56 years at the third SWAN annual visit (1999-2000). Least squared geometric mean concentrations were compared across race/ethnicity, education, financial hardship, smoking, secondhand smoking, seafood intake and rice intake groups. Overall exposure patterns of multiple metals were derived using k-means clustering method.

Results: The percentage of women with detectable concentrations of metals ranged from 100% for arsenic, cesium, molybdenum and zinc, to less than 5% for platinum; 15 metals had detection rates of 70% or more. Asian women, both Chinese and Japanese, had higher urinary concentrations of arsenic, cadmium, copper, mercury, molybdenum, lead and thallium, compared with other race/ethnic groups, independent of sociodemographic, lifestyle, dietary, and geographic characteristics. Seafood and rice intake were important determinants of urinary arsenic, cesium, mercury, molybdenum and lead levels. Two distinct overall exposure patterns- "high" vs. "low" -- were identified. Women in the "high" overall exposure pattern were more likely to be Asians, current smokers, and to report high consumption of seafood and rice. Black women were less likely to have the high exposure pattern.

Conclusions: Metal exposure of midlife women differs by racial/ethnic, sociodemographic, lifestyle, dietary, and geographic characteristics. Asian women may be experiencing the highest exposures to multiple metals compared with other racial/ethnic groups in the United States.

1. Introduction

Metals occur naturally in the environment and are widely used in industrial, agricultural, and manufacturing processes. Individuals are commonly exposed to metals found in soil, water, air, dust, food, and consumer products (Alloway 2013; Bosch et al. 2016; Mohod and Dhote 2013). Arsenic, cadmium, lead and mercury are among the most toxic environmental pollutants. Although a remarkable reduction in environmental sources of such toxic metals has been achieved in the United States (U.S.) over the last several decades (Calafat 2012), low-to-moderate chronic exposure has been associated with numerous health outcomes, including cardiovascular diseases, kidney diseases, metabolic diseases, neurocognitive outcomes, some cancers, and mortality (Lanphear et al. 2018; Mohammed Abdul et al. 2015; Navas-Acien et al. 2007; Satarug et al. 2009; Zahir et al. 2005). Other metals, like cobalt, copper, manganese, molybdenum, vanadium and zinc, are necessary for multiple biochemical pathways and required for certain enzymes (Fraga 2005). Given the role of essential elements in human nutrition, deficiencies are frequently associated with diseases; excessively high concentrations of such metals may also have toxic effects (Fraga 2005).

Exposure to metals is unequally distributed, and this unequal distribution is often related to sociodemographic, lifestyle, dietary and geographic factors. For example, lead body burden varies across socioeconomic and racial/ethnic groups (Hu et al. 1996; Theppeang et al. 2008). Exposure to arsenic, lead, cadmium, mercury, cesium, thallium and antimony has been observed as a function of poverty-income-ratio among the U.S. adults (Tyrrell et al. 2013). Exposures to arsenic, cadmium, mercury and lead with has also been linked to lifestyle factors, such as smoking and food/dietary intake, especially seafood and rice (Castro-González and Méndez-Armenta 2008; Gilbert-Diamond et al. 2011; Wang et al. 2017).

Few studies have evaluated exposures to metals in midlife women despite the growing appreciation of the importance of this lifestage to health and wellbeing. The menopausal transition is characterized by a shift in women's sex hormone profile owing to permanent changes in ovarian function which is associated with increased risk of chronic diseases, most notably cardio-metabolic disorders (Davis et al. 2012b; Kim 2012; Polotsky and Polotsky 2010; Stuenkel 2017). Exposure to toxic metals during this window of susceptibility may increase the risk of adverse health consequences associated with ovarian aging. For example, menopause has been suggested to play an important role in the mobilization of lead from bone into the circulation due to an increased bone turnover rate (Hernandez-Avila et al. 2000; Tsaih et al. 2001). Bone lead stores accrued from cumulative environmental exposures for decades are the major endogenous source of lead (Tsaih et al. 2001). Lead exposure has been associated with health outcomes such as hypertension and coronary heart disease (Ding et al. 2016; Korrick et al. 1999). To date, however, no study has examined midlife women's metal exposure profile comprehensively or identified characteristics of highly exposed subpopulations.

Exposures to different metals may be correlated due to common environmental sources or similarities in metabolic pathways (Pang et al. 2016). Given the complexity in the correlations, simultaneous exposure to multiple metals may result in effects that can depart from a simple summation of the effects of single metals (Park et al. 2014, 2017; Wang et al. 2018). Identification of overall exposure patterns is critical for evaluating the associations between metal mixtures and health outcomes. These issues have been recognized by the National Institute of Environmental Health Sciences (NIEHS), which has set understanding the field of exposures to mixtures of environmental chemicals as one of the goals of the 2018-2023 Strategic Plan (NIEHS 2018).

In this paper, we report on measurements of 21 metals in urine samples collected from the Study of Women's Health Across the Nation (SWAN), a multi-site, multi-race/ethnic cohort of women aged 45-56 years at the time of urine collection. The overall objectives were (1) to examine the distributions of urinary concentrations of metals, (2) to identify subgroups exposed to different patterns of metals using the k-means clustering method, a nonparametric clustering method seeking a minimum error sum of squares, which could suggest specific exposure patterns of metals (Jain 2010), and (3) to evaluate associations of demographic, socioeconomic, lifestyle, dietary and geographical characteristics with each urinary metal, as well as with exposure patterns of multiple metals, in this diverse population of midlife women.

2. Methods

2.1 Study population

Women in the present study were participants in the SWAN, an ongoing, a multi-site, multi-ethnic, community-based longitudinal study of the natural history of menopause designed to address the effect of the menopausal transition on subsequent health and to identify risk factors for age-related chronic diseases (Sowers et al. 2000). Between 1996 and 1997, 3,302 women were enrolled from seven study sites, including Boston, MA; Chicago, IL; southeast Michigan, MI; Los Angeles, CA; Oakland, CA; Newark, NJ; and Pittsburgh, PA. Each site enrolled white women and women from one minority group (black women from Boston, Chicago, Southeast Michigan, and Pittsburgh; Chinese women from Oakland; Japanese women from Los Angeles; Hispanic women from Newark). Black, Chinese, Japanese, and Hispanic women comprised greater proportions of the SWAN population than their respective proportions in the general U.S population, reflecting the study design to oversample these groups (Sowers et al. 2000). Eligibility criteria for enrollment into the SWAN cohort included the following: age 42

to 52 years, intact uterus and at least one ovary, no use of exogenous hormones affecting ovarian function in the past 3 months, at least one menstrual period in the previous 3 months, and self-identification with a site's designated racial/ethnic groups. Institutional review board approval was obtained at each study site, and all participants provided signed informed consent at each study visit.

The SWAN Multi-Pollutant Study used urine samples from the SWAN Repository collected during the third SWAN follow-up visit (visit 03, 1999-2000) for environmental exposure assessment. A subset of 1,400 SWAN participants from the five SWAN sites who provided urine samples to the SWAN Repository (Boston, southeast Michigan, Los Angeles, Oakland and Pittsburgh) were assayed for metal concentration determinations. Women from Chicago and Newark were excluded because urine samples were not collected in these two sites. This subpopulation, by design, included self-identified white, black, Chinese, and Japanese but not Hispanic women who were recruited exclusively from Newark. Among these five sites, women for whom urine samples were not available were less educated and more likely to be current smokers or obese than women with available urine. For this analysis, we excluded 2 participants with insufficient urine samples such that one or more metal concentrations could not be determined, and 63 participants with missing information on core covariates (smoking, secondhand smoking, education, financial hardship, seafood intake, rice intake, and urinary creatinine concentrations), leaving 1,335 eligible participants (95.6%) for analysis. When compared to the 65 excluded women, women eligible for this analysis were similar with respect to age and racial distributions. An overview of our sampling procedure is illustrated in **Supplemental Figure II.1**.

2.2 Urinary metals

Urine specimens were collected prior to 11 am in the morning. First morning voided urine was collected. Aliquoted specimens were frozen and stored in ultra-low freezers at -80 ˚C until they were later analyzed for the metal content. All specimens were collected and stored in the SWAN Repository (http://swanrepository.com/) using a systematic protocol. A total of 21 metals including total arsenic, barium, beryllium, cadmium, cobalt, chromium, cesium, copper, mercury, manganese, molybdenum, nickel, lead, platinum, antimony, tin, thallium, uranium, vanadium, tungsten and zinc were measured in the urine samples. All the urinary metals were analyzed with high-resolution inductively coupled plasma-mass spectrometry (ICP-MS) (Thermo Scientific iCAP RQ, Waltham, MA) by the Applied Research Center of NSF International (Ann Arbor, Michigan), a part of the Michigan Children's Health Exposure Analysis Resource (M-CHEAR) Laboratory Hub. We used the CDC method 3018.3 (CDC 2012b), with modifications for the expanded metals panel. All standards, quality controls (QCs), blanks, rinse solution and urine samples were diluted 10-fold in a diluent consisting of 2% HNO_3 solution containing the internal standards and gold. The samples were analyzed in two analysis modes - standard (default) for the majority of the metals, and kinetic energy discrimination (KED) for vanadium, chromium, arsenic, molybdenum and cadmium. The following QC procedures was conducted in parallel with sample analyses: (a) second source standards and spike and surrogate recoveries were tested periodically; (b) linearity and drift checks were performed with each sample batch; and (c) metal internal standards were used on each sample. Each sample run contained a minimum of 4 calibration standards and a blank. The coefficients of variation were 2.4-34.8% for the low QC pools; 1.6-4.0% for the high QC pools; and 1.8-4.0% for the laboratory fortified blank. The limits of detection (LODs) of each metal were determined during the method validation by running a dilution matrix blank 10 times and then calculating the standard

deviation of the instrument response. The limit of detection was then defined by calculating three times the standard deviation. Metal concentrations below their LODs were assigned the LOD divided by the square root of 2.

2.3 Covariates

Sociodemographic factors including age, race/ethnicity, educational attainment, and financial hardship were assessed at the SWAN baseline examination (1996-1997). Race/ethnicity was classified into self-identified white, black, Chinese, or Japanese. Education was categorized as graduated from high school or less, attended some college, and graduated from 4-year college or higher degree. Financial hardship was derived from the question "How hard is it for you to pay for the very basics like food, housing, medical care and heating?" with the 3-level response indicating "very hard", "somewhat hard", and "not hard at all" (Hall et al. 2008). Lifestyle variables including cigarette smoking and secondhand smoking at home, work, and other social settings were collected at SWAN visit 03 with a self-administered questionnaire. Cigarette smoking was categorized as never, former, or current smoking. Total person-hours of secondhand smoking exposure per week was calculated and categorized as 0 hour per week, less than 5 hours per week and more or equal than 5 hours per week. Dietary intake was collected at the SWAN baseline, using a detailed semi-quantitative food frequency questionnaire (FFQ) adopted from Block FFQ (Block et al. 1986). The 103-food item FFQ included 4 seafood items (fried fish/fish sandwich, tuna fish/tuna salad, shellfish, and other fish) and 1 rice item (rice/dishes made with rice). For analysis, weekly seafood intake was computed by summing the frequency of intake for the 4 fish items. To obtain comparable numbers of participants in each group of food intake, we categorized seafood intake into tertiles as less than 1 time per week, 1 to 1.9 times per week, and greater than or equal to 2 times per week. Rice intake was categorized

into tertiles as less than 1.5 times per week, 1.5 to 3.4 times per week, and greater than or equal to 3.5 times per week. Total energy intake was obtained from the FFQ based on each food intake. Urine creatinine was determined by the Cobas Mira analyzer (Horiba ABX, Montpellier, France) at SWAN visit 03 as makers of urine dilution.

2.4 Statistical analysis

Means and percentages of participant characteristics were calculated and examined by race/ethnic groups. Detection rate, geometric mean and distribution percentiles for both the volume-based (μg/L) and creatinine-adjusted (μg/g creatinine) concentrations of each urinary metal were calculated. Pairwise Spearman correlations among urinary creatinine-adjusted metal concentrations were calculated and presented via a correlation-matrix heat map. To compare metal exposure profiles in SWAN to the concentrations in the U.S. general population, median creatinine-adjusted concentrations of urinary metals in white and black women aged 45-56 years (the age range of SWAN women) from the National Health and Nutrition Examination Survey (NHANES) 1999-2000 were calculated. The complex survey design of NHANES was considered using the R 'survey' package. We were not able to compare metal concentrations in Chinese and Japanese women between SWAN and NHANES due to the limited number of Asian Americans included in the NHANES 1999-2000 cycle (the category "Non-Hispanic Asian" was not available until NHANES survey cycle 2011-2012) (CDC/NCHS 2018).

We used analysis of covariance (ANCOVA) to examine the influence of race/ethnicity, education, financial hardship, smoking, secondhand smoking, seafood and rice intake on each natural log-transformed urinary metal concentration, given the right-skewed concentration distributions. This model enabled us to compare the expected least square geometric mean (LSGM) metal concentrations for selected determinants (e.g., across race/ethnic groups), which

were adjusted statistically so that participants had comparable levels of all other covariates in the model. To control for potential confounding, age and study sites were adjusted in all models (Santoro et al. 2011). All models were adjusted for urine creatinine to account for variations in dilution in spot urine samples (Barr et al. 2005; O'Brien et al. 2016). When seafood intake and rice intake were included in the model, we also adjusted for total energy intake (Willett et al. 1997). We calculated LSGM metal concentrations across the study sites within white and black women respectively, to assess potential geographical differences in metal exposures. Geographical differences could not be evaluated within the Chinese or Japanese groups as they were sampled at only one site by design. We also calculated and compared the LSGM metal concentrations between white and Chinese women within the Oakland site, and between white and Japanese women within the Los Angeles site, to examine potential race/ethnic differences in metal exposures within these sites.

K-means clustering was implemented to identify subgroups of SWAN participants with different overall exposure patterns of urinary metals. K-means clustering is a commonly used nonparametic clustering method partitioning quantitative variables towards different centroids seeking a minimum total within-cluster variation (Jain 2010). This approach creates a single variable with k categories as different clusters where participants within the same cluster are as similar as possible and participants from different clusters are as dissimilar as possible, in terms of the quantitative variables (i.e., urinary metal concentrations). The k-means algorithm (1) randomly selected k centroids in a space of urinary metals and assigned each participant to the closest centroid by minimizing the distance to the corresponding centroid (within-cluster sum of squares), and (2) updated the centroids as the average of all data points in a cluster and again assigned each participant to the closet centroid. Step (2) was iterated until the cluster

assignments stopped changing (convergence). Each cluster represents a subpopulation with a specific metals exposure pattern. All log-transformed urinary metals were standardized to make variables comparable before the k-means analysis. The number of optimal clusters (k) was determined based on (1) cubic clustering criterion (Warren and Sarie 1983); (2) Elbow method (plotting total within-cluster sum of square vs. number of clusters); and (3) average Silhouette method (Rousseeuw 1987).

To identify those risk factors most strongly associated with exposure to individual metals, as well as to the overall exposure patterns, variable selection was performed using backward elimination with an initial model including race, education, financial hardship, smoking and secondhand smoking and a threshold of $p < 0.05$ for retaining the variables in linear regression for individual metals, and logistic regression for the overall k-means clustering exposure patterns, respectively. Age, study site, and total energy intake were forced into all models to control for confounding. Urinary creatinine was forced into all linear regression but not into the logistic regression models since the overall exposure patterns were derived based on the creatinine adjusted-metal concentrations. Regression analyses and k-means clustering were performed only for metals for which the detection rate was ≥ 70%.

To examine analytical consistency and robustness of our findings, we substituted specific gravity for urinary creatinine to adjust for urine dilution in all regression analyses, as a sensitivity analysis. Specific gravity was measured using a handheld digital refractometer (ATAGO model PAL-10S, Tokyo, Japan) at SWAN visit 03. All analyses were conducted using R, version 3.4.0.

3. Results

3.1 Characteristics of study population

Table II.1 presents participants' characteristics by racial groups and for the total study population. Participants had a mean age of 49.4 years, ranging from 45 to 56 years, which was not significantly different across the four racial groups ($P = 0.12$). There were significant differences in education, financial hardship, smoking, secondhand smoking, seafood and rice intake between race/ethnic groups ($Ps < 0.001$). Generally, black women reported the lowest socioeconomic status as indicated by education and financial hardship. The prevalence of current cigarette smokers was highest in black women and lowest in Chinese women. Black women also reported higher exposure to secondhand smoking than women of other race/ethnicities. Chinese and Japanese women consumed seafood and rice more frequently than white or black women.

3.2 Analysis of individual metals

The distributions of all 21 metal concentrations (μg/L urine and μg/g creatinine), LODs and detection rates are summarized in **Supplemental Table II.1**. The percentage of women with detectable concentrations of an individual metal ranged from 2.6 to 100%. Six metals had detection rates less than 70% (beryllium: 16.2%, chromium: 24.3%, platinum: 2.6%, uranium: 33.0%, vanadium: 37.2%, tungsten: 29.6%). The median number of metals detected in SWAN participants was 16. **Figure II.1** shows the Spearman correlation matrix of the 15 creatinine-adjusted metal concentrations for which detection rates were greater or equal than 70%. In general, most metals were modestly and positively correlated with each other. Comparisons of creatinine-adjusted median concentrations of urinary barium, cadmium, cobalt, cesium, mercury, molybdenum, lead, antimony, and thallium in white and black women from SWAN and NHANES 1999-2000 within the same age range are displayed in **Figure II.2**.

The LSGM concentrations of 15 urinary metals detectable in more than 70% of the participants are shown in **Supplemental Table II.2**, stratified by race/ethnicity, education,

financial hardship, smoking, and secondhand smoking status. Concentration differences for 12 out of 15 metals were observed between race/ethnicity groups. Both Chinese and Japanese women had higher concentrations of arsenic, cadmium, copper, mercury, molybdenum, lead and thallium than white or black women. The most pronounced differences between Asian and white/black women were for arsenic and cadmium. For example, on average, arsenic concentrations were 95.9% and 121.2% higher in Chinese than in white and black women, respectively, while cadmium concentrations were 93.0% higher in Japanese than in white or black women. The highest LSGM concentrations of cobalt, cesium and nickel were also detected in Chinese participants. Women with higher education had lower concentrations of cadmium, antimony and zinc, but higher concentrations of mercury than women with less education. Current smoking status was positively associated with cadmium and lead and inversely associated with cobalt and nickel concentrations. Higher concentrations of mercury and lead were found also among women who had higher exposure to secondhand smoking. No significant differences were observed between metal concentrations and financial hardship.

The LSGM metal concentrations are presented in **Table II.2** after further adjustment for seafood and rice intake. Higher seafood intake was significantly associated with higher concentrations of arsenic, mercury, molybdenum and lead. Rice intake was also positively associated with arsenic, cesium and mercury concentrations. Women who consumed two or more seafood meals per week had 55.4% higher mean concentrations of total arsenic in urine than did women who reported eating seafood less than 1 time per week. For rice, those who consumed 3.5 or more rice meals per week, on average, had 65.5% higher concentrations of urinary total arsenic compared with participants in the lowest category of rice intake (<1 time per week). To note, seafood and rice intake were only weakly correlated (Spearman correlation $\rho = 0.25$) (data

not shown). The race/ethnic differences in LSGM concentrations of arsenic, cesium, mercury and molybdenum were attenuated but remained significant (*Ps* < 0.05) after further adjusting for seafood and rice intake (**Table II.2, Supplemental Table II.2**). Similar race/ethnic differences in metal exposures were observed when LSGM concentrations were compared between white and Chinese women within the Oakland site, and between white and Japanese women within the Los Angeles site (**Supplemental Table II.3**).

The LSGM concentrations within white and black women are presented in **Table II.3**, stratified by SWAN study sites. In both racial groups, women in Boston had the highest average concentrations of arsenic, cadmium, cesium, mercury and lead; and those at the Pittsburgh site had the highest concentrations of barium, nickel and thallium. Women in southeast Michigan had the lowest average concentrations of arsenic, cadmium and lead.

After backward elimination, race/ethnicity was selected as a significant predictor for most metals (**Supplemental Table II.4**). Higher education level remained as a correlate of higher urinary cadmium and mercury concentrations. Being a former or current smoker was significantly associated with both higher cadmium and lead concentrations. Seafood intake remained as an independent predictor of arsenic, cadmium, cesium, mercury and lead. Rice intake was associated also with elevated arsenic, cesium, copper, mercury, molybdenum and nickel concentrations.

3.3 Analysis of exposure patterns of metals

Two clusters of metal exposures were derived by k-means clustering based on the cubic clustering criterion, Elbow method and average Silhouette method (**Supplemental Figure II.2**), which were labeled as “high” (n = 562) and “low” (n = 773) for the overall metal exposure patterns. **Figure II.3** shows the mean of each standardized log-transformed creatinine adjusted

metal concentration corresponding to the high and low clusters (geometric means can be found in **Supplemental Table II.5**). Note that standardized concentrations were comparable within each cluster. No cluster had a particularly high or low concentration of specific metals. Odds ratios in the full logistic regression model are presented in **Supplemental Table II.6**. After backward elimination, being black was associated with higher odds of being clustered into the "low" overall exposure group, while being Chinese or Japanese, being a current smoker, and being in the highest category of seafood intake and rice intake were significantly (*Ps* < 0.05) associated with higher odds of being clustered into the "high" group (**Table II.4**).

Use of specific gravity instead of urinary creatinine in models for urine dilution adjustment did not alter our findings significantly (**Supplemental Table II.7**).

4. Discussion

In this study, we evaluated concentrations of 21 metals in urine samples, identified two overall exposure patterns, and examined demographic, socioeconomic, lifestyle, and dietary factors associated with both individual metals and metals exposure patterns, in a large population-based multi-racial/ethnic, multi-site cohort of midlife women in the U.S.. Participants sorted into two clusters, suggesting two distinct overall exposure patterns to mixtures of multiple metals in the general environment. Interestingly, each exposure pattern showed homogeneous distributions of individual metals (standardized concentrations). This similarity could be partly explained by the positive correlations among most of the metals we measured in urine samples (**Figure II.1**). One recent study of profiles of environmental chemical mixture exposure among pregnant women using the same k-means clustering method revealed a similar exposure cluster in which some women were consistently exposed to high concentrations of metals (Kalloo et al. 2018). Understanding the exposure patterns of multiple metals is an important first step before

evaluating the association between metal mixtures and health outcomes. Our study suggests that k-means clustering is a useful tool to identify exposure clusters in the population.

We observed significant race/ethnic differences in the urinary concentrations of metals, i.e., higher concentrations of arsenic, cadmium, copper, mercury, molybdenum, lead, and thallium in Asian women. Similarly, findings in the U.S. general population for arsenic, cadmium, lead, and mercury suggest that Asians had the highest adjusted geometric mean biomarker levels of these metals in NHANES 2011-2012 (Awata et al. 2017b). However, most previous epidemiological studies have focused primarily on "priority toxic metals" while the racial/ethnic differences in metals such as copper and thallium, as well as the overall exposure patterns, have not been adequately captured. Some of the differences in metal concentrations between race/ethnic groups may be related to diet, such as higher intake of seafood and rice reported by Chinese and Japanese study participants. Regular seafood intake, particularly fish and shellfish, contributes to overexposure to methyl mercury, total arsenic, organic arsenic (arsenobetaine and arsenocholine), and lead (Awata et al. 2017a; Bae et al. 2013; Burger and Gochfeld 2005; Castro-González and Méndez-Armenta 2008; Falcó et al. 2006; Storelli 2008). Rice consumption has also gained recent attention as a potential source of arsenic exposure (Awata et al. 2017a; Azizur Rahman et al. 2008; Davis et al. 2012a; Gilbert-Diamond et al. 2011; Melkonian et al. 2013). Intake of seafood and rice is an important exposure pathway for explaining racial/ethnic differences in, at least, arsenic and mercury. Stronger associations of seafood and rice intake with both arsenic and mercury were observed within the two Asian populations compared to other race/ethnic populations in this study (data not shown). The seafood intake assessment in this study was based on FFQ, adapted to include ethnic specific foods. However, other seafood items might not have been captured in the standard FFQ that were

often served in the Asian diet, such as seaweed, which might be important determinants of the unequal metal distributions across racial groups (Lee et al. 2012). Different metal contamination levels by different types of rice (i.e., white vs. brown rice) may also explain our findings, if the types of rice were different between high and low consumption groups (Consumer Reports 2014). However, the FFQ used in the current study did not distinguish specific types of rice. Furthermore, Chinese women had even higher concentrations of cobalt, cesium and nickel than Japanese women. The fact that intake of seafood and rice was not different between Chinese and Japanese suggests that other environmental exposure sources or pathways, may differ across Asian populations or that different levels of unmeasured confounding are present within Asian subgroups. The observed differences between Asian and other race/ethnic groups could be confounded by geographic location as both Chinese and Japanese women were sampled only in California, but not at any of the Midwest or Northeast SWAN sites. One recent study conducted in six U.S. cities reported higher arsenic concentrations for participants in the Los Angeles compared to the other cities (Jones et al. 2018). However, we observed distinct race/ethnic differences in metal exposures when LSGM concentrations were compared between white and Chinese women within the Oakland site, and between white and Japanese women within the Los Angeles site (**Supplemental Table II.3**).

Additionally, we found that geographic location was an important predictor of metal concentrations. White and black women in Boston had higher concentrations of arsenic, cadmium, cesium, mercury and lead than those at other study sites. A higher seafood intake in Boston could potentially account for the observed high metal concentrations. This was supported by the significantly higher seafood intake among white and black women at Boston site compared with other sites in our study population (data not shown). A possible alternative

explanation that should be considered is metal contamination of drinking water because of an aging infrastructure. For example, lead in the water supply has been attributed to dilapidated drinking water infrastructures, including lead jointed pipelines, end-of-life polyvinyl chloride pipes and household plumbing in communities with aging infrastructures (Hanna-Attisha et al. 2016; Harvey et al. 2015). Lead exposure could also be higher due to older housing stock with lead-based paint another important exposure source (Aschengrau et al. 1997).

High exposure to barium, nickel and thallium were consistently observed in both white and black women in Pittsburgh. Barium is commonly used in metal alloys, colorant in paints, x-ray contrast medium, and naturally occurs in groundwater (ATSDR 2007b). Barium concentrations in the drinking water were around 10 times higher in regions of Kentucky, northern Illinois, New Mexico, and Pennsylvania in the U.S. (ATSDR 2007b). Thus drinking water from groundwater sources might be a common route of high exposure to barium among participants in Pittsburgh. Nickel is used in the manufacturing of electronics, metal alloys and batteries. It is released to the atmosphere by combustion of fuel oil, municipal incineration, and industries involved in nickel refining, steel production, and other nickel alloy production (ATSDR 2005). Based on the emission data in the EPA 1996 National Toxics Inventory database, Pennsylvania had one of the highest average concentrations of nickel in ambient air among the states in the U.S. (ATSDR 2005). Thallium is another toxic metal that has been widely used in electronics manufacturing in the U.S.. Its exposure occurs primarily from industrial processes such as coal-burning and smelting (Peter and Viraraghavan 2005). Therefore, higher urinary nickel and thallium concentrations among women at Pittsburgh might be attributed to inhalation of contaminated ambient air.

Our study found that several other characteristics are also important predictors of metal concentrations. The observed decreasing trend in cadmium and antimony with increasing education levels accords with previous findings, indicating the role of socioeconomic status in determination of high exposure to environmental toxicants (Tyrrell et al. 2013). However, higher socioeconomic status is not always associated with lower exposure to toxic metals. For example, higher mercury concentrations have been observed in participants with higher education levels because individuals of higher socioeconomic position tend to have higher regular seafood consumption (Awata et al. 2017b; Buchanan et al. 2015; Mortensen et al. 2014). We also observed significant positive associations of cigarette smoking with cadmium and lead concentrations, again demonstrating that cigarette smoking is one of the main sources of cadmium and lead in the general population (ATSDR 2012a; Hu et al. 1996; Richter et al. 2013). Secondhand smoking also contributed to increased urinary lead concentration, providing support for its role as a modifiable source of lead exposure not only in children and adolescents reported previously (Apostolou et al. 2012), but in midlife women.

In this study, the creatinine-adjusted median concentrations of most metals were comparable to the concentrations in women of the same age range (45-56 years) from NHANES 1999-2000. Median concentrations of molybdenum in white and black women and of mercury in black women in SWAN women seem to be a little bit higher than those in NHANES. Seafood intake was shown to be a significant source of both mercury and molybdenum in this study. Higher seafood consumption, especially in an area like Boston that reported the highest seafood intake in our analysis, might account for the high metal concentrations in SWAN participants compared with those in NHANES. Significantly higher LSGM concentrations of mercury and molybdenum were also observed among women at the Boston site compared with other sites in

our study (**Table II.3**). However, we cannot rule out other possible exposure pathways that may account for the observed differences. Because our understanding of sources of metal exposure remains incomplete, these findings prompt follow-up in a future study.

Our study has several limitations. First, the metals we measured in this study have various half-lives in the human body. Urinary concentrations of metals with short half-lives such as arsenic, barium, cobalt, cesium, and thallium mainly reflect recent exposures (ATSDR 1992, 2004a, 2004b, 2007b, 2007c) and may depend on the participants' food consumption within a few days previous to the urine sample collection (Navas-Acien et al. 2011). In contrast, other metals such as cadmium are not rapidly excreted and have very long half-lives from years to decades (ATSDR 2012a). As health endpoints related to metals are likely affected by exposures over time-periods longer than a few days, information on the temporal variability of urinary metals concentrations, especially for those with short half-lives, is needed to characterize average metals exposures over time in epidemiological studies. Second, urine may not be an optimal biological matrix for some metals, such as lead. However, urinary lead adjusted for creatinine has been suggested as a good proxy for plasma lead, where plasma lead is the most toxicologically active lead component but is difficult to measure accurately due to the extremely low concentrations and possible contamination from various sources (Tsaih et al. 1999, 2001). Third, in our study only total arsenic concentrations were measured; arsenic speciation data were not available. The source and toxicity of different arsenic species vary. Major sources of inorganic arsenic in the general population are contaminated drinking water and rice intake (Gilbert-Diamond et al. 2011; Hughes et al. 2011). Inorganic arsenic has been associated with adverse health outcomes such as cardiovascular disease, diabetes, and some cancers (Chen et al. 2013; Maull et al. 2012; Meliker et al. 2010; Steinmaus et al. 2014). Seafood intake is a major

source of organic arsenic (Jones et al. 2016), which is generally considered to have low toxicity (Cullen and Reimer 1989). Arsenic speciation would improve assessment of arsenic exposures and associated health risks. Fourth, seafood and rice intake in this study was obtained from an FFQ administered at the SWAN baseline (1996-1997), whereas the urine samples were collected at SWAN visit 03 (1999-2000). This FFQ would not capture possible dietary changes that may have occured during the 3-year gap before urine sample collection although rapid diet changes are not very likely in this age group (Weismayer et al. 2006). Dietary assessments were self-reported, and thus subject to recall bias. However, statistical adjustment for self-reported energy intake in our regression models helped to reduce the influence of response biases since measurement error in both energy intake and food intake estimates are correlated (Subar et al. 2015). Finally, participants in our study were midlife women. Thus the present findings may not be generalizable to men or women at different lifestages.

This study also has numerous strengths. We systematically examined a suite of 21 metals in urine samples in midlife women for whom scant exposure data are available. We used a data-driven clustering approach to summarizing information of multiple environmental exposures into distinct metal clusters. This approach proved to be a useful tool as it identified different overall exposure patterns of metals and the underlying grouping of metals that may be useful for future evaluations of metal-mixtures health effects. Further, the wide geographical and racial/ethnic coverage of the SWAN participants enabled us to compare differences in biomarkers levels across multiple groups and increased the generalizability of our findings.

In conclusion, we observed marked differences in distributions of a comprehensive set of metals and in the overall metal exposure patterns, by race, education, smoking, secondhand smoking, seafood intake, rice intake and geographic sites, among midlife women from the U.S.

general population, as represented by participants of SWAN. Chinese and Japanese women, had higher urinary concentrations of arsenic, cadmium, copper, mercury, molybdenum, lead, thallium, compared with other race/ethnic groups. Women in the "high" overall exposure pattern were more likely to be Asians and less likely to be black. We confirmed that seafood intake and rice intake were important dietary sources of toxic metals including arsenic, lead, cadmium and mercury, which could also explain the observed racial differences in arsenic and mercury. Education, smoking, secondhand smoking and geographic sites were significant predictors of urinary concentrations of different sets of metals. Additional studies are needed to examine other potential sources and characterizations of metal exposures, to better understand racial/ethnic inequalities in environmental metal exposures. Further research is also needed to investigate whether the observed race/ethnic differences in metal exposures may contribute to differences in health outcomes.

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Table II. 1. Characteristics of SWAN participants providing urine samples for analysis of metals by race/ethnicity.

Characteristics	Total population (n=1,335)	White (n=675)	Black (n=291)	Chinese (n=170)	Japanese (n=199)
	N (%)	N (%)	N (%)	N (%)	N (%)
Mean age (range), year	49.4 (44.9, 56.0)	49.5 (44.9, 56.0)	49.3 (45.0, 55.7)	49.7 (45.0, 55.7)	49.9 (45.0, 55.6)
Education					
High school or less	239 (17.9)	82 (12.2)	83 (28.5)	42 (24.7)	32 (16.1)
Some College	437 (32.7)	206 (30.5)	121 (41.6)	39 (22.9)	71 (35.7)
College and Post-graduate	659 (49.4)	387 (57.3)	87 (29.9)	89 (52.4)	96 (48.2)
Financial hardship					
Severe	83 (6.2)	32 (4.7)	37 (12.7)	8 (4.7)	6 (3.0)
Moderate	331 (24.8)	151 (22.4)	95 (32.7)	37 (21.8)	48 (24.1)
Minor	921 (69.0)	492 (72.9)	159 (54.6)	125 (73.5)	145 (72.9)
Smoking status					
Never	840 (62.9)	390 (57.8)	161 (55.3)	159 (93.5)	130 (65.3)
Former	360 (27.0)	227 (33.6)	72 (24.7)	9 (5.3)	52 (26.1)
Current	135 (10.1)	58 (8.6)	58 (19.9)	2 (1.2)	17 (8.5)
Secondhand smoking					
0 hour/week	681 (51.0)	309 (45.8)	109 (37.5)	135 (79.4)	128 (64.3)
<5 hours/week	352 (26.4)	209 (31.0)	75 (25.8)	25 (14.7)	43 (21.6)
≥5 hours/week	302 (22.6)	157 (23.3)	107 (36.8)	10 (5.9)	28 (14.1)
Seafood intake					
<1 time/week	382 (28.6)	236 (35.0)	81 (27.8)	31 (18.2)	34 (17.1)
1-1.9 times/week	462 (34.6)	232 (34.4)	99 (34.0)	64 (37.7)	67 (33.7)
≥2 times/week	491 (36.8)	207 (30.7)	111 (38.1)	75 (44.1)	98 (49.3)
Rice intake					
<1.5 times/week	470 (35.2)	318 (47.1)	137 (47.1)	7 (4.1)	8 (4.0)
1.5-3.4 times/week	511 (38.3)	323 (47.9)	117 (40.2)	28 (16.5)	43 (21.6)
≥3.5 times/week	354 (26.5)	34 (5.0)	37 (12.7)	135 (79.4)	148 (74.4)

Table II. 2. Model adjusted least-squares geometric mean concentrations[a] (LSGMs, μg/L) and 95% confidence intervals of urinary metals by characteristics of the SWAN participants, including seafood and rice intake.

LSGMs (μg/L)[b]	As	Ba	Cd	Co	Cs	Cu	Hg	Mn	Mo	Ni	Pb	Sb	Sn	Tl	Zn
Race/ethnicity															
White	14.2 (12.5, 16.2)	1.93 (1.74, 2.13)	0.43 (0.39, 0.47)	0.61 (0.56, 0.66)	4.93 (4.64, 5.23)	9.74 (9.16, 10.4)	1.14 (1.04, 1.25)	1.03 (0.95, 1.12)	40.7 (37.6, 43.9)	3.70 (3.46, 3.97)	0.84 (0.77, 0.91)	0.09 (0.08, 0.10)	1.07 (0.95, 1.20)	0.12 (0.11, 0.13)	270 (249, 292)
Black	12.6 (10.7, 14.8)	1.36 (1.19, 1.54)	0.43 (0.38, 0.49)	0.51 (0.46, 0.56)	3.67 (3.40, 3.95)	8.66 (8.01, 9.37)	0.89 (0.79, 1.00)	0.92 (0.82, 1.02)	31.8 (28.8, 35.0)	2.66 (2.44, 2.90)	0.80 (0.72, 0.89)	0.08 (0.08, 0.09)	1.07 (0.92, 1.23)	0.11 (0.10, 0.13)	342 (308, 378)
Chinese	27.9 (21.4, 36.2)	1.83 (1.49, 2.26)	0.76 (0.62, 0.92)	0.73 (0.62, 0.87)	6.88 (6.09, 7.78)	11.5 (10.1, 13.0)	1.29 (1.06, 1.57)	0.99 (0.84, 1.18)	50.5 (43.1, 59.3)	4.18 (3.63, 4.82)	1.14 (0.96, 1.35)	0.10 (0.08, 0.11)	0.73 (0.58, 0.93)	0.15 (0.13, 0.19)	360 (305, 426)
Japanese	24.6 (19.4, 31.2)	1.83 (1.52, 2.22)	0.83 (0.69, 0.99)	0.59 (0.51, 0.69)	4.17 (3.73, 4.66)	10.2 (9.07, 11.5)	1.31 (1.09, 1.57)	1.02 (0.87, 1.20)	50.2 (44.3, 59.3)	3.72 (3.27, 4.24)	0.88 (0.75, 1.03)	0.07 (0.06, 0.09)	0.91 (0.73, 1.13)	0.14 (0.12, 0.17)	340 (292, 396)
P-value	*<.0001*	*<.0001*	*<.0001*	*0.0007*	*<.0001*	*0.003*	*0.0004*	*0.24*	*<.0001*	*<.0001*	*0.008*	*0.08*	*0.03*	*0.07*	*<.0001*
Education															
≤ High school	17.7 (15.2, 20.6)	1.70 (1.51, 1.93)	0.62 (0.55, 0.69)	0.61 (0.55, 0.68)	4.87 (4.53, 5.24)	10.1 (9.34, 10.9)	1.01 (0.90, 1.13)	1.01 (0.91, 1.12)	42.4 (38.6, 46.6)	3.51 (3.23, 3.81)	0.90 (0.81, 0.99)	0.09 (0.08, 0.10)	0.87 (0.76, 1.00)	0.13 (0.11, 0.14)	342 (310, 378)
Some College	20.0 (17.5, 22.9)	1.83 (1.64, 2.04)	0.61 (0.55, 0.68)	0.60 (0.55, 0.65)	4.70 (4.41, 5.01)	9.97 (9.33, 10.7)	1.21 (1.09, 1.33)	0.97 (0.88, 1.06)	43.7 (40.2, 47.5)	3.56 (3.31, 3.83)	0.90 (0.83, 0.99)	0.09 (0.08, 0.09)	1.01 (0.90, 1.15)	0.14 (0.13, 0.16)	336 (308, 366)
≥ College	18.5 (16.1, 21.3)	1.63 (1.46, 1.82)	0.52 (0.47, 0.58)	0.60 (0.55, 0.66)	4.74 (4.44, 5.06)	9.85 (9.20, 10.5)	1.23 (1.11, 1.37)	0.99 (0.90, 1.09)	42.2 (38.8, 46.0)	3.49 (3.24, 3.76)	0.92 (0.84, 1.01)	0.08 (0.07, 0.09)	0.92 (0.82, 1.05)	0.13 (0.12, 0.14)	301 (276, 330)
P for trend	*0.34*	*0.10*	*0.01*	*0.26*	*0.58*	*0.38*	*0.0007*	*0.49*	*0.46*	*0.20*	*0.20*	*0.01*	*0.81*	*0.79*	*0.0008*
Financial hardship															
Severe	17.3 (13.7, 22.0)	1.69 (1.40, 2.04)	0.58 (0.49, 0.70)	0.62 (0.53, 0.72)	4.71 (4.21, 5.26)	10.1 (9.00, 11.3)	1.07 (0.90, 1.28)	0.99 (0.85, 1.16)	43.3 (37.5, 50.1)	3.60 (3.17, 4.09)	0.95 (0.81, 1.11)	0.09 (0.08, 0.10)	0.91 (0.73, 1.13)	0.13 (0.11, 0.16)	342 (294, 398)
Moderate	19.1 (16.7, 21.8)	1.67 (1.50, 1.86)	0.61 (0.55, 0.68)	0.60 (0.55, 0.66)	4.85 (4.55, 5.17)	10.1 (9.49, 10.8)	1.14 (1.03, 1.26)	1.03 (0.94, 1.12)	42.1 (38.8, 45.7)	3.51 (3.26, 3.77)	0.92 (0.84, 1.00)	0.08 (0.08, 0.09)	0.96 (0.85, 1.09)	0.14 (0.12, 0.15)	316 (290, 345)
Minor	19.9 (17.9, 22.0)	1.80 (1.66, 1.96)	0.55 (0.51, 0.60)	0.59 (0.56, 0.63)	4.76 (4.53, 5.00)	9.65 (9.18, 10.2)	1.22 (1.13, 1.32)	0.95 (0.89, 1.02)	42.9 (40.2, 45.7)	3.45 (3.26, 3.65)	0.86 (0.80, 0.92)	0.08 (0.08, 0.09)	0.93 (0.85, 1.02)	0.13 (0.12, 0.14)	320 (299, 342)
P for trend	*0.44*	*0.19*	*0.10*	*0.63*	*0.67*	*0.18*	*0.10*	*0.19*	*0.89*	*0.51*	*0.08*	*0.32*	*0.84*	*0.26*	*0.78*
Smoking															

LSGMs (μg/L)[b]	As	Ba	Cd	Co	Cs	Cu	Hg	Mn	Mo	Ni	Pb	Sb	Sn	Tl	Zn
Never	18.6 (16.6, 20.9)	1.65 (1.50, 1.80)	0.45 (0.41, 0.49)	0.65 (0.61, 0.70)	4.66 (4.42, 4.92)	10.0 (9.47, 10.6)	1.21 (1.11, 1.31)	0.95 (0.88, 1.02)	44.8 (41.8, 48.0)	3.74 (3.52, 3.98)	0.80 (0.74, 0.86)	0.08 (0.07, 0.09)	0.95 (0.85, 1.05)	0.14 (0.13, 0.15)	310 (288, 333)
Former	18.9 (16.4, 21.9)	1.70 (1.51, 1.90)	0.54 (0.48, 0.60)	0.60 (0.55, 0.66)	4.87 (4.55, 5.21)	10.1 (9.36, 10.8)	1.09 (0.98, 1.22)	1.02 (0.92, 1.12)	42.8 (39.1, 46.7)	3.53 (3.27, 3.82)	0.88 (0.80, 0.97)	0.08 (0.07, 0.09)	0.82 (0.72, 0.93)	0.13 (0.12, 0.15)	320 (296, 357)
Current	18.6 (15.2, 22.7)	1.83 (1.56, 2.14)	0.82 (0.70, 0.95)	0.56 (0.49, 0.64)	4.79 (4.36, 5.26)	9.82 (8.91, 10.8)	1.13 (0.97, 1.31)	1.00 (0.88, 1.15)	40.9 (36.2, 46.2)	3.30 (2.96, 3.67)	1.06 (0.93, 1.20)	0.09 (0.08, 0.10)	1.06 (0.88, 1.26)	0.13 (0.11, 0.15)	344 (302, 390)
P for trend	*0.66*	*0.29*	*<.0001*	*0.002*	*0.21*	*0.55*	*0.17*	*0.10*	*0.16*	*0.005*	*<.0001*	*0.12*	*0.70*	*0.54*	*0.08*
Secondhand smoking															
0 hr/wk	19.2 (16.8, 21.9)	1.67 (1.50, 1.85)	0.61 (0.55, 0.67)	0.59 (0.54, 0.65)	4.75 (4.46, 5.06)	9.91 (9.29, 10.6)	1.12 (1.01, 1.23)	1.04 (0.96, 1.14)	44.7 (41.2, 48.5)	3.44 (3.20, 3.70)	0.86 (0.79, 0.94)	0.09 (0.08, 0.09)	0.92 (0.82, 1.04)	0.14 (0.12, 0.15)	316 (290, 344)
<5 hrs/wk	17.8 (15.3, 20.9)	1.76 (1.56, 1.99)	0.56 (0.50, 0.63)	0.62 (0.57, 0.69)	4.73 (4.40, 5.08)	10.3 (9.60, 11.1)	1.14 (1.02, 1.28)	0.97 (0.88, 1.08)	40.7 (37.1, 44.7)	3.59 (3.31, 3.90)	0.92 (0.83, 1.02)	0.08 (0.07, 0.09)	0.95 (0.82, 1.08)	0.13 (0.11, 0.14)	319 (289, 352)
≥5 hs/wk	19.3 (16.6, 22.3)	1.73 (1.54, 1.95)	0.59 (0.52, 0.65)	0.60 (0.55, 0.66)	4.83 (4.52, 5.18)	9.64 (8.98, 10.4)	1.17 (1.05, 1.31)	0.95 (0.86, 1.05)	43.0 (39.3, 46.9)	3.53 (3.26, 3.82)	0.94 (0.85, 1.03)	0.09 (0.08, 0.10)	0.94 (0.82, 1.07)	0.13 (0.12, 0.15)	344 (313, 377)
P for trend	*0.82*	*0.30*	*0.58*	*0.25*	*0.70*	*0.72*	*0.30*	*0.09*	*0.47*	*0.52*	*0.005*	*0.37*	*0.20*	*0.47*	*0.06*
Seafood intake															
<1 /wk	15.3 (13.2, 17.7)	1.69 (1.51, 1.90)	0.57 (0.51, 0.63)	0.60 (0.55, 0.66)	4.52 (4.22, 4.84)	9.83 (9.15, 10.6)	1.03 (0.92, 1.15)	0.96 (0.87, 1.05)	40.6 (37.1, 44.3)	3.44 (3.18, 3.72)	0.87 (0.79, 0.96)	0.08 (0.08, 0.09)	0.91 (0.80, 1.03)	0.13 (0.11, 0.14)	324 (295, 356)
1-1.9 /wk	17.9 (15.7, 20.6)	1.64 (1.47, 1.82)	0.57 (0.51, 0.63)	0.60 (0.55, 0.65)	4.93 (4.62, 5.25)	9.76 (9.13, 10.4)	1.17 (1.05, 1.29)	0.97 (0.89, 1.06)	43.4 (40.0, 47.2)	3.49 (3.24, 3.75)	0.88 (0.81, 0.96)	0.08 (0.07, 0.09)	1.00 (0.89, 1.13)	0.13 (0.12, 0.15)	328 (301, 358)
≥2 /wk	23.8 (20.9, 27.2)	1.84 (1.66, 2.04)	0.62 (0.56, 0.69)	0.61 (0.56, 0.67)	4.87 (4.58, 5.18)	10.3 (9.67, 11.0)	1.24 (1.12, 1.37)	1.04 (0.96, 1.14)	44.4 (40.9, 48.2)	3.64 (3.39, 3.90)	0.97 (0.89, 1.06)	0.09 (0.08, 0.09)	0.90 (0.80, 1.01)	0.14 (0.13, 0.15)	326 (299, 354)
P for trend	*<.0001*	*0.11*	*0.13*	*0.47*	*0.13*	*0.13*	*0.002*	*0.10*	*0.04*	*0.11*	*0.04*	*0.52*	*0.93*	*0.09*	*0.74*
Rice intake															
<1.5 /wk	15.8 (13.5, 18.4)	1.69 (1.49, 1.91)	0.56 (0.50, 0.63)	0.59 (0.54, 0.65)	4.59 (4.26, 4.94)	9.79 (9.08, 10.6)	1.04 (0.93, 1.17)	0.99 (0.89, 1.10)	40.9 (37.2, 45.0)	3.43 (3.15, 3.73)	0.88 (0.79, 0.97)	0.08 (0.07, 0.09)	0.87 (0.76, 1.00)	0.13 (0.11, 0.14)	323 (293, 357)
1.5-3.4 /wk	16.00	1.68	0.57	0.58	4.60	9.49	1.11	0.99	40.2	3.34	0.89	0.08	0.90	0.13	336

LSGMs (μg/L)[b]	As	Ba	Cd	Co	Cs	Cu	Hg	Mn	Mo	Ni	Pb	Sb	Sn	Tl	Zn
	(13.8, 18.5)	(1.49, 1.89)	(0.51, 0.63)	(0.52, 0.63)	(4.29, 4.93)	(8.83, 10.2)	(0.99, 1.24)	(0.90, 1.09)	(36.8, 44.0)	(3.08, 3.61)	(0.81, 0.98)	(0.07, 0.09)	(0.79, 1.03)	(0.11, 0.14)	(306, 370)
≥3.5 /wk	26.1	1.80	0.63	0.65	5.15	10.6	1.29	0.99	47.5	3.81	0.95	0.09	1.04	0.14	319
	(22.3, 30.5)	(1.59, 2.04)	(0.56, 0.70)	(0.59, 0.72)	(4.79, 5.54)	(9.86, 11.5)	(1.15, 1.45)	(0.89, 1.10)	(43.2, 52.2)	(3.51, 4.15)	(0.86, 1.05)	(0.08, 0.10)	(0.90, 1.19)	(0.13, 0.16)	(288, 352)
P for trend	*<.0001*	*0.98*	*0.07*	*0.99*	*0.002*	*0.56*	*0.003*	*0.55*	*0.09*	*0.65*	*0.14*	*0.97*	*0.29*	*0.06*	*0.60*

[a] All models were adjusted for age, race, education, financial hardship, smoking, secondhand smoking, seafood intake, rice intake, total energy intake, study sites and urinary creatinine.

[b] For values greater than 1, 3 significant figures were shown; for values less than 1, values were shown to 2 decimal places.

Table II. 3. Model adjusted least-squares geometric mean concentrations[a] (LSGMs, μg/L) and 95% confidence intervals of urinary metals in white and black women by study sites.

LSGMs[b,c] (μg/L)	As	Ba	Cd	Co	Cs	Cu	Hg	Mn	Mo	Ni	Pb	Sb	Sn	Tl	Zn
White (n=675)															
Michigan	6.85 (5.30, 8.87)	1.72 (1.41, 2.08)	0.29 (0.24, 0.36)	0.58 (0.50, 0.69)	3.62 (3.18, 4.13)	9.84 (8.80, 11.0)	0.83 (0.69, 1.00)	0.95 (0.80, 1.14)	40.3 (34.3, 47.3)	3.43 (2.99, 3.93)	0.58 (0.49, 0.68)	0.11 (0.09, 0.12)	0.99 (0.79, 1.25)	0.09 (0.07, 0.11)	299 (252, 356)
Boston	15.9 (12.5, 20.4)	1.56 (1.30, 1.87)	0.60 (0.50, 0.72)	0.52 (0.44, 0.61)	5.13 (4.53, 5.82)	9.57 (8.60, 10.7)	1.57 (1.31, 1.88)	1.37 (1.16, 1.62)	30.4 (33.8, 45.9)	2.81 (2.47, 3.20)	1.15 (0.98, 1.35)	0.09 (0.08, 0.11)	1.08 (0.87, 1.35)	0.12 (0.10, 0.15)	231 (195, 272)
Oakland	11.6 (8.98, 15.0)	1.82 (1.50, 2.20)	0.37 (0.30, 0.45)	0.44 (0.37, 0.52)	4.90 (4.30, 5.58)	8.47 (7.58, 9.47)	0.78 (0.64, 0.94)	1.05 (0.89, 1.25)	31.6 (27.0, 37.1)	3.61 (3.15, 4.13)	0.71 (0.61, 0.84)	0.08 (0.07, 0.10)	0.92 (0.73, 1.16)	0.12 (0.10, 0.14)	239 (201, 284)
Los Angeles	14.0 (11.0, 17.7)	1.69 (1.42, 2.02)	0.32 (0.27, 0.39)	0.59 (0.51, 0.69)	4.81 (4.27, 5.43)	9.06 (8.17, 10.0)	0.93 (0.78, 1.11)	0.84 (0.72, 0.99)	38.1 (32.9, 44.2)	4.08 (3.60, 4.63)	0.68 (0.58, 0.79)	0.09 (0.08, 0.10)	1.04 (0.84, 1.29)	0.10 (0.08, 0.12)	263 (224, 308)
Pittsburgh	11.3 (8.87, 14.5)	2.18 (1.82, 2.63)	0.55 (0.46, 0.66)	0.71 (0.61, 0.83)	4.21 (3.72, 4.77)	10.5 (9.43, 11.7)	1.17 (0.97, 1.40)	0.91 (0.77, 1.07)	36.8 (31.5, 42.9)	4.48 (3.93, 5.11)	0.93 (0.79, 1.08)	0.09 (0.08, 0.10)	1.39 (1.11, 1.74)	0.13 (0.10, 0.15)	286 (243, 338)
P-value	*<.0001*	*0.007*	*<.0001*	*<.0001*	*<.0001*	*0.004*	*<.0001*	*<.0001*	*0.04*	*<.0001*	*<.0001*	*0.08*	*0.006*	*0.01*	*0.02*
Black (n=291)															
Michigan	9.54 (7.51, 12.1)	1.69 (1.38, 2.06)	0.42 (0.36, 0.50)	0.72 (0.62, 0.83)	3.99 (3.63, 4.38)	11.0 (9.60, 12.6)	0.99 (0.83, 1.19)	1.04 (0.93, 1.18)	38.2 (33.2, 43.9)	3.23 (2.81, 3.71)	0.81 (0.69, 0.96)	0.12 (0.10, 0.14)	1.51 (1.21, 1.88)	0.09 (0.07, 0.11)	498 (436, 570)
Boston	18.4 (14.1, 24.1)	1.29 (1.03, 1.62)	0.69 (0.57, 0.82)	0.54 (0.46, 0.64)	4.68 (4.22, 5.20)	12.3 (10.6, 14.4)	1.35 (1.10, 1.65)	1.35 (1.19, 1.55)	42.0 (35.9, 49.1)	2.45 (2.10, 2.87)	1.08 (0.90, 1.29)	0.09 (0.07, 0.11)	1.23 (0.96, 1.57)	0.14 (0.11, 0.19)	429 (370, 499)
Pittsburgh	15.4 (11.2, 21.2)	2.06 (1.57, 2.69)	0.44 (0.36, 0.55)	0.68 (0.56, 0.82)	3.87 (3.42, 4.38)	11.3 (9.41, 13.5)	1.27 (1.00, 1.61)	0.93 (0.80, 1.09)	39.5 (32.9, 47.5)	3.84 (3.19, 4.61)	1.08 (0.87, 1.34)	0.10 (0.08, 0.13)	1.44 (1.07, 1.92)	0.21 (0.16, 0.29)	413 (346, 493)
P-value	*0.0001*	*0.02*	*<.0001*	*0.02*	*0.02*	*0.46*	*0.02*	*0.0004*	*0.61*	*0.0003*	*0.009*	*0.06*	*0.40*	*<.0001*	*0.08*

[a] All models were adjusted for age, race, education, financial hardship, smoking, secondhand smoking, seafood intake, rice intake, total energy intake, and urinary creatinine.

[b] Chinese and Japanese were not sampled in multiple sites by study design.

[c] For values greater than 1, 3 significant figures were shown; for values less than 1, values were shown to 2 decimal places.

Table II. 4. Estimated cumulative odds ratio (95% confidence intervals) of being clustered into the "high" exposure pattern[a] by selected determinants in backward elimination[b].

Selected variables[c]	Odds ratio	95% CI	P-value[d]
Black vs. white women	0.39	0.26, 0.56	<.0001
Chinese vs. white women	2.10	1.19, 3.69	0.01
Japanese vs. white women	2.32	1.39, 3.90	0.001
Former vs. never smoker	1.03	0.78, 1.37	0.84
Current vs. never smoker	2.25	1.47, 3.44	0.0002
Seafood intake 1-1.9 /wk vs. <1 time/wk	1.31	0.96, 1.77	0.09
Seafood intake ≥2 vs. <1 time/wk	1.83	1.34, 2.50	0.0001
Rice intake 1.5-3.4 /wk vs. <1.5 times/wk	1.07	0.80, 1.44	0.65
Rice intake ≥3.5 vs. <1.5 times/wk	1.68	1.09, 2.59	0.02

[a] Participants with "high" vs. "low" exposure patterns were clustered by k-means clustering method.

[b] Initial model included race, education, financial hardship, smoking, secondhand smoking, seafood intake and rice intake. Age, study sites, and total energy intake, were forced in model selection.

[c] Reference groups: race: white women; smoking: never smoker; seafood intake: <1 time/week; rice intake: <1.5 times/week.

[d] *Ps* <0.05 for all selected variables in backward elimination.

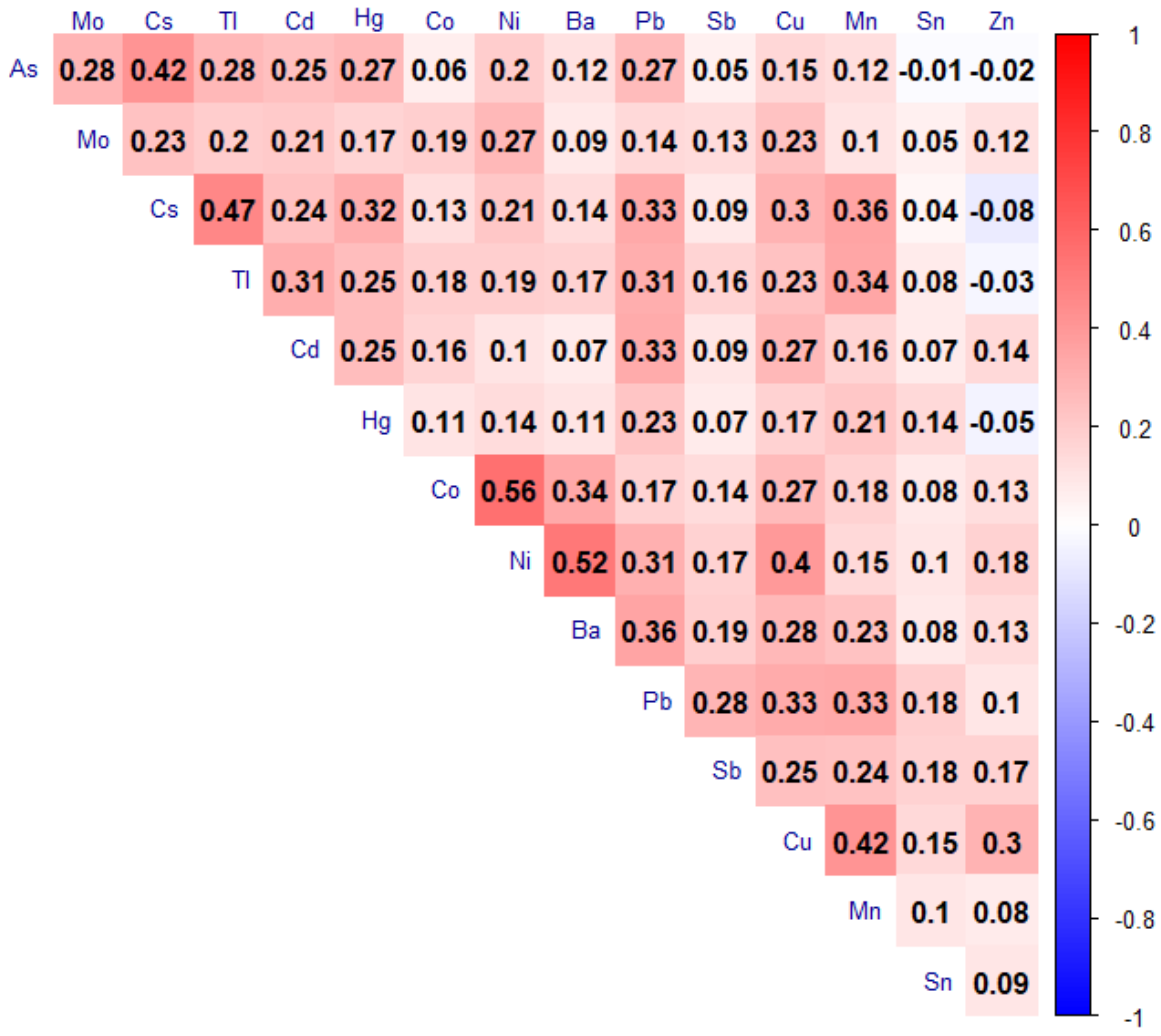


Figure II. 1. Spearman correlation matrix of urinary creatinine-adjusted metal concentrations.
As: arsenic, Ba: barium, Cd: cadmium, Co: cobalt, Cs: cesium, Cu: copper, Hg: mercury, Mn: manganese, Mo: molybdenum, Ni: nickel, Pb: lead, Sb: antimony, Sn: tin, Tl: thallium, Zn: zinc.

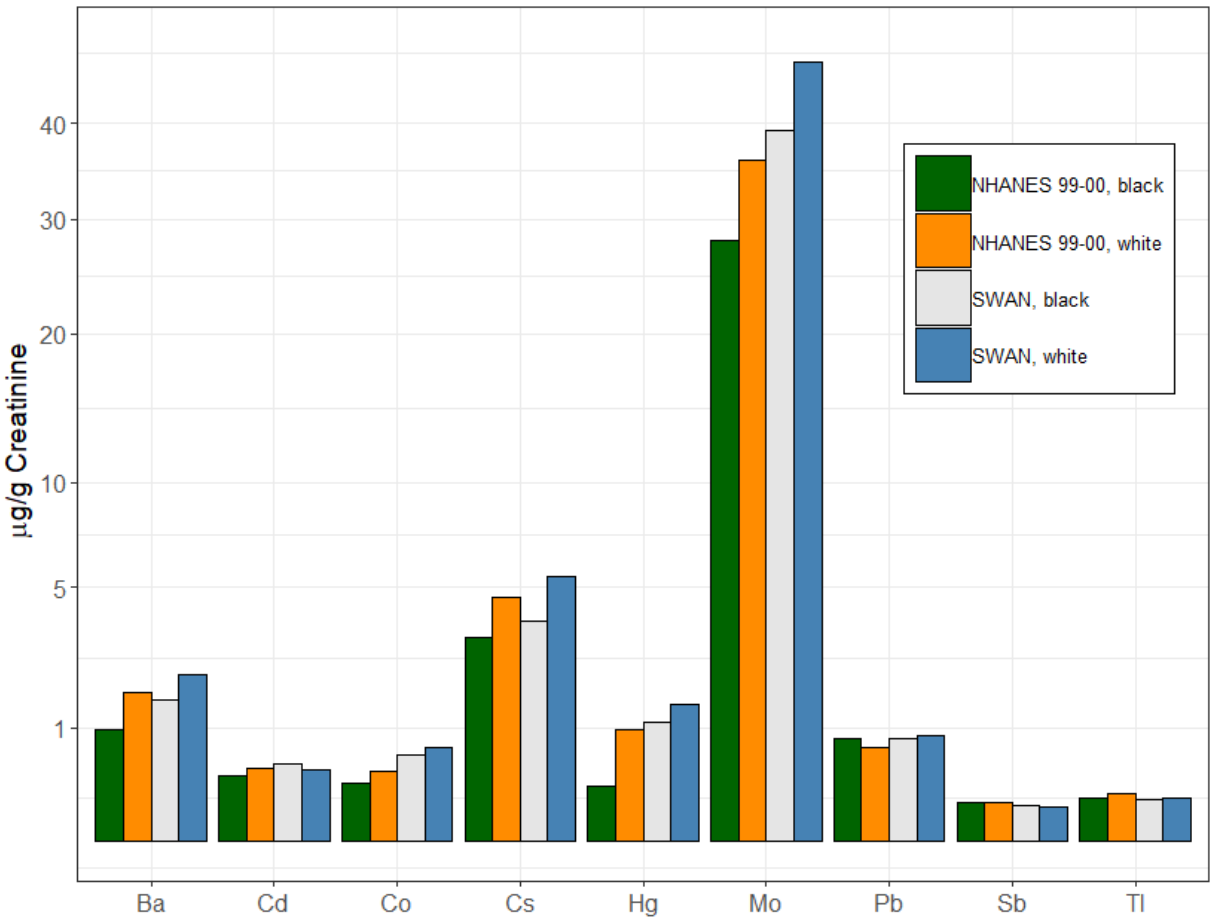


Figure II. 2. Comparisons of creatinine-adjusted median concentrations of urinary metals in white and black women from SWAN and NHANES 1999-2000.
NHANES: National Health and Nutrition Examination Survey. Ba: barium, Cd: cadmium, Co: cobalt, Cs: cesium, Hg: mercury, Mo: molybdenum, Pb: lead, Sb: antimony, Tl: thallium.

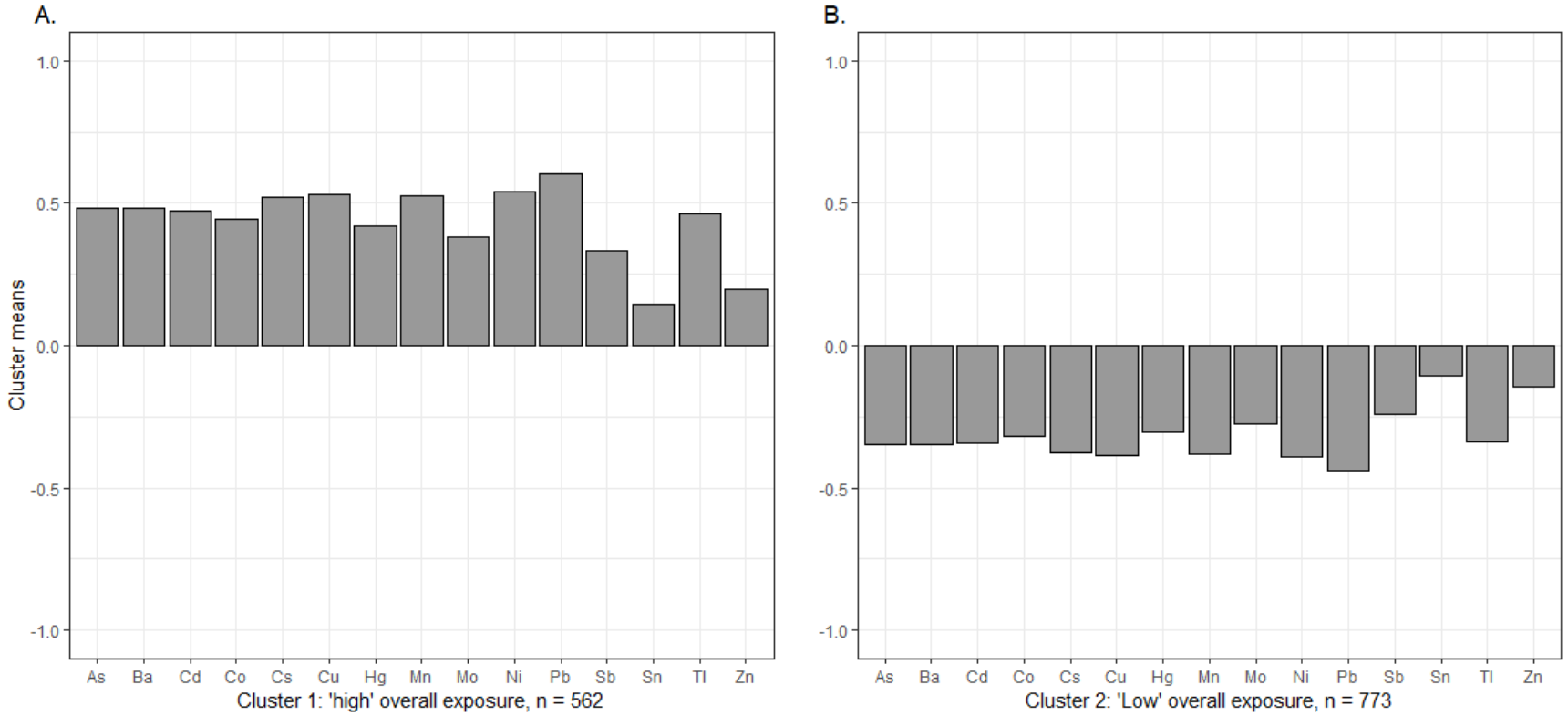


Figure II. 3. Cluster means of the 15 standardized log-transformed urinary metals using k-means in the SWAN data.
Y-axis (cluster means) represents the mean standardized natural log-transformed urinary creatinine adjusted metal concentrations. Cluster 1: "high" overall metal exposure pattern; cluster 2: "low" overall metal exposure pattern. As: arsenic, Ba: barium, Cd: cadmium, Co: cobalt, Cs: cesium, Cu: copper, Hg: mercury, Mn: manganese, Mo: molybdenum, Ni: nickel, Pb: lead, Sb: antimony, Sn: tin, Tl: thallium, Zn: zinc.

Supplemental Table II. 1. Unadjusted (μg/L) and urinary creatinine adjusted (μg/g) metal concentrations in SWAN participants.

Metals	LOD[a]	% > LOD[b]	GM (GSD)[c]	Selected percentiles					
				5th	25th	50th	75th	90th	95th
Arsenic	0.3	100	17.11 (3.58)	2.55	6.84	15.03	38.75	94.71	151.13
			20.23 (3.39)[d]	*3.96*	*8.03*	*16.89*	*43.73*	*107.23*	*184.35*
Barium	0.1	99.5	1.72 (2.50)	0.37	0.99	1.78	2.97	5.02	7.31
			2.04 (2.38)	*0.53*	*1.16*	*2.00*	*3.47*	*5.79*	*8.51*
Beryllium	0.04	16.2	n.d.[e]	< LOD	< LOD	< LOD	< LOD	0.05	0.08
			n.d.	*< LOD*	*< LOD*	*< LOD*	*< LOD*	*0.12*	*0.19*
Cadmium	0.06	94.5	0.41 (2.81)	< LOD	0.22	0.44	0.80	1.42	1.98
			0.48 (2.34)	*< LOD*	*0.29*	*0.49*	*0.84*	*1.32*	*1.83*
Cobalt	0.05	99.2	0.60 (2.28)	0.15	0.37	0.62	0.95	1.70	2.27
			0.71 (1.92)	*0.27*	*0.46*	*0.67*	*1.05*	*1.68*	*2.30*
Chromium	0.4	24.3	n.d.	< LOD	< LOD	< LOD	< LOD	0.84	1.58
			n.d.	*< LOD*	*< LOD*	*< LOD*	*< LOD*	*1.38*	*2.50*
Cesium	0.01	100	4.67 (2.02)	1.51	3.03	4.73	7.32	10.44	14.06
			5.52 (1.77)	*2.51*	*3.97*	*5.32*	*7.50*	*11.03*	*14.20*
Copper	2.5	96.6	9.45 (2.04)	2.99	6.07	9.53	14.47	21.75	29.36
			11.18 (1.67)	*5.96*	*8.35*	*10.45*	*13.54*	*19.12*	*27.01*
Mercury	0.05	99.7	1.18 (2.57)	0.25	0.66	1.23	2.37	3.71	5.22
			1.40 (2.28)	*0.35*	*0.87*	*1.43*	*2.47*	*3.79*	*5.02*
Manganese	0.08	99.7	0.96 (2.14)	0.33	0.59	0.90	1.47	2.46	3.40
			1.14 (2.22)	*0.37*	*0.67*	*1.03*	*1.78*	*3.15*	*4.87*
Molybdenum	0.3	100	41.76 (2.28)	9.86	24.92	43.88	71.73	113.74	144.31
			49.39 (1.92)	*17.83*	*34.18*	*48.65*	*70.75*	*105.28*	*139.33*
Nickel	0.8	95.9	3.56 (2.11)	0.89	2.35	3.77	5.85	8.49	10.66
			4.21 (1.83)	*1.56*	*2.89*	*4.20*	*6.10*	*8.99*	*11.12*
Lead	0.1	97.8	0.76 (2.35)	0.19	0.46	0.78	1.26	2.06	2.74
			0.90 (2.05)	*0.32*	*0.57*	*0.87*	*1.33*	*2.14*	*2.85*
Platinum	0.05	2.6	n.d.	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD
			n.d.	*< LOD*	*< LOD*	*< LOD*	*< LOD*	*< LOD*	*< LOD*
Antimony	0.04	78.8	0.08 (2.20)	< LOD	0.04	0.08	0.13	0.21	0.30
			0.09 (2.05)	*< LOD*	*0.06*	*0.09*	*0.14*	*0.21*	*0.30*
Tin	0.1	96.8	0.97 (3.00)	0.16	0.49	0.94	1.78	3.57	6.67
			1.14 (2.57)	*0.32*	*0.63*	*1.00*	*1.83*	*3.74*	*7.06*
Thallium	0.02	92.2	0.13 (2.57)	< LOD	0.08	0.15	0.23	0.33	0.40
			0.15 (2.32)	*< LOD*	*0.10*	*0.16*	*0.23*	*0.36*	*0.48*
Uranium	0.01	33.0	0.01 (1.97)	< LOD	< LOD	< LOD	0.01	0.03	0.04
			0.01 (2.44)	*< LOD*	*< LOD*	*< LOD*	*0.02*	*0.04*	*0.07*
Vanadium	0.6	37.2	0.69 (2.14)	< LOD	< LOD	< LOD	1.05	2.39	3.42
			0.82 (2.56)	*< LOD*	*< LOD*	*< LOD*	*1.45*	*3.13*	*4.41*
Tungsten	0.2	29.6	n.d.	< LOD	< LOD	< LOD	0.23	0.43	0.64
			n.d.	*< LOD*	*< LOD*	*< LOD*	*0.33*	*0.67*	*1.04*
Zinc	2	100	283 (2)	56	167	308	532	810	1033
			335 (2)	117	228	345	503	714	927

[a] LOD: limit of detection.
[b] % > LOD: detection rate
[c] GM: geometric mean; GSD: geometric standard deviation
[d] Italic type denotes measure in μg/g creatinine
[e] n.d.: not determined

Supplemental Table II. 2. Model adjusted least-squares geometric mean concentrationsa (LSGMs, µg/L) and 95% confidence intervals of urinary metals by characteristics of the SWAN participants.

LGSMs[b] (µg/L)	As	Ba	Cd	Co	Cs	Cu	Hg	Mn	Mo	Ni	Pb	Sb	Sn	Tl	Zn
Race															
White	12.3 (10.9, 13.8)	1.89 (1.73, 2.08)	0.42 (0.38, 0.46)	0.60 (0.55, 0.64)	4.75 (4.50, 5.02)	9.48 (8.96, 10.0)	1.07 (0.98, 1.17)	1.03 (0.95, 1.11)	38.8 (36.2, 41.7)	3.58 (3.36, 3.81)	0.82 (0.76, 0.88)	0.08 (0.07, 0.09)	1.02 (0.92, 1.13)	0.12 (0.11, 0.13)	272 (252, 293)
Black	11.5 (9.79, 13.5)	1.35 (1.19, 1.52)	0.42 (0.38, 0.47)	0.50 (0.45, 0.55)	3.58 (3.32, 3.85)	8.51 (7.88, 9.18)	0.85 (0.75, 0.95)	0.92 (0.83, 1.02)	30.7 (27.9, 33.8)	2.59 (2.38, 2.81)	0.79 (0.71, 0.88)	0.08 (0.07, 0.09)	1.03 (0.90, 1.19)	0.11 (0.10, 0.13)	342 (310, 378)
Chinese	37.2 (28.9, 48.0)	1.92 (1.58, 2.34)	0.80 (0.67, 0.97)	0.77 (0.66, 0.91)	7.31 (6.50, 8.21)	12.1 (10.7, 13.6)	1.42 (1.18, 1.72)	1.01 (0.86, 1.19)	54.8 (47.1, 63.8)	4.44 (3.88, 5.07)	1.19 (1.01, 1.40)	0.10 (0.08, 0.12)	0.78 (0.63, 0.98)	0.16 (0.14, 0.20)	353 (302, 414)
Japanese	32.1 (25.4, 40.5)	1.92 (1.61, 2.30)	0.87 (0.74, 1.03)	0.62 (0.54, 0.71)	4.38 (3.94, 4.88)	10.7 (9.59, 12.0)	1.42 (1.20, 1.68)	1.04 (0.90, 1.21)	55.1 (47.9, 63.3)	3.93 (3.47, 4.40)	0.92 (0.79, 1.07)	0.08 (0.07, 0.09)	0.96 (0.78, 1.17)	0.15 (0.13, 0.18)	333 (288, 385)
P-value	*<.0001*	*<.0001*	*<.0001*	*<.0001*	*<.0001*	*<.0001*	*<.0001*	*0.28*	*<.0001*	*<.0001*	*<.0001*	*0.09*	*0.11*	*0.0002*	*<.0001*
Education															
≤ High school	18.9 (16.2, 22.1)	1.72 (1.53, 1.95)	0.63 (0.56, 0.70)	0.62 (0.56, 0.68)	4.94 (4.60, 5.32)	10.2 (9.48, 11.0)	1.02 (0.91, 1.15)	1.01 (0.92, 1.12)	43.3 (39.4, 47.5)	3.56 (3.28, 3.87)	0.90 (0.82, 1.00)	0.10 (0.08, 0.11)	0.88 (0.77, 1.01)	0.13 (0.11, 0.15)	340 (308, 374)
Some College	21.7 (18.9, 24.9)	1.87 (1.68, 2.08)	0.62 (0.56, 0.69)	0.61 (0.56, 0.66)	4.75 (4.46, 5.06)	10.1 (9.49, 10.8)	1.22 (1.11, 1.35)	0.98 (0.90, 1.07)	44.5 (41.0, 48.3)	3.61 (3.36, 3.88)	0.91 (0.84, 1.00)	0.09 (0.08, 0.10)	1.02 (0.90, 1.15)	0.14 (0.13, 0.16)	333 (305, 362)
≥ College	20.2 (17.6, 23.3)	1.66 (1.49, 1.86)	0.53 (0.48, 0.59)	0.61 (0.56, 0.67)	4.80 (4.50, 5.12)	9.96 (9.32, 10.7)	1.26 (1.13, 1.40)	1.00 (0.92, 1.10)	42.9 (39.5, 46.7)	3.52 (3.27, 3.79)	0.93 (0.85, 1.02)	0.08 (0.07, 0.09)	0.93 (0.82, 1.05)	0.13 (0.12, 0.15)	299 (274, 327)
P for trend	*0.13*	*0.15*	*0.02*	*0.29*	*0.50*	*0.47*	*0.0004*	*0.38*	*0.55*	*0.22*	*0.14*	*0.01*	*0.81*	*0.69*	*0.0006*
Financial hardship															
Severe	19.0 14.9, 24.2)	1.74 (1.44, 2.10)	0.59 (0.50, 0.71)	0.63 (0.54, 0.73)	4.74 (4.24, 5.29)	10.3 (9.18, 11.6)	1.08 (0.91, 1.29)	1.00 (0.86, 1.17)	44.0 (38.1, 50.8)	3.65 (3.21, 4.14)	0.96 (0.83, 1.12)	0.09 (0.08, 0.10)	0.91 (0.73, 1.12)	0.13 (0.11, 0.16)	338 (291, 392)
Moderate	20.7 (18.1, 23.7)	1.70 (1.53, 1.88)	0.62 (0.56, 0.69)	0.61 (0.56, 0.67)	4.94 (4.64, 5.25)	10.3 (9.63, 11.0)	1.17 (1.06, 1.29)	1.03 (0.95, 1.13)	43.1 (39.7, 46.7)	3.55 (3.31, 3.82)	0.93 (0.85, 1.01)	0.08 (0.08, 0.09)	0.98 (0.87, 1.10)	0.14 (0.13, 0.15)	314 (289, 342)
Minor	21.1 (19.0, 23.5)	1.82 (1.68, 2.00)	0.56 (0.52, 0.61)	0.60 (0.56, 0.64)	4.82 (4.59, 5.06)	9.75 (9.27, 10.3)	1.25 (1.16, 1.35)	0.96 (0.90, 1.03)	43.6 (40.9, 46.4)	3.49 (3.30, 3.69)	0.87 (0.81, 0.93)	0.08 (0.08, 0.09)	0.94 (0.86, 1.03)	0.13 (0.12, 0.14)	319 (299, 341)
P for trend	*0.59*	*0.23*	*0.09*	*0.59*	*0.65*	*0.16*	*0.09*	*0.16*	*0.89*	*0.55*	*0.07*	*0.29*	*0.83*	*0.26*	*0.87*
Smoking															
Never	20.5	1.68	0.46	0.66	4.74	10.2	1.24	0.96	45.9	3.81	0.82	0.08	0.96	0.14	308

LGSMs[b] (μg/L)	As	Ba	Cd	Co	Cs	Cu	Hg	Mn	Mo	Ni	Pb	Sb	Sn	Tl	Zn
	(18.3, 23.0)	(1.54, 1.83)	(0.42, 0.50)	(0.62, 0.71)	(4.50, 5.00)	(9.64, 10.7)	(1.14, 1.35)	(0.89, 1.03)	(42.9, 49.1)	(3.58, 4.04)	(0.76, 0.88)	(0.07, 0.09)	(0.87, 1.06)	(0.13, 0.15)	(287, 331)
Former	20.6	1.73	0.55	0.61	4.93	10.2	1.12	1.03	43.6	3.58	0.89	0.09	0.83	0.13	322
	(17.8, 23.8)	(1.54, 1.93)	(0.49, 0.61)	(0.56, 0.67)	(4.61, 5.28)	(9.52, 10.9)	(1.00, 1.24)	(0.93, 1.13)	(40.0, 47.6)	(3.32, 3.87)	(0.81, 0.98)	(0.08, 0.10)	(0.73, 0.94)	(0.12, 0.15)	(294, 353)
Current	19.7	1.86	0.83	0.57	4.82	9.93	1.14	1.01	41.3	3.32	1.06	0.09	1.06	0.13	341
	(16.1, 24.1)	(1.58, 2.17)	(0.71, 0.96)	(0.50, 0.64)	(4.39, 5.29)	(9.01, 10.9)	(0.98, 1.32)	(0.89, 1.15)	(36.6, 46.6)	(2.98, 3.70)	(0.93, 1.21)	(0.08, 0.10)	(0.89, 1.27)	(0.11, 0.15)	(300, 387)
P for trend	*0.88*	*0.27*	*<.0001*	*0.002*	*0.28*	*0.53*	*0.10*	*0.10*	*0.12*	*0.004*	*<.0001*	*0.12*	*0.67*	*0.46*	*0.09*
Secondhand smoking															
0 hr/wk	20.8	1.69	0.62	0.60	4.82	10.1	1.14	1.05	45.6	3.49	0.88	0.09	0.94	0.14	314
	(18.1, 23.8)	(1.52, 1.88)	(0.56, 0.68)	(0.55, 0.65)	(4.54, 5.13)	(9.43, 10.7)	(1.03, 1.26)	(0.96, 1.14)	(42.1, 49.5)	(3.25, 3.75)	(0.80, 0.95)	(0.08, 0.10)	(0.83, 1.06)	(0.13, 0.16)	(289, 341)
<5 hrs/wk	19.00	1.79	0.57	0.63	4.77	10.5	1.16	0.98	41.4	3.63	0.93	0.08	0.95	0.13	317
	(16.3, 22.2)	(1.59, 2.02)	(0.50, 0.63)	(0.57, 0.69)	(4.45, 5.12)	(9.73, 11.3)	(1.03, 1.30)	(0.89, 1.09)	(37.7, 45.4)	(3.34, 3.94)	(0.84, 1.03)	(0.07, 0.09)	(0.83, 1.09)	(0.12, 0.15)	(288, 349)
≥5 hs/wk	21.1	1.77	0.60	0.61	4.89	9.79	1.19	0.96	43.8	3.57	0.95	0.09	0.94	0.13	340
	(18.2, 24.4)	(1.58, 1.99)	(0.53, 0.66)	(0.56, 0.67)	(4.58, 5.24)	(9.13, 10.5)	(1.07, 1.33)	(0.88, 1.06)	(40.1, 47.8)	(3.31, 3.86)	(0.87, 1.04)	(0.08, 0.10)	(0.83, 1.07)	(0.12, 0.15)	(311, 373)
P for trend	*0.89*	*0.19*	*0.62*	*0.21*	*0.53*	*0.67*	*0.04*	*0.10*	*0.43*	*0.53*	*0.005*	*0.32*	*0.24*	*0.41*	*0.06*

[a] All models were adjusted for age, race, education, financial hardship, smoking, secondhand smoking, study sites and urinary creatinine.

[b] For values greater than 1, 3 significant figures were shown; for values less than 1, values were shown to 2 decimal places.

Supplemental Table II. 3. Model adjusted least-squares geometric mean concentrationsa (LSGMs, μg/L) and 95% confidence intervals of urinary metals between white and Chinese women within Oakland site, and between white and Japanese women within Los Angeles site.

LGSMs[b,c] (μg/L)	As	Ba	Cd	Co	Cs	Cu	Hg	Mn	Mo	Ni	Pb	Sb	Sn	Tl	Zn
Oakland (n=300)															
White (n=130)	13.9 (9.52, 20.4)	1.91 (1.38, 2.63)	0.46 (0.34, 0.62)	0.40 (0.31, 0.53)	5.32 (4.10, 6.91)	7.71 (6.27, 9.48)	1.12 (0.84, 1.48)	0.91 (0.68, 1.20)	31.6 (25.0, 39.9)	3.04 (2.49, 3.70)	0.71 (0.56, 0.89)	0.08 (0.07, 0.11)	0.72 (0.50, 1.02)	0.13 (0.11, 0.16)	219 (169, 284)
Chinese (n=170)	32.0 (21.0, 48.7)	1.89 (1.32, 2.70)	0.81 (0.59, 1.12)	0.46 (0.35, 0.62)	7.72 (5.78, 10.30)	9.32 (7.42, 11.7)	1.34 (0.98, 1.84)	0.83 (0.61, 1.14)	39.0 (30.2, 50.5)	3.84 (3.09, 4.78)	0.94 (0.72, 1.21)	0.09 (0.07, 0.11)	0.52 (0.35, 0.77)	0.17 (0.14, 0.22)	297 (223, 396)
P-value	*<.0001*	*0.96*	*<.0001*	*0.28*	*0.003*	*0.06*	*0.17*	*0.55*	*0.06*	*0.01*	*0.01*	*0.82*	*0.06*	*0.01*	*0.01*
Los Angeles (n=353)															
White (n=154)	16.0 (12.3, 20.8)	1.90 (1.53, 2.35)	0.31 (0.25, 0.38)	0.61 (0.51, 0.73)	4.66 (4.16, 5.21)	8.24 (7.33, 9.25)	1.05 (0.87, 1.28)	0.86 (0.71, 1.05)	38.8 (33.0, 45.7)	3.67 (3.14, 4.28)	0.67 (0.57, 0.80)	0.08 (0.07, 0.09)	0.88 (0.68, 1.13)	0.10 (0.09, 0.12)	242 (203, 288)
Japanese (n=199)	26.8 (20.1, 35.7)	1.85 (1.47, 2.33)	0.55 (0.44, 0.69)	0.58 (0.48, 0.71)	4.24 (3.76, 4.79)	8.60 (7.58, 9.76)	1.25 (1.01, 1.55)	0.95 (0.76, 1.18)	49.5 (41.4, 59.1)	3.50 (2.96, 4.13)	0.68 (0.55, 0.80)	0.08 (0.06, 0.09)	0.73 (0.55, 0.96)	0.12 (0.10, 0.15)	317 (262, 383)
P-value	*0.0006*	*0.82*	*<.0001*	*0.64*	*0.15*	*0.51*	*0.12*	*0.40*	*0.009*	*0.59*	*0.93*	*0.42*	*0.19*	*0.07*	*0.007*

[a] All models were adjusted for age, education, financial hardship, smoking, secondhand smoking, seafood intake, rice intake, total energy intake, and urinary creatinine.

[b] Chinese was only sampled in Oakland site and Japanese was only sampled in Los Angeles site by study design.

[c] For values greater than 1, 3 significant figures were shown; for values less than 1, values were shown to 2 decimal places.

Supplemental Table II. 4. Ratios of geometric means for urinary metals from linear regression with backward elimination.

Ratio (95% CI)	As	Ba	Cd	Co	Cs	Cu	Hg	Mn	Mo	Ni	Pb	Sb	Sn	Tl	Zn
Race															
White	1.00 (Ref)	1.00 (Ref)	1.00 (Ref)	1.00 (Ref)	1.00 (Ref)	1.00 (Ref)	1.00 (Ref)		1.00 (Ref)	1.00 (Ref)	1.00 (Ref)			1.00 (Ref)	1.00 (Ref)
Black	0.88 (0.74, 1.05)	0.71 (0.63, 0.80)	1.03 (0.92, 1.16)	0.84 (0.76, 0.92)	0.75 (0.69, 0.81)	0.90 (0.83, 0.97)	0.77 (0.69, 0.87)		0.79 (0.71, 0.87)	0.72 (0.66, 0.78)	0.97 (0.87, 1.08)			0.97 (0.85, 1.10)	1.27 (1.15, 1.40)
Chinese	1.93 (1.47, 2.55)	1.00 (0.82, 1.21)	1.93 (1.62, 2.31)	1.30 (1.11, 1.52)	1.38 (1.20, 1.58)	1.17 (1.02, 1.35)	1.17 (0.96, 1.43)		1.28 (1.09, 1.52)	1.15 (1.00, 1.32)	1.42 (1.21, 1.66)			1.43 (1.20, 1.71)	1.27 (1.09, 1.48)
Japanese	1.73 (1.34, 2.44)	1.01 (0.85, 1.21)	2.10 (1.79, 2.45)	1.04 (0.91, 1.19)	0.85 (0.76, 0.96)	1.05 (0.93, 1.18)	1.15 (0.96, 1.37)		1.27 (1.09, 1.48)	1.01 (0.88, 1.15)	1.09 (0.95, 1.26)			1.28 (1.08, 1.52)	1.23 (1.08, 1.42)
Education															
≤ High school			1.00 (Ref)				1.00 (Ref)								
Some College			0.99 (0.88, 1.11)				1.21 (1.08, 1.36)								
≥ College			0.83 (0.74, 0.93)				1.26 (1.12, 1.42)								
Smoking															
Never			1.00 (Ref)	1.00 (Ref)							1.00 (Ref)		1.00 (Ref)		
Former			1.19 (1.07, 1.31)	0.92 (0.85, 1.00)							1.09 (1.01, 1.18)		0.90 (0.80, 1.01)		
Current			1.82 (1.59, 2.09)	0.85 (0.76, 0.96)							1.35 (1.19, 1.53)		1.13 (0.95, 1.35)		
Secondhand smoking															
0 hr/wk												1.00 (Ref)			
<5 hrs/wk												0.92 (0.85, 1.01)			
≥5 hs/wk												1.09 (1.00, 1.20)			

Ratio (95% CI)	As	Ba	Cd	Co	Cs	Cu	Hg	Mn	Mo	Ni	Pb	Sb	Sn	Tl	Zn
Seafood intake															
<1 /wk	1.00 (Ref)				1.00 (Ref)		1.00 (Ref)				1.00 (Ref)				
1-1.9 /wk	1.17 (1.02, 1.35)				1.09 (1.03, 1.16)		1.14 (1.03, 1.26)				1.01 (0.92, 1.11)				
≥2 /wk	1.55 (1.35, 1.78)				1.07 (1.01, 1.14)		1.21 (1.08, 1.36)				1.13 (1.01, 1.23)				
Rice intake															
<1.5 /wk	1.00 (Ref)				1.00 (Ref)	1.00 (Ref)	1.00 (Ref)		1.00 (Ref)	1.00 (Ref)					
1.5-3.4 /wk	1.02 (0.89, 1.17)				1.00 (0.94, 1.06)	0.97 (0.92, 1.03)	1.06 (0.96, 1.17)		1.00 (0.92, 1.08)	0.98 (0.91, 1.05)					
≥3.5 /wk	1.67 (1.37, 2.03)				1.13 (1.02, 1.24)	1.09 (0.99, 1.21)	1.23 (1.05, 1.44)		1.19 (1.05, 1.34)	1.13 (1.01, 1.26)					

[a] *P* <0.05 for all selected variables in backward elimination. Age, study sites, total energy intake, and urinary creatinine were forced in model selection.

Supplemental Table II. 5. Geometric mean (GMs, μg/g) of urinary creatinine adjusted metal concentrations by overall exposure patterns.

GM (GSD)[a]	As	Ba	Cd	Co	Cs	Cu	Hg	Mn	Mo	Ni	Pb	Sb	Sn	Tl	Zn
Low exposure pattern (n=773)	13.24 (2.75)	1.51 (2.19)	0.36 (2.14)	0.58 (1.84)	4.45 (1.65)	9.17 (1.50)	1.09 (2.27)	0.84 (1.88)	41.35 (1.85)	3.33 (1.71)	0.66 (1.80)	0.08 (2.00)	1.04 (2.47)	0.11 (2.16)	305 (1.89)
High exposure pattern (n=562)	36.43 (3.46)	3.10 (2.23)	0.72 (2.22)	0.95 (1.81)	7.45 (1.70)	14.69 (1.69)	1.98 (2.01)	1.73 (2.25)	63.38 (1.85)	5.84 (1.70)	1.39 (1.93)	0.12 (1.98)	1.31 (2.67)	0.22 (2.19)	381 (1.89)

[a] GM: geometric mean; GSD: geometric standard deviation.

Supplemental Table II. 6. Estimated cumulative odds ratio (95% confidence intervals) of being clustered into the "high" exposure pattern[a].

Covariates[b]	Odds ratio	95% CI	P-value
Black vs. white women	0.38	0.26, 0.55	<.0001
Chinese vs. white women	2.13	1.21, 3.77	0.009
Japanese vs. white women	2.33	1.39, 3.92	0.001
Some college vs. high school or less	0.91	0.62, 1.31	0.60
College and above vs. high school or less	0.97	0.67, 1.40	0.86
Financial hardship: moderate vs. severe	0.68	0.39, 1.19	0.17
Financial hardship: minor vs. severe	0.63	0.37, 1.07	0.09
Former vs. never smoker	1.03	0.77, 1.36	0.86
Current vs. never smoker	2.02	1.29, 3.17	0.002
Secondhand smoking <5 vs. 0 hrs/wk	1.29	0.96, 1.74	0.09
Secondhand smoking ≥5 vs. 0 hrs/wk	1.33	0.94, 1.89	0.10
Seafood intake 1-1.9 /wk vs. <1 time/wk	1.32	0.97, 1.80	0.08
Seafood intake ≥2 vs. <1 time/wk	1.82	1.33, 2.49	0.0002
Rice intake 1.5-3.4 /wk vs. <1.5 times/wk	1.10	0.81, 1.48	0.55
Rice intake ≥3.5 vs. <1.5 times/wk	1.73	1.12, 2.67	0.01
Boston vs. Michigan	3.37	2.11, 5.34	<.0001
Oakland vs. Michigan	2.65	1.52, 4.60	0.0006
Los Angeles vs. Michigan	1.84	1.07, 3.16	0.03
Pittsburgh vs. Michigan	3.18	1.99, 5.07	<.0001

[a] Participants with "high" vs. "low" exposure patterns were clustered by k-means clustering method.

[b] Reference groups: race: white women; education: ≤ high school; financial hardship: severe; smoking: never smoker; secondhand smoking: 0 hours/week; seafood intake: <1 time/week; rice intake: <1.5 times/week; study sites: Michigan. Age and total energy intake were adjusted as continuous variables.

Supplemental Table II. 7. Model adjusted least-squares geometric mean concentrations[a] (LSGMs, µg/L) and 95% confidence intervals of urinary metals by characteristics of the SWAN participants, including seafood and rice intake, adjusting for specific gravity.

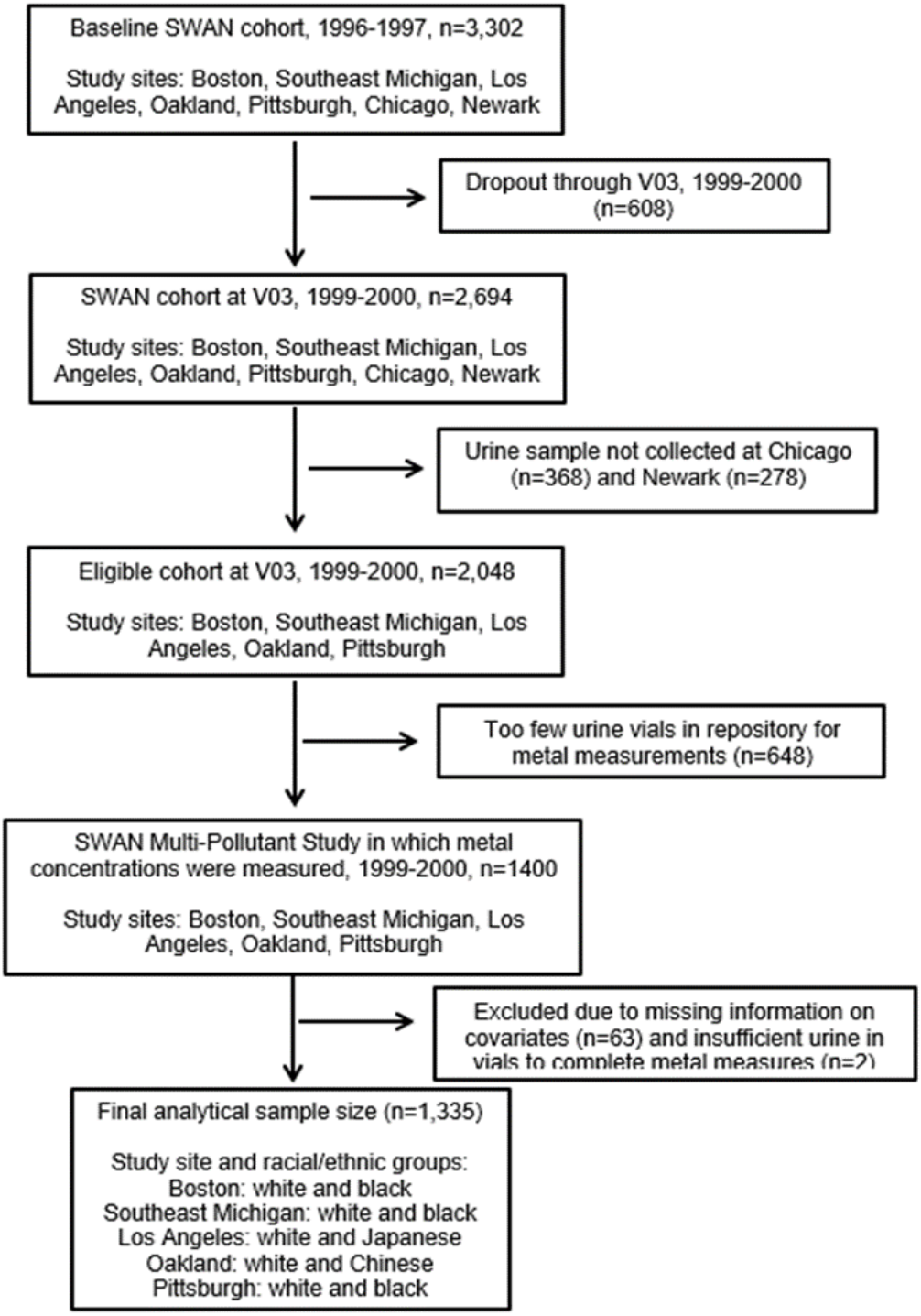
LGSMs[b] (µg/L)	As	Ba	Cd	Co	Cs	Cu	Hg	Mn	Mo	Ni	Pb	Sb	Sn	Tl	Zn
Race															
White	13.5 (11.9, 15.3)	1.86 (1.69, 2.05)	0.41 (0.37, 0.45)	0.59 (0.54, 0.64)	4.71 (4.44, 5.01)	9.24 (8.69, 9.84)	1.09 (0.99, 1.20)	1.00 (0.92, 1.09)	38.9 (36.0, 42.1)	3.53 (3.31, 3.76)	0.80 (0.73, 0.86)	0.08 (0.08, 0.09)	1.02 (0.91, 1.14)	0.12 (0.11, 0.13)	256 (237, 278)
Black	14.0 (12.0, 16.4)	1.47 (1.30, 1.66)	0.49 (0.44, 0.56)	0.56 (0.51, 0.62)	4.10 (3.80, 4.42)	9.73 (9.00, 10.5)	1.00 (0.88, 1.12)	0.98 (0.88, 1.08)	35.8 (32.5, 39.5)	2.95 (2.72, 3.20)	0.89 (0.81, 0.99)	0.09 (0.08, 0.10)	1.21 (1.05, 1.40)	0.13 (0.11, 0.14)	390 (353, 431)
Chinese	26.4 (20.4, 34.2)	1.77 (1.45, 2.16)	0.71 (0.58, 0.87)	0.70 (0.60, 0.83)	6.58 (5.81, 7.44)	10.9 (9.56, 12.3)	1.23 (1.01, 1.51)	0.97 (0.82, 1.15)	48.3 (41.2, 56.6)	3.99 (3.50, 4.54)	1.09 (0.92, 1.29)	0.09 (0.08, 0.11)	0.70 (0.55, 0.88)	0.15 (0.12, 0.18)	342 (290, 403)
Japanese	24.0 (19.0, 30.3)	1.82 (1.52, 2.18)	0.80 (0.66, 0.96)	0.58 (0.50, 0.67)	4.09 (3.65, 4.58)	9.94 (8.84, 11.2)	1.28 (1.07, 1.54)	1.01 (0.87, 1.19)	50.5 (43.7, 58.4)	3.65 (3.24, 4.12)	0.86 (0.74, 1.01)	0.07 (0.06, 0.08)	0.89 (0.72, 1.11)	0.14 (0.12, 0.17)	334 (287, 388)
P-value	*<.0001*	*0.004*	*<.0001*	*0.15*	*<.0001*	*0.08*	*0.13*	*0.94*	*0.0007*	*<.0001*	*0.002*	*0.02*	*0.002*	*0.04*	*<.0001*
Education															
≤ High school	17.1 (14.7, 19.9)	1.68 (1.49, 1.89)	0.60 (0.53, 0.68)	0.60 (0.55, 0.66)	4.76 (4.43, 5.13)	9.77 (9.07, 10.5)	0.98 (0.87, 1.11)	1.00 (0.90, 1.10)	41.6 (37.9, 45.7)	3.41 (3.16, 3.69)	0.87 (0.79, 0.96)	0.08 (0.08, 0.09)	0.86 (0.75, 0.98)	0.12 (0.11, 0.14)	333 (303, 367)
Some College	20.0 (17.5, 22.9)	1.84 (1.66, 2.04)	0.62 (0.55, 0.69)	0.60 (0.55, 0.66)	4.73 (4.43, 5.04)	10.0 (9.36, 10.7)	1.21 (1.09, 1.35)	0.97 (0.89, 1.06)	44.1 (40.6, 47.9)	3.57 (3.33, 3.82)	0.91 (0.83, 0.99)	0.09 (0.08, 0.10)	1.02 (0.90, 1.15)	0.14 (0.13, 0.16)	339 (311, 369)
≥ College	18.8 (16.4, 21.6)	1.66 (1.49, 1.85)	0.53 (0.48, 0.59)	0.61 (0.56, 0.67)	4.83 (4.52, 5.16)	10.0 (9.34, 10.7)	1.26 (1.13, 1.40)	1.00 (0.91, 1.10)	43.2 (39.7, 47.1)	3.55 (3.31, 3.80)	0.94 (0.85, 1.02)	0.08 (0.07, 0.09)	0.94 (0.83, 1.07)	0.13 (0.12, 0.15)	309 (283, 338)
P for trend	*0.72*	*0.49*	*0.01*	*0.67*	*0.70*	*0.69*	*0.0008*	*0.84*	*0.77*	*0.54*	*0.21*	*0.27*	*0.53*	*0.86*	*0.10*
Financial hardship															
Severe	17.0 (13.5, 21.5)	1.69 (1.41, 2.02)	0.57 (0.47, 0.69)	0.62 (0.53, 0.72)	4.70 (4.20, 5.26)	10.0 (8.90, 11.2)	1.07 (0.90, 1.29)	0.99 (0.85, 1.16)	43.6 (37.7, 50.4)	3.56 (3.16, 4.01)	0.94 (0.80, 1.10)	0.09 (0.08, 0.10)	0.91 (0.73, 1.13)	0.13 (0.11, 0.16)	343 (296, 399)
Moderate	19.1 (16.8, 21.8)	1.68 (1.52, 1.86)	0.62 (0.56, 0.69)	0.61 (0.56, 0.66)	4.89 (4.59, 5.21)	10.2 (9.6, 10.9)	1.15 (1.04, 1.28)	1.03 (0.94, 1.13)	42.5 (39.2, 46.2)	3.53 (3.30, 3.77)	0.92 (0.84, 1.01)	0.08 (0.08, 0.09)	0.97 (0.86, 1.10)	0.14 (0.12, 0.15)	320 (294, 348)
Minor	19.7 (17.8, 21.8)	1.81 (1.67, 1.95)	0.55 (0.51, 0.60)	0.59 (0.55, 0.63)	4.73 (4.50, 4.97)	9.58 (9.11, 10.1)	1.21 (1.12, 1.32)	0.95 (0.89, 1.02)	42.7 (40.1, 45.5)	3.44 (3.26, 3.62)	0.85 (0.79, 0.91)	0.08 (0.07, 0.09)	0.93 (0.85, 1.02)	0.13 (0.12, 0.14)	318 (298, 339)
P for trend	*0.35*	*0.21*	*0.08*	*0.39*	*0.58*	*0.09*	*0.17*	*0.16*	*0.81*	*0.35*	*0.05*	*0.20*	*0.62*	*0.21*	*0.47*
Smoking															
Never	18.3	1.63	0.45	0.65	4.61	9.87	1.20	0.94	44.4	3.69	0.79	0.08	0.93	0.13	306

LGSMs[b] (μg/L)	As	Ba	Cd	Co	Cs	Cu	Hg	Mn	Mo	Ni	Pb	Sb	Sn	Tl	Zn
	(16.3, 20.4)	(1.49, 1.78)	(0.41, 0.49)	(0.60, 0.69)	(4.37, 4.87)	(9.34, 10.4)	(1.10, 1.31)	(0.87, 1.01)	(41.4, 47.5)	(3.48, 3.90)	(0.73, 0.85)	(0.07, 0.09)	(0.84, 1.03)	(0.12, 0.15)	(285, 329)
Former	18.5	1.67	0.52	0.59	4.77	9.82	1.07	1.01	41.9	3.45	0.86	0.08	0.80	0.13	318
	(16.0, 21.3)	(1.49, 1.86)	(0.47, 0.59)	(0.54, 0.65)	(4.45, 5.11)	(9.15, 10.5)	(0.96, 1.20)	(0.91, 1.11)	(38.4, 45.8)	(3.21, 3.71)	(0.78, 0.95)	(0.07, 0.09)	(0.70, 0.91)	(0.11, 0.14)	(290, 348)
Current	19.0	1.88	0.84	0.58	4.94	10.1	1.17	1.03	42.6	3.39	1.08	0.09	1.10	0.13	358
	(15.6, 23.2)	(1.62, 2.19)	(0.72, 0.98)	(0.51, 0.66)	(4.49, 5.43)	(9.16, 11.1)	(1.00, 1.36)	(0.90, 1.17)	(37.7, 48.1)	(3.07, 3.75)	(0.95, 1.23)	(0.08, 0.10)	(0.92, 1.32)	(0.12, 0.16)	(316, 406)
P for trend	*0.62*	*0.13*	*<.0001*	*0.01*	*0.13*	*0.90*	*0.21*	*0.08*	*0.35*	*0.02*	*<.0001*	*0.06*	*0.98*	*0.70*	*0.02*
Secondhand smoking															
0 hr/wk	19.3	1.68	0.61	0.60	4.80	9.99	1.13	1.05	45.3	3.46	0.87	0.09	0.94	0.14	320
	(16.9, 22.0)	(1.52, 1.86)	(0.55, 0.68)	(0.55, 0.65)	(4.51, 5.11)	(9.36, 10.7)	(1.02, 1.25)	(0.96, 1.15)	(41.8, 49.2)	(3.24, 3.70)	(0.80, 0.95)	(0.08, 0.09)	(0.83, 1.06)	(0.13, 0.15)	(295, 348)
<5 hrs/wk	17.8	1.78	0.56	0.63	4.76	10.4	1.15	0.98	41.2	3.61	0.92	0.08	0.96	0.13	322
	(15.3, 20.6)	(1.59, 2.00)	(0.50, 0.63)	(0.57, 0.69)	(4.43, 5.12)	(9.65, 11.2)	(1.02, 1.29)	(0.89, 1.08)	(37.6, 45.2)	(3.35, 3.90)	(0.84, 1.02)	(0.07, 0.09)	(0.84, 1.10)	(0.12, 0.14)	(293, 355)
≥5 hs/wk	18.8	1.71	0.57	0.59	4.76	9.43	1.16	0.94	42.4	3.45	0.92	0.09	0.91	0.13	339
	(16.3, 21.6)	(1.53, 1.91)	(0.51, 0.64)	(0.54, 0.64)	(4.44, 5.09)	(8.78, 10.1)	(1.04, 1.29)	(0.86, 1.04)	(38.8, 46.3)	(3.21, 3.71)	(0.83, 1.01)	(0.08, 0.10)	(0.80, 1.04)	(0.11, 0.14)	(309, 371)
P for trend	*0.62*	*0.45*	*0.40*	*0.96*	*0.74*	*0.35*	*0.43*	*0.02*	*0.11*	*0.75*	*0.17*	*0.79*	*0.75*	*0.17*	*0.23*
Seafood intake															
<1 /wk	15.6	1.73	0.58	0.62	4.65	10.1	1.06	0.97	41.9	3.53	0.89	0.09	0.94	0.13	336
	(13.5, 18.0)	(1.55, 1.93)	(0.52, 0.65)	(0.57, 0.68)	(4.34, 4.98)	(9.39, 10.8)	(0.95, 1.19)	(0.88, 1.07)	(38.4, 45.8)	(3.28, 3.79)	(0.81, 0.98)	(0.08, 0.10)	(0.82, 1.07)	(0.11, 0.14)	(306, 367)
1-1.9 /wk	17.7	1.63	0.56	0.60	4.89	9.64	1.16	0.96	43.2	3.45	0.87	0.08	0.99	0.13	326
	(15.5, 20.3)	(1.47, 1.81)	(0.50, 0.62)	(0.55, 0.65)	(4.58, 5.21)	(9.02, 10.3)	(1.05, 1.29)	(0.88, 1.05)	(39.8, 47.0)	(3.22, 3.70)	(0.80, 0.95)	(0.07, 0.09)	(0.88, 1.12)	(0.12, 0.14)	(299, 355)
≥2 /wk	23.2	1.81	0.60	0.60	4.78	10.1	1.22	1.04	43.7	3.55	0.95	0.08	0.88	0.14	319
	(20.4, 26.4)	(1.64, 2.00)	(0.54, 0.67)	(0.55, 0.65)	(4.49, 5.09)	(9.43, 10.7)	(1.10, 1.35)	(0.95, 1.13)	(40.3, 47.4)	(3.32, 3.79)	(0.87, 1.03)	(0.08, 0.09)	(0.78, 1.00)	(0.12, 0.15)	(294, 347)
P for trend	*<.0001*	*0.39*	*0.57*	*0.55*	*0.48*	*0.98*	*0.02*	*0.18*	*0.41*	*0.86*	*0.21*	*0.66*	*0.29*	*0.23*	*0.30*
Rice intake															
<1.5 /wk	16.0	1.72	0.58	0.61	4.70	10.0	1.07	1.01	42.2	3.50	0.90	0.09	0.90	0.13	334
	(13.7, 18.7)	(1.53, 1.94)	(0.51, 0.65)	(0.55, 0.67)	(4.37, 5.06)	(9.29, 10.8)	(0.95, 1.21)	(0.91, 1.11)	(38.4, 46.4)	(3.24, 3.79)	(0.81, 0.99)	(0.08, 0.09)	(0.78, 1.03)	(0.11, 0.14)	(303, 368)
1.5-3.4 /wk	16.1	1.71	0.57	0.59	4.67	9.61	1.13	1.00	41.1	3.38	0.90	0.08	0.92	0.13	344
	(13.9, 18.6)	(1.52, 1.91)	(0.51, 0.65)	(0.53, 0.64)	(4.36, 5.01)	(8.94, 10.3)	(1.01, 1.27)	(0.91, 1.10)	(37.6, 45.0)	(3.14, 3.64)	(0.82, 1.00)	(0.08, 0.09)	(0.81, 1.05)	(0.12, 0.15)	(314, 377)
≥3.5 /wk	24.9	1.74	0.59	0.62	4.95	10.2	1.24	0.97	45.6	3.65	0.91	0.08	0.99	0.14	304
	(21.4, 29.0)	(1.55, 1.96)	(0.52, 0.67)	(0.57, 0.69)	(4.60, 5.32)	(9.42, 11.0)	(1.10, 1.40)	(0.87, 1.07)	(41.5, 50.1)	(3.38, 3.94)	(0.82, 1.00)	(0.07, 0.09)	(0.86, 1.14)	(0.12, 0.15)	(276, 355)

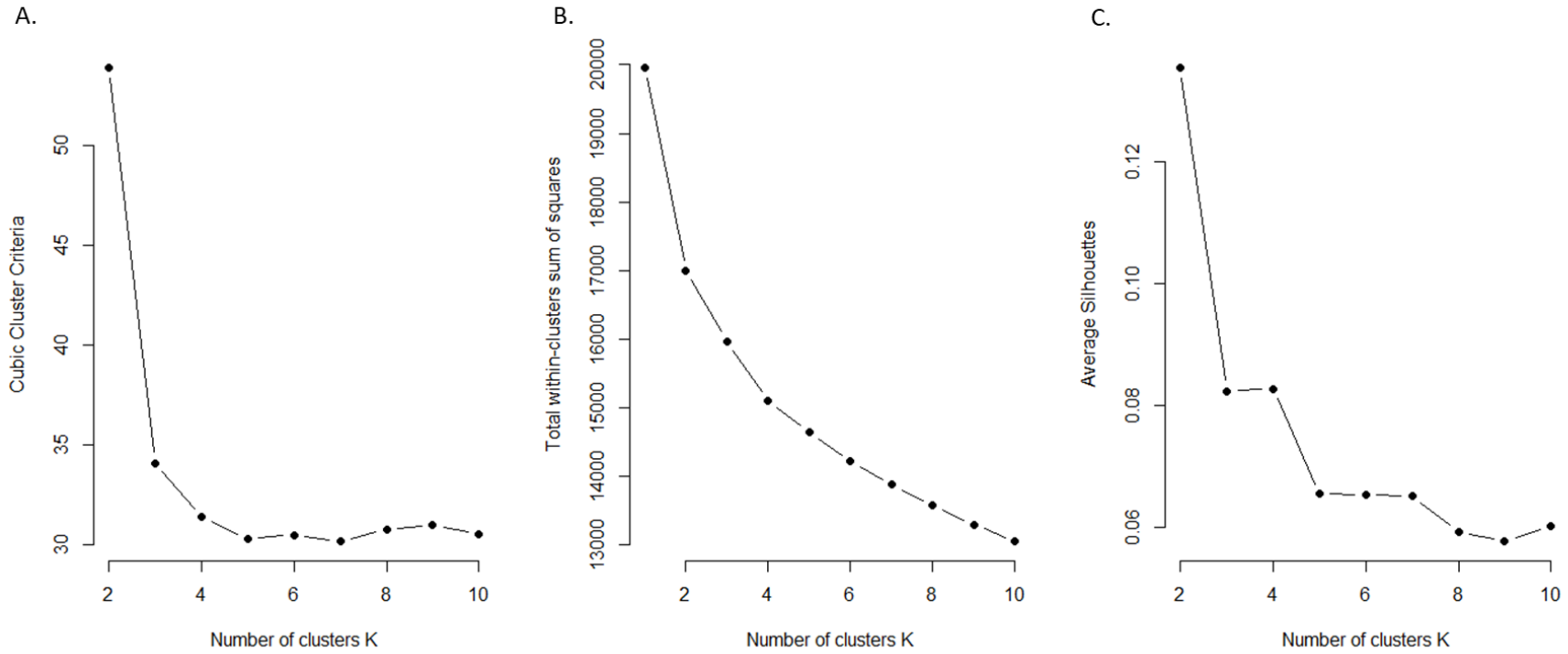
LGSMs[b] (μg/L)	As	Ba	Cd	Co	Cs	Cu	Hg	Mn	Mo	Ni	Pb	Sb	Sn	Tl	Zn
P for trend	*0.0004*	*0.91*	*0.63*	*0.99*	*0.48*	*0.83*	*0.05*	*0.69*	*0.41*	*0.79*	*0.82*	*0.96*	*0.37*	*0.42*	*0.39*

[a] All models were adjusted for age, race, education, financial hardship, smoking, secondhand smoking, seafood intake, rice intake, total energy intake, study sites and specific gravity.

[b] For values greater than 1, 3 significant figures were shown; for values less than 1, values were shown to 2 decimal places.



Supplemental Figure II. 1. Schematic diagram of the SWAN Multi-Pollutant Study and analytic sample.



Supplemental Figure II. 2. Determination of optimal number of clusters in k-means clustering.
(A) Cubic clustering criterion. This figure contains the cubic clustering criterion values from k=2 to k=10. Note the local maxima at k=2, indicating two is optimal estimate for the number of clusters. (B) Elbow method. This figure plots the total within-cluster sum of square against number of clusters (1 to 10 in our case). The location of a bend (knee) in the plot is generally considered as an indicator of the appropriate number of clusters. (C) Average Silhouette method. This figure shows how well each participants lies within its cluster by average silhouette width. A high average silhouette width indicates a good clustering. The optimal number of clusters is the one that maximizes the average silhouette over a range of possible values (which from 1 to 10 in our case).

Chapter III. Urinary Metals and Incident Diabetes in Midlife Women: the Study of Women's Health Across the Nation (SWAN)

Xin Wang, Carrie A. Karvonen-Gutierrez, William H. Herman, Bhramar Mukherjee, Siobán D. Harlow, Sung Kyun Park

Abstract

Objective: Environmental exposure to metals may play a role in the pathogenesis of diabetes, however, evidence from human studies is limited. We prospectively evaluated the associations of 20 urinary metal concentrations and their mixtures with incident diabetes in the Study of Women's Health Across the Nation, a multi-site, multi-ethnic cohort study of midlife women.

Research Design and Methods: The sample included 1,237 white, black, Chinese and Japanese-American women, aged 45-56 years, free of diabetes at baseline (1999-2000) who were followed through 2016. Concentrations of 20 metals (arsenic, barium, beryllium, cadmium, cobalt, chromium, cesium, copper, mercury, manganese, molybdenum, nickel, lead, antimony, tin, thallium, uranium, vanadium, tungsten and zinc) were measured in urine specimens using high-resolution inductively coupled plasma-mass spectrometry at baseline. Incident diabetes was identified annually. Exposure to metal mixtures were captured using k-means clustering.

Results: After multivariable adjustment, the hazard ratios (HR) (95% CI) of diabetes associated with each doubling increase in urinary metal concentrations were 1.13 (1.04, 1.23) for arsenic and 1.22 (1.09, 1.37) for lead, in Cox proportional hazards models after controlling for multiple comparison. A doubling in urinary excretion of zinc was associated with higher diabetes risk (adjusted HR 1.47, 95% CI: 1.27, 1.70). Two distinct exposure patterns to metal mixtures- "high" vs. "low"-were identified. Adjusted HR of diabetes associated with "high" exposure pattern compared with "low" was 1.38 (1.09, 1.75).

Conclusions: Exposure to arsenic and lead, an increase urinary excretion of zinc, as well as a high overall exposure to metal mixtures were associated with elevated diabetes risks. Future studies should further investigate the underlying mechanisms by which metals may influence diabetes.

1. Introduction

To date, most epidemiologic studies of type 2 diabetes mellitus risk have focused on the potential impact of genetics, unhealthy diets, and sedentary lifestyles. The potential contributions of environmental toxicants to the epidemic of diabetes have received less attention. The general population is commonly exposed to metals through food, drinking water, and ambient air. Dietary intake of toxic metals has been a significant public health concern, in particular for populations with consumption of contaminated drinking water and/or rice (Hanna-Attisha et al. 2016; Wang et al. 2019b). Exposure to metals may play a role in the induction or exacerbation of diabetes: arsenic has been associated with diabetes in a number of studies (Maull et al. 2012). A National Toxicology Program systematic review suggests that there is "limited to sufficient" evidence for an association between arsenic and diabetes in high exposure areas (≥150 μg arsenic/L in drinking water) but 'insufficient' evidence in lower exposure areas (<150 μg arsenic/L in drinking water) (Maull et al. 2012). Other metals including cadmium and lead have been examined in relation to diabetes risk but the studies have been limited, inconsistent and mostly cross-sectional (Li et al. 2017; Menke et al. 2015). Associations of environmental exposure to most other metals with diabetes have not been investigated.

Toxic metals such as arsenic, cadmium and lead, are well-known inducers of oxidative stress in a variety of tissues and cell types (Ercal et al. 2001). The accumulation of these metals in pancreatic islets is hypothesized to lead to impaired function and apoptotic death of β-cells via the induction of oxidative stress (Lu et al. 2011; Patra et al. 2011). Arsenic and cadmium have also been demonstrated to interfere with gene expression involving signal transduction and gene transcription related to insulin pathways, leading to insulin resistance (Han et al. 2003; Mohammed Abdul et al. 2015). On the other hand, deficiency in essential metals such as zinc

attributed to excessive excretion in urine has been related to dysregulation of insulin secretion and glucose transportation (Jansen et al. 2009). Metal exposures could also be associated with obesity. A recent cross-sectional study found that exposure to mixtures of metals was associated with BMI and waist circumference in the U.S. general population (Wang et al. 2018). These findings suggest a need to investigate the role of metal exposures to diabetes risk in humans, especially in a well characterized prospective cohort study.

In this study, we report on the associations of 20 urinary metals with the incidence of diabetes over 16 years of follow-up in the Study of Women's Health Across the Nation (SWAN), a multi-site, multi-ethnic prospective cohort study of midlife women. We have previously identified two distinct exposure patterns to metal mixtures in SWAN (Wang et al. 2019b). The present study was designed to further assess the role of metal mixtures in diabetes risk.

2. Methods

2.1 Study population

Women in the present study were participants in SWAN, an ongoing, multi-site, multi-ethnic, community-based longitudinal study of the natural history of menopause designed to address the effect of the menopausal transition on subsequent health and to identify risk factors for age-related chronic diseases (Sowers et al. 2000). In 1996 to 1997, 3,302 women were enrolled from seven study sites where white women and women from one specified minority group were recruited (black women from Boston, MA, Pittsburgh, PA, southeast Michigan, MI, and Chicago, IL; Hispanic women from Newark, NJ; Chinese women from Oakland, CA; and Japanese women from Los Angeles, CA). Black, Chinese, Japanese, and Hispanic women comprised greater proportions of the SWAN population than their respective proportions in the

general U.S. population, reflecting the study design to oversample these groups (Sowers et al. 2000). Eligibility criteria for enrollment into the SWAN cohort included the following: age 42 to 52 years, intact uterus and at least one ovary, no use of exogenous hormones affecting ovarian function in the past 3 months, at least one menstrual period in the previous 3 months, and self-identification with a site's designated racial/ethnic groups. These women returned for regular examinations annually, and approximately 75% of still living participants completed the 15th SWAN follow-up visit (2015-2016). Institutional review board approval was obtained at each study site, and all participants provided signed informed consent at each study visit.

To evaluate associations between urinary metals and risk of diabetes, we used data from the SWAN Multi-Pollutant Substudy (SWAN-MPS), which was initiated to examine the associations of multiple environmental chemicals with metabolic and reproductive health outcomes in midlife women (Wang et al. 2019b). This substudy used urine samples from the SWAN Repository collected during the third SWAN follow-up visit (1999-2000) for environmental exposure assessment. A subset of 1,400 SWAN participants from the five SWAN sites who provided urine samples to the SWAN Repository (Boston, southeast Michigan, Los Angeles, Oakland and Pittsburgh) were assayed for metal concentrations. Therefore, this substudy, by design, included self-identified white, black, Chinese, and Japanese women, but not Hispanic women. After excluding 82 participants with prevalent diabetes at the baseline for the Multi-Pollutant Substudy (1999-2000), 1,318 women in the sample were at risk of developing diabetes. In addition, we excluded 1 participant who provided an insufficient quantity of urine, and 80 participants who had no information on key covariates (education, household income, BMI, physical activity, and variables used in the application of the inverse probability weighting method), leaving a final analytic sample of 1,237 women including 11,715 observations followed

from 1999 to 2016. An overview of our analytic sample is illustrated in **Supplemental Figure III. 1**.

2.2 Diabetes ascertainment

Fasting serum glucose level was determined by hexokinase method (Boehringer Mannheim Diagnostics, Indianapolis, IN, USA). At any follow-up visit, participants with one or more of the following were defined as having incident diabetes: (1) fasting serum glucose level ≥ 126 mg/dL; (2) self-reported use of insulin or oral medications for diabetes; (3) self-reported physician diagnosis of diabetes. The vast majority of the diabetes cases in this life stage are considered type 2 diabetes

2.3 Urinary metals

Details regarding urinary metal measurements and associated quality control procedures in the SWAN-MPS have been described previously (Wang et al. 2019b). Baseline concentrations of the following 20 metals including total arsenic, barium, beryllium, cadmium, cobalt, chromium, cesium, copper, mercury, manganese, molybdenum, nickel, lead, antimony, tin, thallium, uranium, vanadium, tungsten, and zinc were measured in these urine samples using high-resolution inductively coupled plasma-mass spectrometry (ICP-MS) (Thermo Scientific iCAP RQ, Waltham, MA) following the CDC method 3018.3 (CDC 2012b), with modifications for the expanded metals panel, by the Applied Research Center of NSF International (Ann Arbor, Michigan). The limits of detection (LOD) and detection rates are presented in **Supplemental Table III. 1**. Participants with metal concentration below LOD were assigned a value equal to LOD divided by the square root of 2. Pairwise Spearman correlations among urinary metal concentrations were calculated.

2.4 Covariates

Sociodemographic variables including age, self-reported race/ethnicity, and education level, and family history of diabetes were assessed through a self-administered questionnaire at the SWAN baseline examination (1996-1997). At each study visit, annual household income, smoking status, alcohol drinking, menopausal status, and use of exogenous hormones were self-reported. BMI was calculated as weight in kilograms divided by the square of height in meters. Waist circumference was measured to the nearest 0.1 cm with a measuring tape placed horizontally around the participant at the narrowest part of the torso. Blood pressures were measured twice with a minimum 2-min rest period between measures with each participant in a seated position according to a standardized protocol. We calculated mean of systolic blood pressure (SBP) by averaging up the two measures. Blood samples were taken to measure serum levels of total cholesterol, high-density lipoprotein (HDL) cholesterol, and triglycerides. Physical activity was measured by a total score indicating the activity levels during the previous 12 months. Activity was assessed with a modified version of the Kaiser Physical Activity Survey (Sternfeld et al. 2000), which consists of 38 questions with primarily Likert-scale responses about physical activity in various domains, including sports/exercise, household/caregiving, and daily routine. Domain-specific indices were derived by averaging the ordinal responses to questions in each domain, resulting in values from 1 to 5. Thus, the total physical activity score ranged from 3 to 15 with 15 indicating the highest level of activity. Dietary seafood and rice intake and zinc intake from diet and supplements were collected using a detailed semi-quantitative food frequency questionnaire (FFQ) adopted from the Block FFQ (Wang et al. 2019b). Total energy intake was obtained from the FFQ based on each food intake. Urinary specific gravity was determined using a handheld digital refractometer (ATAGO model PAL-10S, Tokyo, Japan) at the same time as metal measurements as a marker of urine dilution.

2.5 Statistical analysis

Cox proportional hazards models were used to estimate the hazard ratios (HRs) and 95% confidence intervals (CIs) of incident diabetes in relation to each metal concentration. Given the highly skewed distributions of urinary metal concentrations, logarithmic transformations with base 2 were applied to all metal concentrations so that shapes of exposure-outcome relationships were closer to being log-linear, and the HRs and 95% CIs were interpreted as effects of a two-fold increase in each urinary metal concentration. For metals with low detection rate (beryllium, chromium, uranium, vanadium, tungsten, **Supplemental Table III.1**), HRs were calculated comparing participants with metal concentration above the LOD to those with value below the LOD. In our study, platinum was also measured, but most participants (98.9%) had concentrations below the LOD, and that metal was excluded from this analysis.

Potential confounders were adjusted progressively in the Cox models. Initial regression models included adjustment for age, race/ethnicity, study site, and specific gravity (log-transformed), while subsequent models further adjusted for education, annual household income, BMI and waist circumference at baseline, SBP at baseline, smoking, alcohol drinking, serum lipids (total cholesterol, HDL cholesterol, and triglyceride) levels at baseline, physical activity score, family history of diabetes, total energy intake, menopausal status, and hormone therapy (full model). We decided not to include time-varying BMI, waist circumference, SBP, and serum lipids in our primary analysis because of its role as a potential diabetes risk factor and the fact that it could be affected by metal exposures at baseline (Wang et al. 2018). For arsenic, cadmium and, mercury, we additionally adjusted for seafood and rice intake in the full model, which have been identified as important determinants of their urinary concentrations in our previous study [1]. For zinc, we additionally adjusted for total zinc intake from food and supplements in the full

model to better capture the potential effect of urinary zinc excretion on diabetes that is independent of dietary zinc intake. For other essential metals, such as copper, no dietary intake was adjusted due to a lack of data. Given the relatively large number of associations that were evaluated, we adjusted P values for multiple comparison at a false discovery rate (FDR) of 0.05 using the Benjamini–Hochberg Method (Benjamini and Hochberg 1995). We also calculated HRs in relation to specific gravity-adjusted metal concentrations (urinary metal concentration × (1.017-1)/specific gravity -1), where 1.017 was the median level of specific gravity in our analytic sample, as a sensitivity analysis.

To quantify the differences in diabetes risks between subgroups corresponding to different exposure patterns to metal mixtures, a nonparametric clustering method, k-means clustering, was applied. Details regarding k-means clustering in identification of exposure patterns to metal mixtures in SWAN has been described previously (Wang et al. 2019b). Briefly, this approach creates a single variable with k categories representing different clusters where participants within the same cluster are as similar as possible and participants from different clusters are as dissimilar as possible, in terms of their urinary metal concentrations. K-means clustering was performed for the metals of which the detection rate was ≥70%. All log-transformed specific gravity-adjusted urinary metal concentrations were standardized to make variables comparable before the k-means clustering. The number of optimal clusters (k) was determined based on cubic clustering criterion, Elbow method, and interpretability. HRs and 95% CIs of diabetes incidence were estimated between subgroups (clusters) with different exposure patterns to metal mixtures using the Cox proportional hazards models. We also calculated survival probability of diabetes of participants in different subgroups throughout 16 years of follow-up using adjusted survival curves recommended by Hernán (Hernán 2010) and

displayed results graphically. Briefly, a discrete-time hazards model with adjustment of confounding factors was fitted to estimate the conditional survival probability of diabetes under different exposure patterns to metal mixtures in a counterfactual causal framework (Hernán 2010).

We recognized that selection bias may have existed, as selection into the SWAN-MPS was potentially affected by women's metal exposures, their related diabetes risk factors, or potential confounders before or at the time of enrollment. Also, selective loss to follow-up that occurred after the metal measurements were obtained may have biased estimates of associations between metals and diabetes if the likelihood of continuation in the follow-up was influenced by metal exposures and risk factors for diabetes. To mitigate these biases, we assigned weights to participants based on inverse probability weighting (IPW), to create a pseudo population representative of the original cohort, as well as to address informative attrition in our analyses. Directed acyclic graphs illustrating the potential selection bias and details of estimation of IPW are described in the **supplementary Methods**, **Supplemental Figure III. 2** and **Supplemental Figure III. 3**. All analyses were conducted by SAS, version 9.4 (SAS Institute, Inc., Cary, North Carolina).

3. Results

Among 1,237 SWAN participants free of diabetes at baseline, 102 developed diabetes during 17,005 person-years of follow-up, with an incidence of 6.0 per 1,000 person-years. Women with incident diabetes were more likely to be black, from Michigan, to have higher BMI and lower education levels, and to be current or former smokers, and hormone users (**Table III. 1**). The percentage of women with detectable concentrations of an individual metal ranged from 15.7 to 100% (**Supplemental Table III.1**). Five metals had detection rates less than 70%

(beryllium, 15.7%; chromium, 24.3%; uranium, 33.3%; vanadium, 37.4%; tungsten, 29.0%). Participants with incident diabetes had higher copper, manganese, nickel, lead, tin, zinc and lower cesium concentrations than those without incident diabetes. Concentrations of most metals were modestly and positively correlated with each other (**Supplemental Figure III. 4**).

Table III. 2 summarizes the associations between urinary metal concentrations (detection rate $\geqslant$70%) and incident diabetes. In the initial models, significant associations were found for urinary tin (p=0.03) and zinc (p<0.0001). After full adjustments of covariates, HRs of diabetes associated with each doubling of urinary metal concentration were 1.24 (95% CI: 1.14, 1.35, p<0.0001) for arsenic, 1.23 (95% CI: 1.08, 1.40, p=0.002) for lead, 1.11 (95% CI:1.01, 1.22, p=0.04) for tin, and 1.31 (95% CI: 1.11, 1.55, p=0.001) for zinc. To adjust for multiple comparison, a significance level of α=0.002 was used, which corresponded to an FDR of 5% using the Benjamini–Hochberg Method. This adjustment for multiple comparison left only arsenic, lead, and zinc as significant independent predictors (p<0.002) for diabetes. No significant association was detected between metals with detection rate <70% and diabetes (**Table III. 3**).

Two distinct clusters of participants were identified based on the exposure profiles of metal mixtures through k-means clustering according to both the cubic clustering criterion and the Elbow method, which was consistent with our previous finding (Wang et al. 2019b). These two clusters were labeled as “high” (n=604) and “low” (n=633) for the exposure patterns. Participants assigned to the “high” cluster had higher overall exposures to all the metals compared to those classified into the “low” cluster (Supplementary Figure 5). Adjusted survival curves of diabetes by these two clusters are shown in Figure 1. After adjustment for potential

confounders, the HR for diabetes was 1.46 (95% CI:1.11, 1.91, p=0.007) for women in the "high" cluster compared to those in the "low" cluster in the Cox model.

Use of specific gravity-adjusted concentrations of urinary metals instead of adjusting for specific gravity in the Cox models for urine dilution adjustment did not alter our findings significantly (**Supplemental Table III.2**).

4. Discussion

In this multi-site, multi-ethnic cohort study of women at midlife, urinary arsenic, lead, and zinc concentrations were associated with incidence of diabetes after adjusting for sociodemographic variables, lifestyle factors, BMI, menopausal status, use of hormones and dietary sources. These associations remained significant after further controlling for multiple comparisons. A metal mixtures analysis revealed that a "high" overall exposure pattern to metals was associated with a higher incidence of diabetes. These results suggest that exposure to metals may be a diabetes risk factor of environmental origin.

Arsenic

We found a positive association between total arsenic in urine and incidence of diabetes. Inorganic arsenic is a toxicant and its common sources include drinking water and certain foods (e.g., rice, seafood) (Wang et al. 2019b). After absorption through the gastrointestinal tract, inorganic arsenic is metabolized into monomethylarsonate (MMA) and dimethylarsinate (DMA), which are excreted into the urine together with inorganic arsenic rapidly (Jones et al. 2016). The sum of inorganic arsenic, MMA, and DMA in the urine mainly reflects inorganic arsenic exposure (Jones et al. 2016). Epidemiologic evidence has supported a possible role of arsenic in diabetes. High exposure to arsenic in drinking water ($\geqslant$50 μg/L) has been associated with

increased risks of diabetes in areas such as Taiwan and Bangladesh where historical problems of arsenic contamination exist (Wang et al. 2014). Association between arsenic and diabetes has also been reported in population with low-moderate exposure (<50 μg/L in drinking water). In the United States, urinary arsenic was noted to be positively associated with diabetes prevalence in the general population (Navas-Acien et al. 2008) and in American Indian adults (Gribble et al. 2012). A diabetogenic effect of arsenic has been supported by mechanistic evidence. Arsenic had been linked with insulin resistance by altering gene expression of a variety of diabetes-related factors and by affecting insulin-stimulated glucose uptake in adipocytes and skeletal muscle cells (Mohammed Abdul et al. 2015; Walton et al. 2004). In the pancreas, arsenic may increase amyloid formation and apoptotic death/damage of pancreatic β cells through the generation of oxidative stress (Lu et al. 2011). Additionally, arsenic has been suggested to substitute phosphate and to interact with sulfhydryl groups, which could impair the glucose transport, interrupt the production of energy, and interfere with the ATP-dependent insulin secretion of β cells (Petrick et al. 2001).

Lead

We found a significant association between urinary lead concentration and incidence of diabetes. Bone lead stores accrued from cumulative environmental exposures for decades are the major endogenous source of lead (Wang et al. 2019a). Urinary lead adjusted for urine dilution has been found to closely reflect lead mobilized from the bone (Wang et al. 2019a). Given the fact that midlife women may experience an increased bone turnover rate (Hernandez-Avila et al. 2000), the observed association could be attributed to a greater mobilization of lead from bone into the circulation. Existing evidence on the influence of lead exposure on diabetes risk has been limited and inconsistent: higher lead concentrations in different biological matrices have been

observed in diabetic patients compared to referents in case-control studies (Afridi et al. 2013; Nagaraj et al. 2009). On the contrary, no association has been found in two cross-sectional studies in both the U.S. and South Korea (Menke et al. 2015; Moon 2013). One recent study in China found that higher blood lead concentration was associated with an increased risk of non-alcoholic fatty liver disease, which commonly coexists with type 2 diabetes and has been suggested as a predictor of diabetes risk (Zhai et al. 2017). Lead is a well-known toxicant that can induce oxidative stress through reactive oxygen species (ROS) generation, where the ROS pathway has been suggested in the pathogenesis of diseases including diabetes (Leff et al. 2018). Lead is also thought to disrupt a variety of intracellular signaling pathways by interfering with calcium homeostasis and calcium cellular uptake, and modulating activity of protein kinase C (Leff et al. 2018).

Zinc

Zinc is an essential nutrient that is necessary for biochemical pathways and required by thousands of proteins for catalytic functions. The human body has no specialized zinc storage system and humans rely on a daily intake of zinc to maintain health. Zinc leaves body mainly in feces and urine (Jansen et al. 2009). Zinc intake has been associated with a lower risk of type 2 diabetes in women (Vashum et al. 2013). In our study, zinc status was assessed from both zinc intake and urinary excretion. We observed a positive association between urinary zinc concentration and risk of diabetes after adjustment for zinc intake from diets and supplements, suggesting urinary zinc excretion independent of dietary sources as a predictor of diabetes. The average intake levels in our participants were greater than the recommended dietary allowance, which is 8 mg/day for women (Maret and Sandstead 2006). Our results suggest that women with excess zinc in urine may be at elevated risk of diabetes regardless of the amount of dietary zinc

intake. Mechanistic studies have demonstrated that zinc plays important role in the biosynthesis, storage, and action of insulin. In pancreatic β cells, zinc has been known to be necessary for insulin synthesis, storage and, secretion, and has accounted for the conformational integrity of insulin in its hexameric crystalline form (Jansen et al. 2009). Excessive urinary excretion of zinc was found to lead to a loss of zinc in β-cells, which accounted for reduced insulin secretion (Jansen et al. 2009). Certain zinc complexes showed an insulin-like effect including attenuating hyperglycemia and increasing lipogenesis in animal models (Jansen et al. 2009). Zinc has also been shown to enhance tyrosine kinase phosphorylation in insulin signal transduction improving binding of insulin to its receptor and glucose transportation (Jansen et al. 2009). Zinc is a structural part of antioxidant enzymes such as superoxide dismutase that could protect insulin and β-cells from being attacked by free radicals (Jansen et al. 2009). Despite this evidence, hyperglycemia, on the other hand, was suggested to interfere with the active transportation of zinc back to renal cells, leading to a loss of this mineral in the urine (Chausmer 1998). This raised the possibility that the observed association could also be explained by the increased urinary excretion of zinc in women who already had relatively high glucose levels at baseline. However, we still observed a positive association between urinary zinc and incident diabetes when we additionally excluded women with fasting glucose levels from 100 to 125 mg/dL (impaired fasting glucose) at the study baseline (data not shown). Our findings from this prospective study suggest that an increased urinary zinc excretion may increase diabetes risk, independent of dietary zinc intake.

Other metals

Our data provided modest evidence for an association between tin and diabetes. Tin is commonly used in coatings for cans and containers, and in electrical, construction, and

transportation (ATSDR 2005). Environmental exposure to tin occurs through food, consumer products, and ambient air (ATSDR 2005). One recent study in the U.S. general population found that urinary tin was positively associated with diabetes prevalence, which supports our findings (Liu et al. 2018). Experimental research suggested the potential role of tin in glucose tolerance and insulin resistance through induction of hepatic inflammation and excess hepatic fat accumulation (Bertuloso et al. 2015). In pancreatic β cells, tin was demonstrated to interfere with glucose-induced insulin secretion, due to its inhibitory effect on the cellular calcium response in the triggering exocytosis of insulin granules (Miura and Matsui 2006).

Our data did not provide evidence to suggest an association between cadmium and diabetes. Previous studies concerning cadmium exposure and diabetes have yielded inconsistent results. In the most updated meta-analysis based on data from 9 cross-sectional studies, the pooled odds ratio of diabetes for the highest versus lowest category of urinary cadmium concentration was 1.02 (95% CI:1.00, 1.05) (Li et al. 2017). In contrast, neither blood nor urinary cadmium was associated with incident diabetes, impaired glucose tolerance, blood glucose levels, insulin production, insulin resistance, or hemoglobin A1c (HbA1c) level, in two small prospective studies in Sweden and Thailand (Barregard et al. 2013; Swaddiwudhipong et al. 2012). It is notable that cigarette smoking was less prevalent in our study population of midlife women compared to participants investigated in previous studies. Cigarette smoking has been found to be a major source of cadmium exposure (Wang et al. 2019b) and has been associated with an increased risk of developing diabetes by triggering free radicals, increasing inflammation, oxidative stress and dyslipidemia, and directly damaging β-cells (Śliwińska-Mossoń and Milnerowicz 2017). However, no significant association between urinary cadmium concentration and diabetes was observed in never smokers, former smokers, or current smokers

when we stratified our analysis by smoking status (data not shown). Further investigations aimed at confirming the association and explaining the inconsistency between populations is warranted. In previous studies in U.S. adults, urinary cobalt, molybdenum, uranium and, tungsten have been positively associated with prevalence of diabetes (Menke et al. 2015). Urinary barium has been associated with higher odds of impaired fasting glucose (Feng et al. 2015) and urinary nickel has been associated with higher odds of prevalent diabetes, higher fasting glucose, higher HbA1c, higher insulin levels, and increased insulin resistance (Liu et al. 2015). A large longitudinal study in the U.S. young adults suggested that people with high mercury exposure in young adulthood may have an elevated risk of diabetes and decreased β cell function later in life (He et al. 2013). On the contrary, mercury exposure was not associated with diabetes risks in both the Health Professionals Follow-up Study and the Nurses' Health Study, the two other large longitudinal studies of the U.S. adults (Mozaffarian et al. 2013). In a recent longitudinal study of Chinese senior adults, plasma antimony was inversely associated with diabetes incidence (Yuan et al. 2018). Our study did not provide enough evidence to suggest associations of urinary barium, beryllium, cobalt, cesium, mercury, manganese, molybdenum, nickel, antimony, uranium, and tungsten with diabetes. The different results obtained by the present study and previous ones might be attributed to differences in study design, sources and duration of exposures, biomarkers of metals, as well as different characteristics of the study participants.

Metal mixtures

Metals are widely dispersed in the environment and people could be exposed to a myriad of metals simultaneously throughout their lifetime. In this study, we identified two clusters of women with distinct metal concentration profiles, suggesting different exposure patterns to mixtures of metals in the environment. Our previous study using the same clustering approach

reported significant differences in sociodemographic, lifestyle, and dietary characteristics between women with different exposure profiles (Wang et al. 2019b). In the present study, higher overall exposure to metal mixtures was associated with an increased risk of diabetes after adjustment for all these factors, suggesting a potential role for exposure to metal mixtures in diabetes. Notably, each exposure pattern showed homogeneous distributions of individual metals (standardized concentrations). No patterns had particularly high or low concentrations of specific metals including arsenic, lead, and zinc, of which associations with diabetes were identified individually. This indicates that there may be other components of metal mixtures distinct from arsenic, lead, and zinc that affect diabetes risk but may not be adequately captured by the single-pollutant approach possibly due to relatively small or non-linear effects. It should be acknowledged that the associations between the exposure to metal mixtures represented by k-means clusters and diabetes risk do not provide an insight into which metals were responsible for these associations or allow for dose-response characterization. Ultimately, future research adopting advanced statistical approaches is needed to quantify the diabetogenic impact of exposure to metal mixtures with high degrees of correlation while disentangling the potential low-dose, non-linear effects, and metal-metal interactions.

Strengths and limitations

The primary strength of our study is that diabetes status, as well as other potential confounding factors including sociodemographic factors, lifestyle factors, and metabolic quantitative traits, were assessed annually or bi-annually over 16 years follow-up. The prospective design minimized the possibility of reverse causation. The ethnically diverse population, as well as comparable metals concentrations in the SWAN cohort compared to women of the same age in the U.S. general population also increases the generalizability of our

findings (Wang et al. 2019b). Another advantage is that we systematically examined a suite of 20 metals in urine samples with high-quality laboratory methods. To the best of our knowledge, the associations between most of the metals included in our study and diabetes have never been investigated in a prospective cohort study.

Our study also has several limitations. First, metals included in the current analysis have very different half-lives in the human body. Urinary concentrations of metals with short half-lives such as arsenic mainly reflect recent exposures (ATSDR 2007). In contrast, metals such as cadmium are not rapidly excreted and have half-lives of years to decades. Therefore, diabetes risk is likely impacted by metal exposures over time-periods longer than a few days, information on the temporal variability of urinary metals concentrations, especially for those with short half-lives, is needed to characterize cumulative metal exposures. Second, we measured all metal concentrations in urine and urine may not be an optimal biological matrix for some metals, such as lead (Ding et al. 2016, 2018). Our future study could be improved with assessments of metal concentrations in other biological matrices including whole blood, serum, and bone. Third, in our study, only total arsenic concentration was measured in urine sample, and data on arsenic speciation was not available. The source and toxicity of different arsenic species vary. The principal sources of exposure to inorganic arsenic for the general population are contaminated drinking water and rice intake. Exposure to inorganic arsenic has been associated with increased diabetes risk. In contrast, fish intake is a major source of organic arsenic, which is generally considered to have low toxicity and a small impact on diabetes risk (ATSDR 2007; Navas-Acien et al. 2008; Thayer et al. 2012). Arsenic metabolites may also influence diabetes that a lower proportion of urinary MMA relative to urinary DMA was associated with an increased incidence of diabetes in a recent prospective cohort study (Kuo et al. 2015). In future studies, arsenic

speciation will be critical to providing a better understanding of arsenic exposures and associated health risks. Fourth, in this study, urinary zinc was adjusted for dietary intake of zinc and zinc supplements in the regression analysis to better capture renal clearance and excretion of zinc. However, the dietary intake of other essential metals was not measured, and we were unable to distinguish between the metals from dietary sources and the metals from internal sources. Fifth, the use of fasting glucose to determine incident diabetes may have missed some cases who would have been considered to have diabetes based on other tests such as HbA1c test and oral glucose tolerance test. However, the use of self-reported physician diagnosis and antidiabetic medication use in diabetes ascertainment reduces the possibility of misclassification. Finally, our results may be subject to selection bias at enrollment into the SWAN-MPS for selective attrition during follow-up. To minimize the possibility of bias in effect estimates, we assigned weights to participants at each follow-up visit using an inverse probability weighting approach.

In conclusion, this prospective cohort study provides evidence of positive associations of urinary concentrations of arsenic and lead, an increase urinary excretion of zinc, as well as a high overall exposure to metal mixtures with the risk of diabetes among midlife women. Our findings may have important public health implications as increasing and widespread exposure to environmental toxicants and their mixtures may be a key contributor to the worldwide epidemics of type 2 diabetes. Our findings also provide impetus to further investigate the underlying mechanisms by which metals and their mixtures may influence diabetes risk.

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Table III. 1. Characteristics at the time of metal measurements according to diabetes status, the Study of Women's Health Across the Nation.

	No diabetes (n=1,135)	**Diabetes (n=102)**
Age (years)[a]	49.5 (47.4, 51.6)	50.0 (47.7, 52.2)
Race/ethnicity		
White	601 (53.0)	39 (38.2)
Black	202 (17.8)	35 (34.1)
Chinese	150 (13.2)	14 (13.7)
Japanese	182 (16.0)	14 (13.7)
Study site		
Michigan	172 (15.2)	33 (32.4)
Boston	187 (16.5)	7 (6.9)
Oakland	263 (23.2)	20 (19.6)
Los Angeles	327 (28.8)	19 (18.6)
Pittsburgh	186 (16.4)	23 (22.6)
Body mass index (kg/m^2)	25.3 (22.2, 30.0)	31.6 (26.9, 37.3)
Waist circumference (cm)	80.6 (72.9, 91.2)	92.3 (82.3, 108.3)
Education		
High school or less	189 (16.7)	20 (19.6)
Some College	343 (30.2)	43 (42.2)
College and above	603 (53.1)	39 (38.2)
Household income		
Less than $19,999	55 (5.0)	10 (0.10)
$20,000 to 49,999	282 (25.8)	26 (0.25)
$50,000 to 99,999	447 (40.9)	47 (0.46)
$100,000 or more	309 (28.3)	19 (0.19)
Smoking status		
Never	737 (64.9)	58 (56.9)
Former	299 (26.3)	29 (28.4)
Current	99 (8.7)	15 (14.7)
Alcohol consumption		
Infrequent	573 (50.5)	63 (61.8)
Moderate	271 (23.9)	25 (24.5)
Heavy	291 (25.6)	14 (13.7)
Physical activity score	8.0 (6.7, 9.1)	7.4 (6.6, 8.5)
Menopausal status		
Pre-menopausal	796 (70.1)	64 (62.8)
Post-menopausal	162 (14.3)	18 (17.6)
Unknown[b]	177 (15.6)	20 (19.6)
Family history of diabetes		
Yes	367 (32.3)	55 (53.9)
No	418 (36.8)	25 (24.5)
Unknown	350 (30.8)	22 (21.6)
Hormone therapy	230 (20.3)	28 (27.5)
Systolic blood pressure (mmHg)	110.0 (1.01.0, 120.0)	120.0 (109.0, 128.0)
Total cholesterol (mg/dL)	197.0 (175.0, 219.0)	190.0 (172.0, 217.0)
HDL cholesterol (mg/dL)	61.0 (51.0, 72.0)	52.0 (45.0, 60.0)
Triglyceride (mg/dL)	94.0 (71.0, 135.0)	137.5 (93.0, 195.0)
Dietary seafood intake (times/week)	1.4 (0.8, 2.5)	1.5 (0.8, 2.8)
Dietary rice intake (times/week)	2.0 (1.0, 5.5)	2.0 (0.6, 5.5)
Total zinc intake (mg/day)	11.0 (7.6, 20.5)	11.4 (8.5, 21.5)
Total energy intake (kCal)	1661 (1324, 2110)	1950 (1475, 2440)

Note: DM, diabetes mellitus; HDL, high-density lipoprotein.

[a] Data are median (interquartile range) or n (%).

[b] Menopausal status unknown due to hormone therapy or hysterectomy.

Table III. 2. Hazard ratios for incident diabetes for two-fold increase in urinary metal concentrations.

Metals	**Initial model[a]**		**Full Model[b]**	
	HR (95% CI)	**P-value**	**HR (95% CI)**	**P-value[c]**
Arsenic	1.06 (0.98, 1.15)	0.13	1.24 (1.14, 1.35)	**<0.0001**
Barium	0.98 (0.88, 1.09)	0.70	0.99 (0.87, 1.12)	0.83
Cadmium	1.00 (0.90, 1.10)	0.92	0.96 (0.86, 1.07)	0.47
Cobalt	0.97 (0.85, 1.10)	0.60	1.00 (0.87, 1.14)	0.98
Cesium	1.12 (0.92, 1.37)	0.25	1.23 (1.00, 1.51)	0.05
Copper	1.06 (0.91, 1.23)	0.47	0.96 (0.82, 1.14)	0.66
Mercury	0.83 (0.75, 0.92)	0.03	0.92 (0.83, 1.03)	0.14
Manganese	1.14 (0.94, 1.37)	0.18	1.10 (0.89, 1.35)	0.38
Molybdenum	0.93 (0.81, 1.07)	0.29	1.05 (0.90, 1.22)	0.56
Nickel	1.08 (0.93, 1.25)	0.33	1.15 (0.98, 1.35)	0.10
Lead	1.12 (0.99, 1.27)	0.07	1.23 (1.08, 1.40)	**0.002**
Antimony	1.03 (0.91, 1.17)	0.61	1.04 (0.91, 1.20)	0.55
Tin	1.10 (1.01, 1.20)	**0.03**	1.11 (1.01, 1.22)	**0.04**
Thallium	1.05 (0.95, 1.16)	0.38	1.04 (0.92, 1.16)	0.54
Zinc	1.48 (1.27, 1.74)	**<0.0001**	1.31 (1.11, 1.55)	**0.001**

Note: all models were constructed by Cox proportional hazards model.

[a] Initial model: adjustment for age, race/ethnicity, study sites, and specific gravity (log-transformed).

[b] Full model: initial model with additional adjustment for education, household income, body mass index, waist circumference, smoking status, alcohol consumption, physical activity score, systolic blood pressure, total cholesterol level, high-density lipoprotein cholesterol level, triglyceride level, family history of diabetes, total energy intake, menopausal status, and use of hormone. In full model, seafood and rice intake was additionally adjusted for arsenic, cadmium, and mercury models; zinc intake from diets and supplements was additionally adjusted for zinc model.

[c] Significance level α=0.002 corresponding to a false discovery rate of 0.05 using the Benjamini–Hochberg Method.

Table III. 3. Hazard ratios for incident diabetes comparing participants with urinary beryllium, chromium, uranium, vanadium, tungsten concentrations above the limits of detection to those below the limits of detection.

Metals	Initial model[a]		Full model[b]	
	HR (95% CI)	P-value	HR (95% CI)	P-value
Beryllium	1.03 (0.74, 1.41)	0.86	1.00 (0.70, 1.40)	0.99
Chromium	0.77 (0.55, 1.06)	0.11	0.71 (0.50, 1.01)	0.06
Uranium	0.84 (0.61, 1.14)	0.27	0.99 (0.70, 1.40)	0.96
Vanadium	0.85 (0.64, 1.12)	0.24	0.77 (0.57, 1.04)	0.09
Tungsten	1.02 (0.73, 1.40)	0.92	1.14 (0.83, 1.60)	0.63

Note: all models were constructed by Cox proportional hazards model. Detection rate: beryllium, 15.7%; chromium, 24.3%; uranium, 33.5%; vanadium, 37.3%; tungsten, 29.2%.

[a] Initial model: adjustment for age, race/ethnicity, study sites, and specific gravity (log-transformed).

[b] Full model: initial model with additional adjustment for education, household income, body mass index, waist circumference, smoking status, alcohol consumption, physical activity score, systolic blood pressure, total cholesterol level, high-density lipoprotein cholesterol level, triglyceride level, family history of diabetes, total energy intake, menopausal status, and use of hormone.

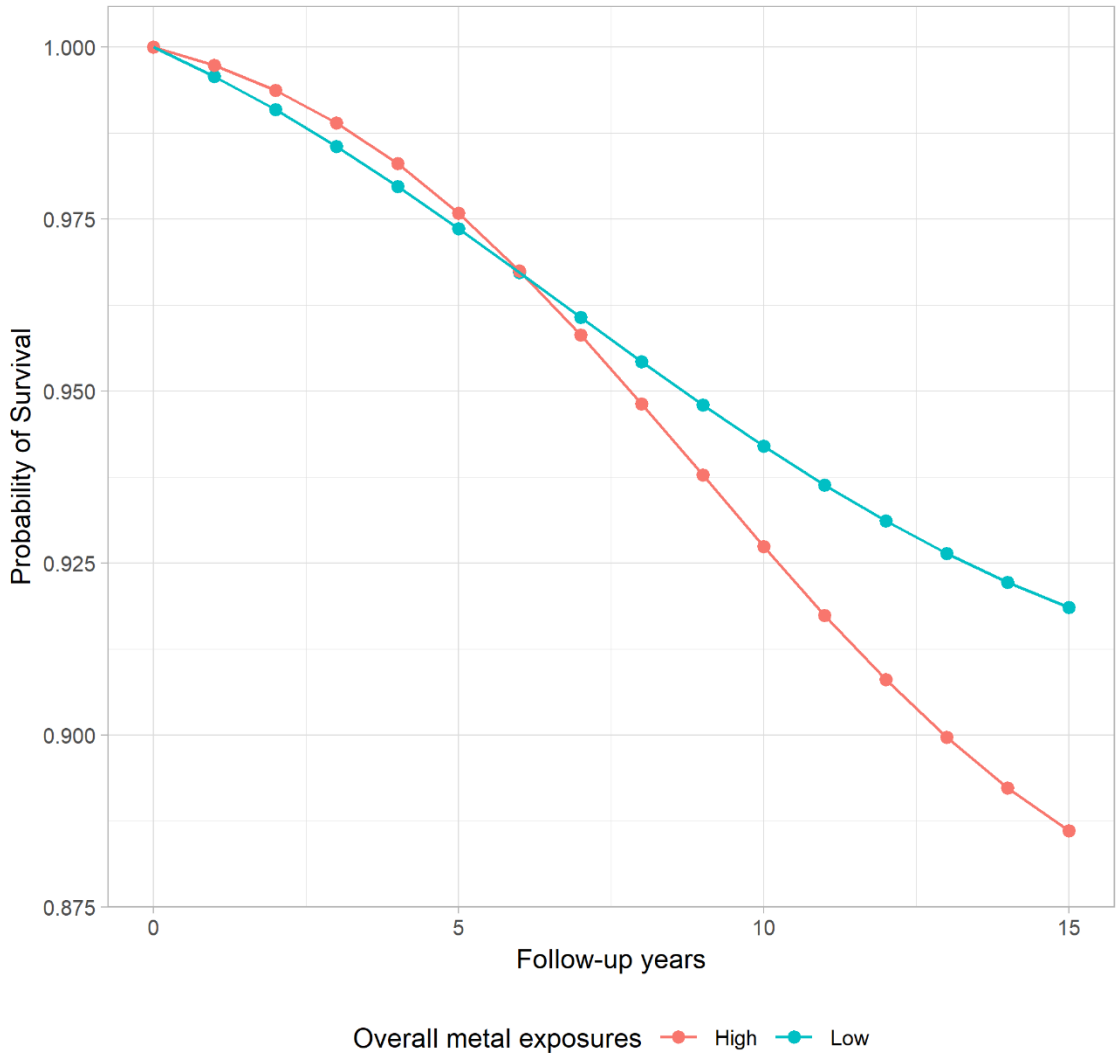


Figure III. 1. Adjusted survival curves of diabetes by two distinct exposure patterns to metal mixtures, adjusting for age, race/ethnicity, study sites, education, household income, body mass index, waist circumference, smoking status, alcohol consumption, physical activity score, systolic blood pressure, total cholesterol level, high-density lipoprotein cholesterol level, triglyceride level, family history of diabetes, total energy intake, menopausal status, use of hormone, dietary intake of seafood and rice, and zinc intake from diets and supplements.

Supplementary Methods

In our study, selection bias may exist, as selection into SWAN the Multi-Pollutant Substudy were probably affected by metal exposures, their related diabetes risk factors, or potential confounders before or at the time of enrollment. On the other hand, selective loss to follow-up or other forms of attrition that occur after metal measurements may also bias estimates of associations between metals and diabetes if continuation in the follow-up is influenced by metal exposures and risk factors of diabetes. We addressed these two types of bias by using the inverse probability weighting (IPW).

Selective participation in the SWAN multi-pollutant substudy

We used Repository samples available from the third SWAN follow-up visit (visit 03, 1999-2000) for metal measurements in our analysis. Women enrolled in SWAN were between the age of 42 to 52 years at the SWAN baseline (visit 00, 1996-1997), which marked a time of increased risk for diabetes (Kim 2012). Some of women who were at high risk of diabetes at the SWAN baseline have been censored before visit 03. Thus, participants susceptible to developing diabetes at the time of metal measurements were possibly different from the source population. On the other hand, at visit 03, only a subpopulation with 1,400 SWAN participants, but not all participants remained in the cohort had urine samples stored in the SWAN Biorepository assayed for metal concentration determinations. In this way, the analysis based on these 1,400 participants is likely to be susceptible to bias attributable to the selective participation in the substudy as shown in the directed acyclic graphs (DAG) (**supplementary Figure 2**).

In the **supplementary Figure 2**, DM represents incidence of diabetes. E_0 and E_3 represent measures of urinary metals at SWAN baseline (visit 00) and third follow-up visit (visit 03). We measure metals only at visit 03, so E_0 is unobserved. Considering the environmental

exposure to metals at one time point is often reasonably correlated with the exposure at other time points, we consider an effect emanating from E_0 to E_3 and terminating in DM to represent a causal effect of metals on diabetes for the purposes of identifying potential bias in our DAG. RF represents metal induced health effects which may affect continuation in the SWAN up to visit 03, substudy participation, and diabetes. L represents both time-fixed and time-varying covariates which may influence both diabetes risks and selection. S_3 with a box drawn around represents remaining uncensored and free of diabetes up to visit 03. S_U with a box drawn around represents urinary metals substudy participation. Selection bias can be found in the DAG. For example, conditioning on S_U opens the path $RF_3 \rightarrow S_U \leftarrow L_3$, introducing an association between E_3 and DM ($E_3 \rightarrow RF_3 \rightarrow S_U \leftarrow L_3 \rightarrow$DM) which is not causal. At the same time, conditioning on S_U blocks some of the association that goes from E_3 to diabetes through RF_3, because conditioning on S_U partially conditions on RF_3.

IPW was used to alleviate the potential bias resulting from the selection into the SWAN multi-pollutant substudy (Hernán et al. 2004). IPW uses information available for participants with and without metal measurements to weight observations from participants with metal measurements, so that the weighted subpopulation is representative of all SWAN participants in the original cohort who were continuing in the cohort and were free of diabetes at the time of metal measurements (visit 03). Probability of continuation in the follow-up study up to visit 03 and probability of selection into the substudy given that participants were not censored at visit 03 were modeled separately. We estimated the probability of continuing in the study up to visit 03 by using pooled logistic regression(Weuve et al. 2012), conditional on covariates (RF, and L) and on being uncensored at the previous visit, which equals to $\prod_{k=1}^{i} \Pr[C_{ik} = 0 \mid C_{i(k-1)} = 0, L_{i(k-1)}, RF_{i(k-1)}]$, where k represents the k^{th} visit (01-03) and C is the censoring indicator.

Given the large number of possible predictors among the relative to the number of persons who dropped out of the study, we used forward selection to inform the variables included in the final models, including age, race/ethnicity, study site, education level, marital status, husband's employment status, smoking, menopausal status, self-rated health, and diagnose of heart attack or angina. The reciprocal of this cumulative probability (W_1) is the weight of remaining free of diabetes and in the study for individual *i* at visit 03. For the probability of selection into the substudy given that participants were not censored at visit 03, we used a single logistic regression model to predict the probability(Weisskopf et al. 2015), which equals to $\Pr[U_i = 1 \mid C_{i,3} = 0, L_{i,3}, RF_{i,3}]$, where U indicates the selection into substudy. Variables included in the final logistic model were determined through forward selection, including age, study site, education level, smoking, menopausal status, total cholesterol level, low density lipoprotein cholesterol level, triglyceride level, and hypertension. The reciprocal of this probability (W_2) is the weight of being selected into the substudy at visit 03. Finally, we calculated a combined weight $W_{substudy} = W_1 \times W_2$, as the inverse of the probability of the conjunction of these two events.

Selective attrition after metal measurements

We hypothesized that women with higher concentrations of toxic metals would experience higher risk of diabetes during 15 years of follow-up after metal measurements. However, given the toxicity of metals such as arsenic, those with high concentrations of toxic metals who remained in the cohort might have other beneficial characteristics (healthier) that protected them from developing diabetes. This is because the risk factors for diabetes especially those health conditions predict the censoring or attrition after the metal measurements. Studies of toxic metals that are also themselves associated with substantial attrition through the related

adverse health outcomes correlated with diabetes. In this way, the selection induces an association between metals and diabetes, even if there is no true effect (see DAG in **Supplemental Figure III. 3**).

Same symbols (E, RF, L, DM) as those in **Supplemental Figure III. 2** were used in **Supplemental Figure III. 3** to represent the same type of variables in the DAG. S with a box drawn around represents continuation in the SWAN study at each visit after visit 03. S is a collider on which we condition through the restriction of our analysis to those remained in the cohort at each visit. Therefore, statistical associations, for example, between E_3 and L_3, RF_3 and L_3, are induced via conditioning on S_4. E_0 is then connected to DM through paths that do not emanate from E_3, such as $E_3 \rightarrow RF_3 \rightarrow S_4 \leftarrow L_3 \rightarrow DM$, which is noncausal. On the other hand, if continuation in SWAN is at least partly driven by RF, then the continuation in the cohort effectively conditions on RF, resulting in bias from conditioning on an intermediate between metal and diabetes.

Similar to the strategy we used to address the selective participation, IPW was used to reduce potential bias resulting from the selective attrition. The intuition behind these weights is that participants with characteristics similar to the observations missing due to attrition are upweighted, so as to represent their original contribution as well as their missing contributions. We modeled and estimate the probability of continuing in the study after visit 03 by using pooled logistic regression, conditional on covariates (RF and L) and on being uncensored at the previous visit, which equals to $\prod_{k=4}^{j} \Pr[C_{ik} = 0 \mid C_{i(k-1)} = 0, E_i, L_{i(k-1)}, RF_{i(k-1)}, Z_i]$, where k represents the k^{th} visit (04-15) and C is the censoring indicator. Age, study site, SWAN visit number, household income, smoking, use of hormone, self-rated health, BMI (linear and quardratic terms), and waist circumference (linear and quardratic terms) were included in the final logistic

model after forward selection. The reciprocal of this cumulative probability of continuing is the non-stabilized weight ($W_{attrition}$). And the weight was applied at the level of observations within individuals.

Combine IPWs for selective participation and selective attrition

We calculated the total $W = W_{substudy} \times W_{atrrition}$ for each participant in the metals-diabetes analysis, as the inverse of the probability of being selected into the SWAN multi-pollutant substudy from the original SWAN cohort and of being uncensored up to a given study visit after metal measurements. To note, including L in the calculation of the weight is not sufficient to control for confounding when evaluating associations between metals and diabetes incidence, and as such, the potential confounders were adjusted as covariates in the Cox proportional hazards model in our primary analysis (Hernán et al. 2004; Weuve et al. 2012).

Supplemental Table III. 1. Medians, interquartile range, and detection rate of urinary metals, the Study of Women's Health Across the Nation.

Metals	LOD*	Percent >LOD	Median concentration (IQR†), µg/L	
			Non-diabetes (n= 1,089)	Incident diabetes (n= 137)
Arsenic	0.3	100	14.24 (6.51, 36.69)	14.28 (6.44, 39.09)
Barium	0.1	99.7	1.79 (0.99, 3.00)	1.75 (1.15, 3.04)
Beryllium	0.04	15.7	<LOD (<LOD, <LOD)	<LOD (<LOD, <LOD)
Cadmium	0.06	94.5	0.47 (0.23, 0.82)	0.48 (0.23, 0.85)
Cobalt	0.05	99.3	0.62 (0.38, 0.97)	0.63 (0.41, 1.00)
Chromium	0.4	24.3	<LOD (<LOD, <LOD)	<LOD (<LOD, <LOD)
Cesium	0.01	100	4.71 (3.05, 7.27)	4.48 (2.75, 7.46)
Copper	2.5	97.1	9.54 (6.12, 13.57)	11.01 (6.73, 16.85)
Mercury	0.05	99.8	1.23 (0.67, 2.45)	1.11 (0.58, 2.02)
Manganese	0.08	99.6	0.92 (0.62, 1.46)	1.06 (0.65, 1.70)
Molybdenum	0.3	100	43.27 (24.39, 69.97)	46.76 (27.93, 74.80)
Nickel	0.8	96.2	3.69 (2.26, 5.79)	4.00 (2.64, 5.97)
Lead	0.1	97.6	0.80 (0.48, 1.27)	0.88 (0.48, 1.46)
Antimony	0.04	78.5	0.08 (0.05, 0.13)	0.10 (0.05, 0.17)
Tin	0.1	96.7	0.94 (0.48, 1.81)	1.08 (0.64, 2.31)
Thallium	0.02	92.4	0.15 (0.08, 0.23)	0.15 (0.08, 0.25)
Uranium	0.01	33.3	<LOD (<LOD, 0.01)	<LOD (<LOD, 0.01)
Vanadium	0.6	37.4	<LOD (<LOD, 1.18)	<LOD (<LOD, 0.81)
Tungsten	0.2	29.0	<LOD (<LOD, 0.21)	<LOD (<LOD, 0.24)
Zinc	2	100	301 (166, 499)	464 (258, 686)

* LOD: limit of detection.
† IQR: interquartile range.

Supplemental Table III. 2. Hazard ratios* for incident diabetes for two-fold increase in specific gravity corrected-urinary metal concentrations.

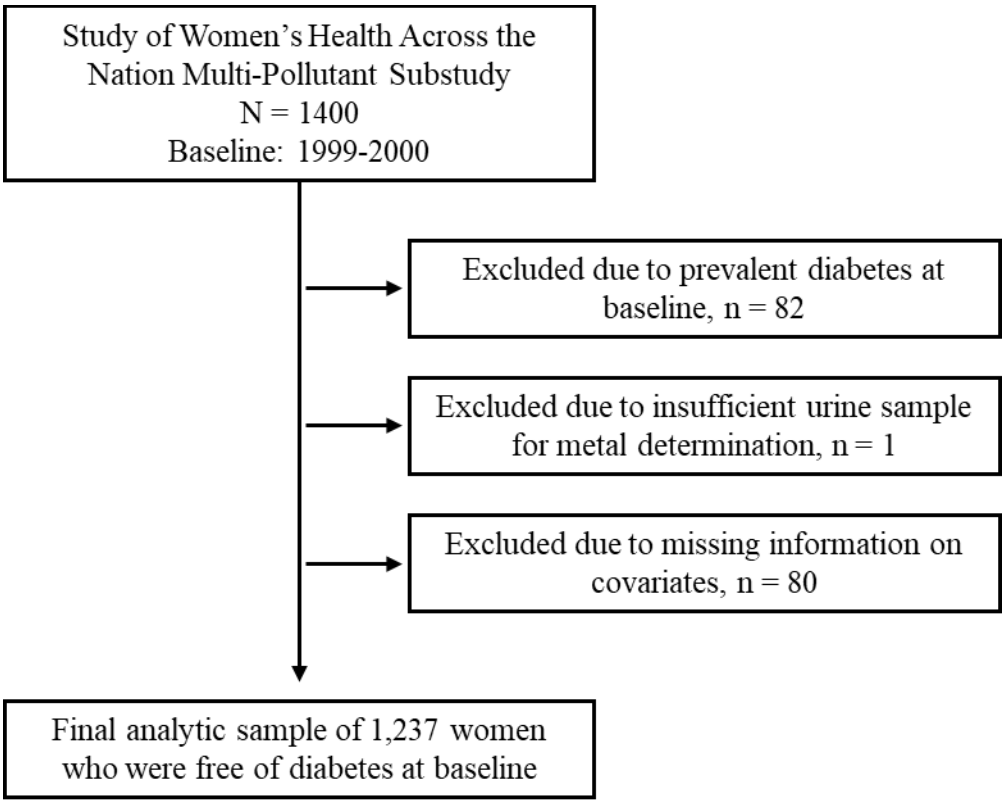
Metals	Model 1†		Model 2‡		Model 3§	
	HR (95% CI)	P-value	HR (95% CI)	P-value	HR (95% CI)	P-value
Arsenic	1.05 (0.97, 1.13)	0.25	1.12 (1.04, 1.22)	0.004	1.15 (1.06, 1.24)	<0.001
Barium	1.00 (0.90, 1.10)	0.96	1.02 (0.93, 1.13)	0.64	1.06 (0.95, 1.17)	0.29
Cadmium	1.01 (0.92, 1.10)	0.92	1.01 (0.91, 1.11)	0.91	1.03 (0.93, 1.14)	0.55
Cobalt	0.97 (0.86, 1.09)	0.60	0.97 (0.86, 1.09)	0.60	1.03 (0.92, 1.17)	0.59
Cesium	0.99 (0.83, 1.18)	0.88	1.03 (0.87, 1.23)	0.71	1.08 (0.90, 1.29)	0.42
Copper	1.04 (0.91, 1.19)	0.55	1.01 (0.88, 1.15)	0.91	0.98 (0.85, 1.12)	0.74
Mercury	0.85 (0.78, 0.93)	0.001	0.87 (0.79, 0.95)	0.03	0.91 (0.82, 1.00)	0.05
Manganese	1.06 (0.95, 1.19)	0.27	1.05 (0.94, 1.18)	0.38	1.06 (0.96, 1.19)	0.30
Molybdenum	1.00 (0.89, 1.14)	0.96	1.02 (0.90, 1.16)	0.74	1.10 (0.97, 1.25)	0.16
Nickel	1.06 (0.93, 1.22)	0.38	1.04 (0.92, 1.19)	0.53	1.13 (0.99, 1.29)	0.07
Lead	1.13 (1.01, 1.26)	0.03	1.16 (1.04, 1.31)	0.01	1.20 (1.07, 1.34)	0.002
Antimony	1.07 (0.96, 1.20)	0.21	1.09 (0.98, 1.23)	0.11	1.10 (0.99, 1.23)	0.08
Tin	1.09 (1.01, 1.18)	0.02	1.08 (1.00, 1.17)	0.05	1.09 (1.01, 1.19)	0.03
Thallium	1.01 (0.92, 1.11)	0.83	1.03 (0.94, 1.13)	0.54	1.01 (0.91, 1.11)	0.90
Zinc	1.58 (1.38, 1.83)	<0.001			1.46 (1.26, 1.69)	<0.001

* All models were constructed by Cox proportional hazards model.

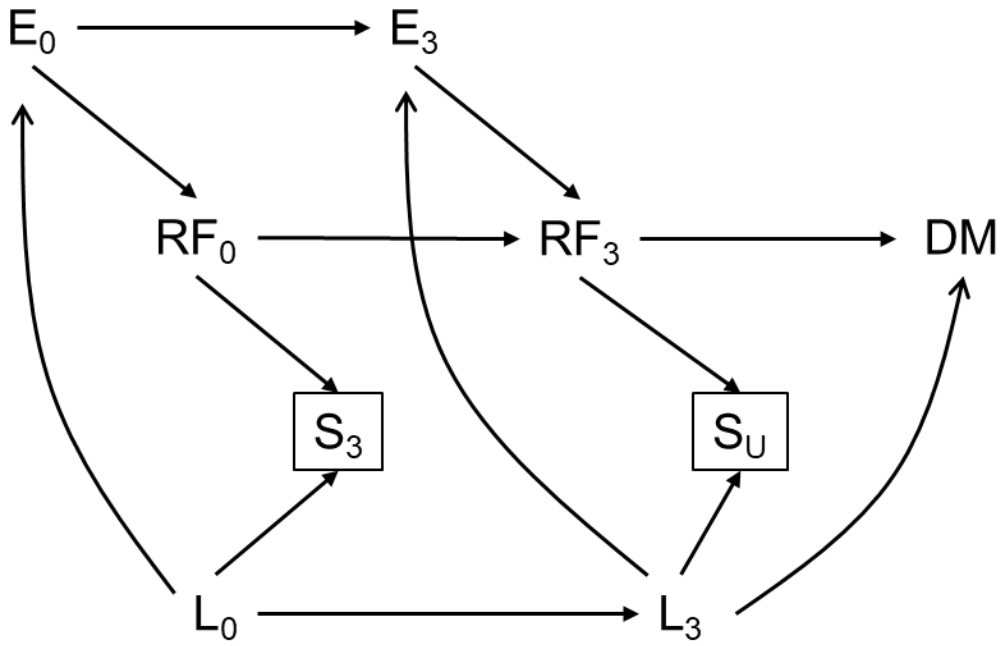
† Model 1: adjustment for age, race/ethnicity, study sites.

‡ Model 2: model 1 with additional adjustment for education, household income, smoking status, alcohol consumption, physical activity score, menopausal status, and use of hormone. In model 2, rice intake and urinary mercury concentration (log-transformed) were additionally adjusted for arsenic, cadmium, and mercury models, except for mercury model that was further adjusted for rice intake only.

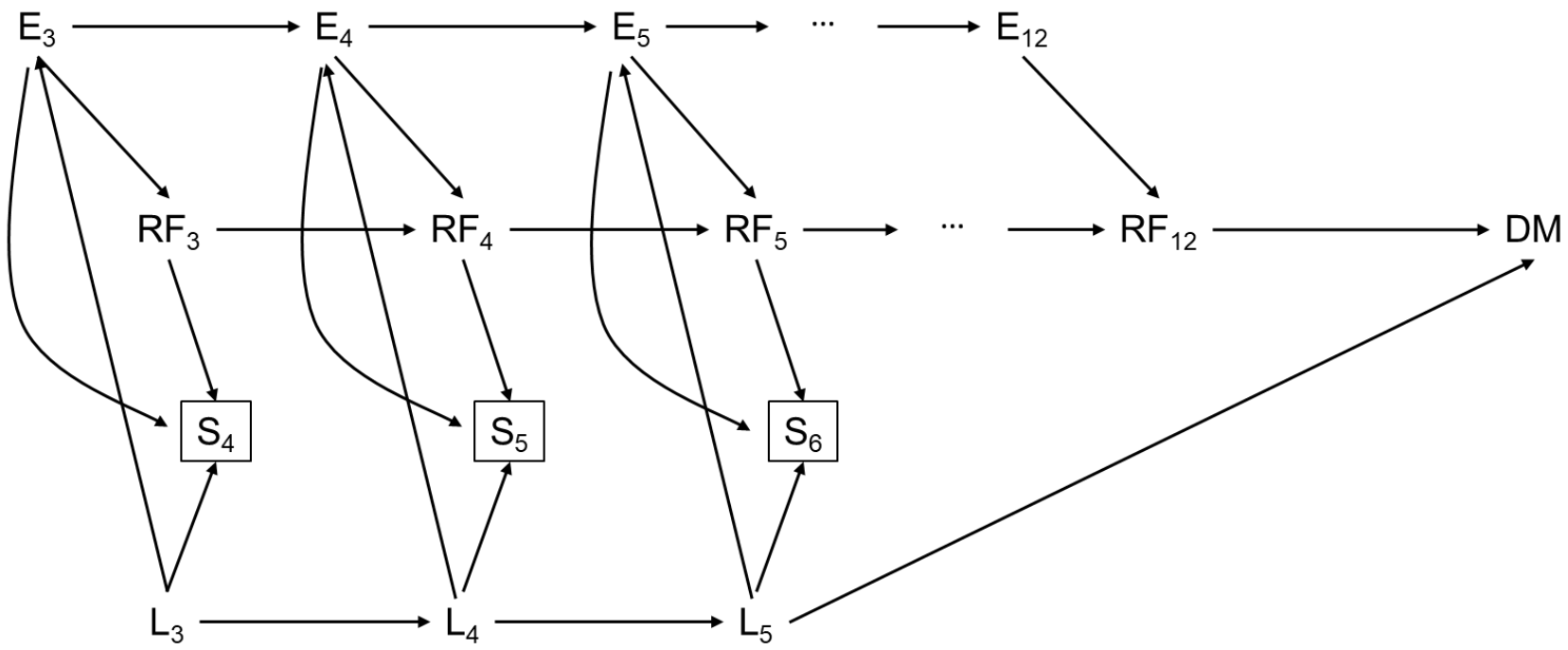
§ Model 3: model 2 with additional adjustment for body mass index at the time of metal measurements.



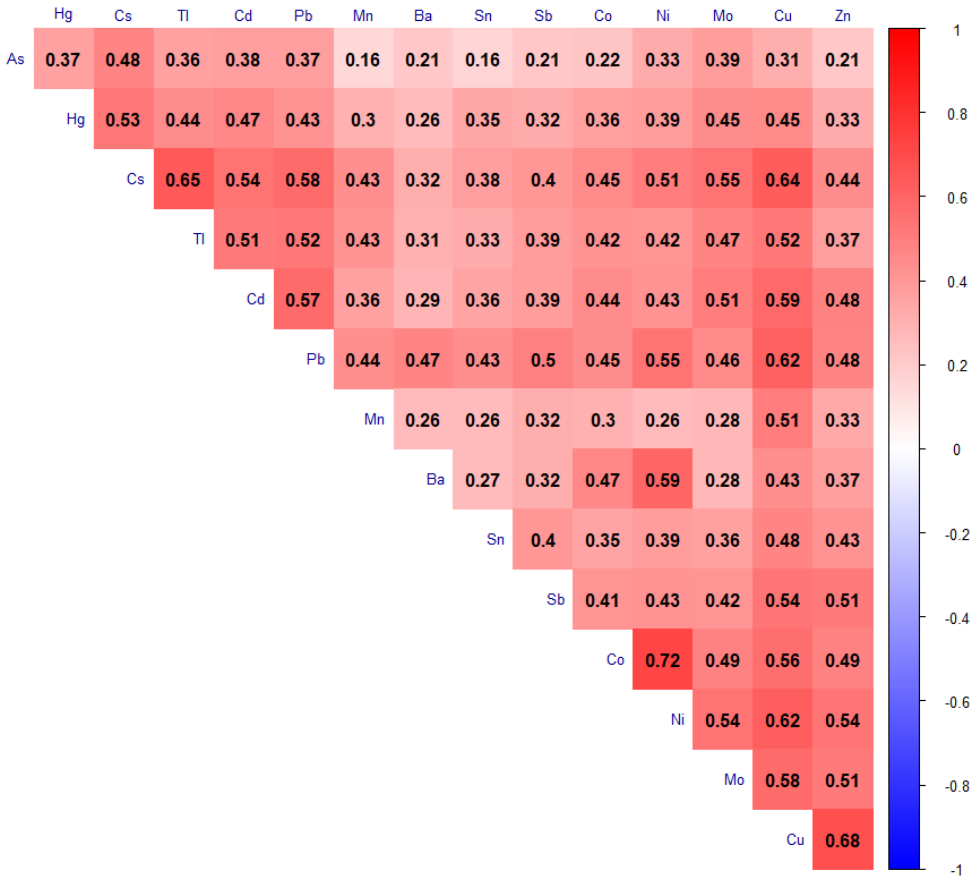
Supplemental Figure III. 1. Schematic diagram of analytic sample.



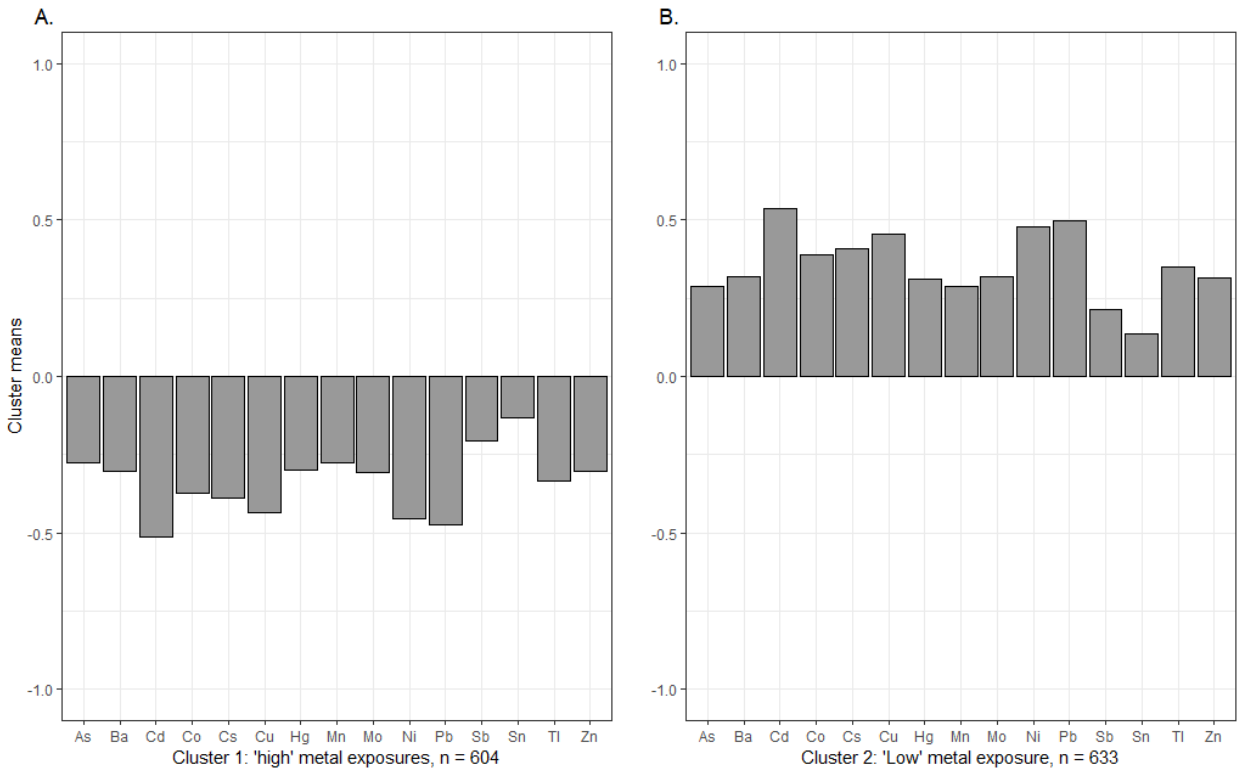
Supplemental Figure III. 2. Directed acyclic graphs illustrating selective participation in the SWAN multi-pollutant substudy.



Supplemental Figure III. 3. Directed acyclic graphs illustrating selective attrition after metal measurements.



Supplemental Figure III. 4. Spearman correlation matrix of metal concentrations.
As: arsenic, Ba: barium, Cd: cadmium, Co: cobalt, Cs: cesium, Cu: copper, Hg: mercury, Mn: manganese, Mo: molybdenum, Ni: nickel, Pb: lead, Sb: antimony, Sn: tin, Tl: thallium, Zn: zinc.



Supplemental Figure III. 5. Cluster means of the 15 standardized log-transformed urinary metals using k-means clustering method.
Y-axis (cluster means) represents the mean standardized log-transformed specific gravity adjusted metal concentrations. Cluster 1: "high" exposure pattern of metal mixtures; cluster 2: "low" exposure pattern of metal mixtures. As: arsenic, Ba: barium, Cd: cadmium, Co: cobalt, Cs: cesium, Cu: copper, Hg: mercury, Mn: manganese, Mo: molybdenum, Ni: nickel, Pb: lead, Sb: antimony, Sn: tin, Tl: thallium, Zn: zinc.

Chapter IV. Urinary Metals and Longitudinal Glucose Homeostasis: the Study of Women's Health Across the Nation (SWAN)

Xin Wang, Carrie A. Karvonen-Gutierrez, William H. Herman, Bhramar Mukherjee, Stuart Batterman, Siobán D. Harlow, Sung Kyun Park

Abstract

Background: Epidemiologic studies on associations between metals and insulin resistance and β-cell dysfunction have been cross-sectional and focused on individual metals.

Objective: We assessed the association between exposure to metal mixtures based on assessment of 15 urinary metals and longitudinal changes in insulin resistance and β-cell function.

Methods: We examined 1,262 women, aged 45-56 years at baseline (1999-2000), who were followed through 2015-2016, from the Study of Women's Health Across the Nation. Urinary concentrations of 15 metals (arsenic, barium, cadmium, cobalt, cesium, copper, mercury, manganese, molybdenum, nickel, lead, antimony, tin, thallium, and zinc) were determined at baseline. Homeostatic model assessments for insulin resistance (HOMA-IR) and β-cell function (HOMA-β) were repeatedly measured over 16 years of follow-up. Adaptive elastic-net (AENET) models were fitted to identify components of metal mixtures associated with longitudinal changes in HOMA-IR and HOMA-β.

Results: In multivariable adjusted AENET models, urinary copper, lead, and zinc were associated with higher HOMA-IR at baseline, whereas molybdenum was associated with lower HOMA-IR at baseline. The estimated changes in baseline HOMA-IR for one-standard deviation increase in log-transformed urinary metal concentrations were 1.57% (-1.09%, 4.29%) for copper, 0.70% (-1.59%, 3.05%) for lead, 5.76% (3.05%, 8.55%) for zinc, and -3.25% (-5.45%, -1.00%) for molybdenum, respectively. Urinary zinc was also associated with a faster rate of increase in HOMA-IR. Urinary arsenic and zinc were associated with lower baseline HOMA- β, whereas cobalt was associated with higher baseline HOMA- β. Arsenic was also associated with a faster rate of decline in HOMA-β.

Conclusion: Exposure to metals including arsenic, cobalt, copper, molybdenum, and lead, and increased urinary excretion of zinc, may be exerting effects on insulin resistance and β-cell dysfunction, which might be mechanisms by which metals affect diabetes risks.

1. Introduction

Type 2 diabetes mellitus (T2DM) is a major global health concern and its incidence has rapidly increased over the past two decades (Magliano et al. 2019). The etiology of T2DM has not been well understood yet and the role of environmental exposures, specifically metals, in the pathogenesis of T2DM has received little attention by the medical community. Metals are widely dispersed in the environment, including soil, water, air, dust, human food chain, as well as manufacturing products (Järup 2003; Tchounwou et al. 2012; Wang et al. 2019b). The general population can be exposed to a myriad of metals through food, drinking water and ambient air throughout their lifetime. Most studies of metabolic diseases to date have focused on the effects of genetics, unhealthy diets and lifestyle, while ignoring the potential effects of environmental toxicants. Nonetheless, exposure to metals needs to be considered one of the leading factors contributing to the global disease burden, including to the burden of T2DM (Thayer et al. 2012). It has been estimated that among the 40 million adults with high exposure to arsenic in the United States, over 4 million would become diabetic attributable to arsenic exposure alone (Smith 2013). In a most recent paper, we found that in addition to arsenic, high concentrations of lead, an excessive urinary excretion of zinc, as well as a high concentrations of urinary metal mixtures were associated with an increased risk of developing T2DM among midlife women with urinary metals concentrations consistent with background levels of environmental exposure to metals in the United States. These findings provided an impetus to investigate the underlying mechanisms by which metal exposures may influence T2DM risk.

The etiopathogenic mechanisms underlying T2DM involves insulin resistance and β-cell dysfunction, which commonly precedes the onset of diabetes by one to two decades (DeFronzo 2004; Warram et al. 1990). Biological studies provided evidence that both non-essential and

essential metals may impact these conditions. For example, non-essential metals such as arsenic, cadmium and lead, are well-known inducers of oxidative stress c. The accumulation of these metals in pancreatic islets is hypothesized to lead to impaired function and apoptotic death of β-cells via the induction of oxidative stress (Lu et al. 2011; Patra et al. 2011). These metals have also been demonstrated to disrupt glucose uptake by interfering insulin intracellular signaling pathways in adipocytes and muscle cells (Han et al. 2003; Kim et al. 2015; Mohammed Abdul et al. 2015). On the other hand, essential metals such as zinc have been known to be necessary for insulin synthesis, storage and secretion in β-cells (Chausmer 1998), and have a preventative role in insulin resistance, for example, zinc complexes showed insulin-like effects (Adachi et al. 2006). Other essential metals including cobalt, copper, manganese, molybdenum, and nickel are required for various biological pathways and appropriate amounts of these metals in human body are necessary for multiple physiological functions (Zoroddu et al. 2019). On the contrary, non-essential metals including arsenic, barium, cadmium, cesium, mercury, lead, antimony, tin, and thallium have no known physiological roles (Zoroddu et al. 2019). Both essential and non-essential metals may exert adverse health effects depending on the level of exposure (Zoroddu et al. 2019).

Only a few epidemiologic studies have examined the associations of metal exposures with insulin resistance and β-cell dysfunction and those studies have yielded inconsistent results (Barregard et al. 2013; Feng et al. 2015; Grau-Perez et al. 2017; He et al. 2013; Moon 2013; Park et al. 2016; Rhee et al. 2013; Wallia et al. 2014). Most studies were cross-sectional and focused on a limited number of metals (Barregard et al. 2013; Feng et al. 2015; Grau-Perez et al. 2017; He et al. 2013; Moon 2013; Park et al. 2016; Rhee et al. 2013; Wallia et al. 2014). In addition, the general population is exposed to metal mixtures (Wang et al. 2019b), however, most previous

studies have been limited to examination of single metals. This narrow focus on individual metals could be partly due to statistical challenges such as the complex correlations among metals, confounding by correlated co-pollutants, and lack of well-established statistical methods to evaluate the combined effects of exposure to metal mixtures (Braun et al. 2016; Park et al. 2017; Wang et al. 2018, 2019c). Quantifying the health impact of exposure to metal mixtures is needed for to enhance our understanding of the role of environmental risk factors in pathogenesis of metabolic diseases including T2DM.

Within this context, we evaluated the associations of 15 urinary metal concentrations with longitudinal changes in homeostatic model assessments for insulin resistance (HOMA-IR) and β-cell function (HOMA-β) over 16 years of follow-up in the Study of Women's Health Across the Nation (SWAN), a multi-site, multi-ethnic prospective cohort study of midlife women. We used a two-stage modeling approach and employed a machine-learning based approach, the adaptive elastic-net (AENET), which was proposed for analyzing high dimensional data while dealing with the collinearity problem (Zou and Zhang 2009), to identify important components of metal mixtures associated with longitudinal changes in HOMA-IR and HOMA-β.

2. Methods

Study population

Participants in the current analysis were from the SWAN, an ongoing, multi-site, multi-ethnic, community-based longitudinal study of the natural history of menopause designed to investigate the natural history of the menopausal transition and its effect on midlife health including risk factors for age-related chronic diseases (Sowers et al. 2000). Between 1996 and 1997, a total of 3,302 women from seven study sites, including Boston, MA; Chicago, IL;

southeast Michigan, MI; Los Angeles, CA; Oakland, CA; Newark, NJ; and Pittsburgh, PA, participated. Each site enrolled white women and women from one minority group (black women from Boston, Chicago, Southeast Michigan, and Pittsburgh; Chinese women from Oakland; Japanese women from Los Angeles; Hispanic women from Newark). Eligibility criteria of enrollment into the SWAN included: age 42 to 52 years; having an intact uterus and at least one ovary; having at least one menstrual period and not taking hormone therapy in the past 3 months; and having self-identified with the site's designated race/ethnic groups. These women returned for regular examinations approximately annually, and approximately 75% of still living participants completed the 15th SWAN follow-up visit (2015-2016). Institutional Review Board approval was obtained at each study site, and all participants provided signed informed consent at each study visit.

To evaluate associations between urinary metals and longitudinal glucose outcomes, we used data from the SWAN Multi-Pollutant Substudy (MPS), which was initiated to examine the associations of multiple environmental chemicals with metabolic and reproductive health outcomes in midlife women (Ding et al. 2020; Wang et al. 2019b). Urinary metal concentrations were assayed in 1,400 SWAN-MPS participants who had stored urine samples available at the third SWAN follow-up visit (V3, 1999-2000, SWAN-MPS baseline). For this analysis, we excluded 39 participants who had no information on key covariates (education, household income, body mass index (BMI), physical activity, total energy intake), and 46 participants with missing information on fasting glucose or insulin levels throughout the entire follow-up, and 53 participants who were taking antidiabetic medications at the SWAN-MPS baseline, yielding 1,262 participants eligible for the present study. We also censored 347 observations in subsequent follow-up visits when the participant started taking antidiabetic medications because

the true untreated levels of the outcome parameters are unknown. A final sample of 1,262 women with 9,527 observations through 2016 was used for data analysis. An overview of our analytic sample is illustrated in **Supplemental Figure IV.1.**

Insulin resistance and β-cell dysfunction determinations

HOMA-IR and HOMA-β are widely used tools to assess insulin resistance and β-cell dysfunction in clinical practices and epidemiological studies (Wallace et al. 2004). HOMA-IR was calculated from fasting glucose and insulin levels according to the following equation: [insulin (μU/mL) × glucose (mmol/L)]/22.5 (Matthews et al. 1985). HOMA-β was calculated as follows: 20 × insulin/[glucose − 3.5] (Matthews et al. 1985). In the SWAN, fasting serum glucose and insulin levels were assayed from serum samples obtained at each follow-up visit (Thurston et al. 2012). Fasting serum glucose level was determined by hexokinase method (Boehringer Mannheim Diagnostics, Indianapolis, IN, USA). Fasting serum insulin was measured by a solid phase radioimmunoassay (Coat-ACount, Diagnostics Product Corp., Los Angeles, CA).

Urinary metals

Urinary metal concentrations were analyzed with high-resolution inductively coupled plasma-mass spectrometry (ICP-MS) (Thermo Scientific iCAP RQ, Waltham, MA) in first morning spontaneously voided urine samples at the Applied Research Center of NSF International (Ann Arbor, Michigan), a part of the Michigan Children's Health Exposure Analysis Resource (M-CHEAR) Laboratory Hub. The analytic methods and quality control procedures have been described previously (Wang et al. 2019b). Urinary concentrations of the following 15 metals were used in the current analysis, including arsenic, barium, cadmium,

cobalt, cesium, copper, mercury, manganese, molybdenum, nickel, lead, antimony, tin, thallium, and zinc. The limits of detection (LOD) and detection rates are presented in **Supplemental Table IV.1**. Participants with metal concentration below the limit of detection (LOD) were assigned a value equal to the LOD divided by the square root of 2. Urinary concentrations of beryllium, chromium, platinum, uranium, vanadium and tungsten were also determined in the SWAN-MPS. However, due to the relatively low detection rates (<40%) as described previously, these metals were excluded from the current analysis (Wang et al. 2019b). Pairwise Spearman correlations among urinary metal concentrations were calculated and presented in a heat map.

Covariates

Sociodemographic variables including age (continuous), race/ethnicity (white, black, Chinese, or Japanese), education level (≤ high school, some college, or college degree/post-college), and annual household income ($19,999 or under, $20,000-$49,999, $50,000-$99,999, or $100,000 or above) were assessed through a self-administered questionnaire. Weight and height were measured using a calibrated balance beam scale and a stadiometer, respectively, and BMI was calculated as weight in kilograms divided by the square of height in meters. Lifestyle variables including smoking (never smoked, former smoked only, or current smoking) and alcohol drinking (use less than once per month, use once per month, or twice or more times per month) were determined based on self-report. Physical activity was evaluated using a modified version of the Kaiser Physical Activity Survey (Sternfeld et al. 2000), and a total score was calculated indicating the activity levels during the previous 12 months in 3 distinct domains: active living, household/caregiving, and sports/exercise. Menopausal status (pre-menopausal, post-menopausal, unknown menopause status due to hormone therapy or hysterectomy) was based on menstrual bleeding patterns and use of exogenous hormones. Dietary intake of seafood

and rice, total zinc intake from diets and supplements, and total energy intake were collected using a detailed semi-quantitative food frequency questionnaire (FFQ) adopted from the Block FFQ (Wang et al. 2019b). Urinary specific gravity was determined using a handheld digital refractometer (ATAGO model PAL-10S, Tokyo, Japan) as a marker of urine dilution.

Statistical analysis

A two-stage modeling approach was used to evaluate the associations of metal mixtures with longitudinal HOMA measures (HOMA-IR and HOMA-β) because there is no available analytical approach that handles correlations for both dependent and independent variables. In stage 1 that accounts for correlations in outcome measurements within each participant, linear mixed effects models were used to capture changes in HOMA measures over the follow-up period. Given the highly skewed distributions of both HOMA-IR and HOMA-β, logarithmic transformations were applied. Time (year) was modeled using a linear term. We decided not to add a quadratic term of time into the models due to the worse model fitting performance based on the Bayesian information criterion. Random intercepts and random slopes of time were included in the models, which allowed the variability of HOMA levels at baseline and their rates of change between each study participant. The participant-specific baseline HOMA levels and participant-specific annualized slopes (rates of changes) were estimated and used as dependent variables in the next stage of analysis.

In stage 2 with the goal of handling multicollinearity among correlated exposure variables, AENET was used to select components of metal mixtures associated with baseline levels of HOMA measures and their rates of changes, respectively. Ordinary least squares (OLS)-based variable selection methods are commonly used but prone to over-fitting and does not work well in the presence of potentially high-dimensional predictors, or when predictors are

highly correlated (multicollinearity) (Tibshirani 1996). To combat this issue, elastic-net (ENET), as one of the shrinkage regression methods, has been introduced (Zou and Hastie 2005). ENET executes variable selection by shrinking coefficients of "unimportant" predictors towards exact zeroes, and has the ability to handle the complex correlations between predictor variables (Zou and Hastie 2005). Adaptive elastic net (AENET), as its name would suggest, is an adaptive version of ENET that not only deals with the collinearity problem over ENET but satisfies the asymptotic normality assumption that allows us to conduct statistical inference and hypothesis testing by providing large sample standard errors and p-values (Zou and Zhang 2009). It should be noted that AENET performs variable selection by shrinking certain coefficients to zero but not based on p-values of coefficients (like forward selection, backward elimination, and stepwise selection). In this study, AENET models were fitted as follows:

$$Y_i = \beta_0 + \sum_{k=1}^{15} \beta_k X_{ki} + \boldsymbol{\beta_z^T Z_i} + \varepsilon_i \ ,$$

where Y_i represents participant-specific baseline HOMA levels or participant-specific rates of changes in HOMA measures estimated from stage 1, X_{ki} denotes urinary concentration of k^{th} metal (we have a total of 15 metals in the initial model to be selected), and $\boldsymbol{Z_i}$ indicates the vector of confounders. Two AENET models were performed to select metals associated with (1) baseline levels of HOMAs, and (2) rates of changes in HOMAs, separately. Given the highly skewed distributions of all metal concentrations, logarithmic transformations were applied because the shapes of dose-response relationships were closer to be linear after log-transformation. To better compare the associations of different metals with HOMA measures, we further standardized the log-transformed urinary metal concentrations by subtracting the mean of the corresponding log-transformed concentrations divided by its standard deviation (SD). All the potential confounders, including age, race/ethnicity, study site, education level, annual household

income, BMI, smoking, alcohol drinking, physical activity score, menopausal status, hormone therapy, dietary intake of seafood and rice, total zinc intake from diets and supplements, total energy intake, and urinary specific gravity were always adjusted for ("forced") in the models. We adjusted for total zinc intake to better capture the potential effects of urinary zinc excretion that are independent of dietary zinc intake. For other essential metals, such as copper, no dietary intake was adjusted for due to lack of data. The AENET penalized parameters were ascertained based on 10-fold cross-validation for minimal prediction errors. The R package 'gcdnet' was used to implement AENET (Yi and Zou 2017).

To better summarize the combined effects of exposure to metal mixtures, we predicted HOMA-IR and HOMA-β at 0-16 years (follow-up intervals) using the coefficient estimates of AENET models for all metal concentrations fixed at their 25^{th}, 50^{th}, 75^{th} and 90^{th} percentiles, respectively, with all other covariates adjusted and results displayed graphically.

Hyperglycemia has been associated with increased urinary zinc excretion (Chausmer 1998). Because participants who had relatively high glucose levels at the SWAN-MPS baseline would already have elevated urinary zinc excretion, the reverse causation may bias the associations between urinary zinc concentration and HOMA measures. To examine the potential impact of this reverse causation on our results, we excluded participants with fasting glucose level ≥ 100 mg/dL (impaired fasting glucose) or HOMA-IR ≥ 4.2 (90th percentile) at SWAN-MPS baseline in the sensitivity analysis. All analyses were conducted by R, version 3.5.3 (www.R-project.org).

3. Results

Descriptive statistics

Characteristics of the study population at baseline are summarized in **Table IV. 1**. The median (interquartile range, IQR) age of the 1,262 participants was 49.6 (47.5, 51.7) years. Most women had at least some college education, had never smoked, and were pre-menopausal at baseline. Geometric means (geometric standard deviations) of HOMA-IR and HOMA-β were 2.1 (1.7) and 154.5 (1.6), respectively, at baseline. The distributions and detection rates of all 15 urinary metal concentrations were summarized in **Supplemental Table IV.1**. Most metals were modestly and positively correlated with each other (**Supplemental Figure IV.2**).

Metal mixtures and HOMA-IR

Table IV. 2 summarizes the associations of selected components of metal mixtures with baseline HOMA-IR and its rate of change in the AENET models. A total of 4 metals including copper, molybdenum, lead, and zinc were selected to be associated with baseline HOMA-IR out of 15 candidate predictors. The beta coefficients for all other metals were shrunk to zero. After multiple adjustment, 1-SD increase in log-transformed urinary metal concentration was associated with 1.57% (95%CI: -1.09%, 4.29%) higher baseline HOMA-IR level for copper, 0.70% (95%CI: -1.59%, 3.05%) higher level for lead, and 5.76% (95% CI: 3.05%, 8.55%) higher level for zinc. Urinary molybdenum concentration was inversely associated with baseline HOMA-IR (mean percent change in HOMA-IR for 1-SD increase in urinary molybdenum concentration = -3.25%, 95%CI: -5.45%, -1.00%). HOMA-IR climbed by 1.51% (95%CI: 1.41%, 1.61%) annually during the follow-up period. Urinary zinc concentration was associated with faster rate of increase in HOMA-IR, that 1-SD increase in urinary zinc concentration was associated with 0.06% (95%CI: -0.03%, 0.15%) increase in the annual rate of increase in HOMA-IR. Beta coefficients of selected non-zero predictors in AENET models are shown in **Supplemental Table IV.2**.

Predicted HOMA-IR levels over time based on the selected non-zero metal predictors in AENET models are shown in **Figure IV. 1**. A simultaneous increase in all metal concentrations was associated with higher HOMA-IR at baseline, as well as with a slightly greater increase over the follow-up period. At the SWAN-MPS baseline, the predicted HOMA-IR was 1.99 when metal concentrations were fixed at their 25th percentiles and 2.16 when concentrations were fixed at their 90th percentiles. At the end of follow-up, the predicted HOMA-IR increased to 2.55 when metal concentrations were fixed at their 25th percentiles and 2.80 when concentrations were fixed at their 90th percentiles.

Metal mixtures and HOMA-β

Table IV. 3 shows the associations of selected components of metal mixtures with baseline HOMA- β and its rate of change in the AENET models. After adjusting for all potential confounders, 1-SD increase in urinary metal concentration was associated with -1.59% (95%CI: -3.63%, 0.50%) lower baseline HOMA- β level for arsenic, and -2.66% (95%CI: -5.07%, -0.30%) lower level for zinc, respectively. In contrast, 1-SD increase in urinary cobalt concentration was associated with 2.22% (95%CI: -0.10%, 4.60%) higher HOMA- β at baseline. HOMA- β declined during the follow-up (-1.00% annually, 95%CI: -1.02%, -0.90%). Urinary arsenic concentration was associated with faster rate of decline in HOMA- β, that 1-SD increase in urinary arsenic concentration was associated with 0.02% more negative change (95%CI: -0.05%, 0%) in HOMA- β annually. Beta coefficients of selected non-zero predictors in AENET models are shown in **Supplemental Table IV. 3**.

Predicted HOMA- β levels over the follow-up period based on the selected non-zero metal predictors in AENET models are shown in **Figure IV. 2**. A higher exposure to metal mixtures was associated with lower HOMA- β at both baseline and the end of follow-up. The

predicted HOMA- β was 159.39 when metal concentrations were fixed at their 25th percentiles and 153.00 when concentrations were fixed at their 90th percentiles at the SWAN-MPS baseline. At the end of the study, the predicted HOMA- β was 135.81 when metal concentrations were fixed at their 25th percentiles and 129.41 when concentrations were fixed at their 90th percentiles.

Sensitivity analysis

In the sensitivity analysis, 186 women who had fasting glucose level ≥ 100 mg/dL or HOMA-IR ≥ 4.2 at the SWAN-MPS baseline were excluded. In this subpopulation, we still observed a positive association between urinary zinc concentration and HOMA-IR, that 1-SD increase in urinary zinc concentration was associated with 4.08% higher HOMA-IR at baseline and 0.08% increase in the annual rate of increase in HOMA-IR, respectively. Similarly, urinary zinc concentration was inversely associated with HOMA-β at baseline, that 1-SD increase in urinary zinc concentration was associated with 1.00% lower HOMA-β at baseline.

4. Discussion

In this study, we evaluated the associations between the concentrations of 15 metals in urine samples and HOMA-IR and HOMA-β in a prospective cohort of 1,262 women over 16 years of follow-up. Using the AENET with a two-stage modeling approach, we identified copper, lead, and zinc as components of metal mixtures in urine that were associated with higher HOMA-IR at baseline, whereas molybdenum was associated with lower HOMA-IR at baseline. Urinary zinc was also associated with a faster rate of increase in HOMA-IR over time. For HOMA- β, arsenic and zinc were identified as components of metal mixtures in urine that were associated with lower levels at baseline, whereas cobalt was associated with higher levels at baseline. Arsenic was also associated with a faster rate of decline in HOMA-β over time, though

the magnitude of the association was modest. An increase in all metal concentrations was predicted to have consistently higher HOMA-IR and lower HOMA-β across the 16 years of the study. These results suggest that metal mixtures may impact insulin resistance and β-cell dysfunction, which are the major players in the pathogenesis of T2DM.

To the best of our knowledge, this study is the first to evaluate the association of exposure to metal mixtures with insulin resistance and β-cell dysfunction. Existing epidemiologic evidence has suggested that metals with high degree of toxicity, particularly arsenic, play a role in dysregulated glucose metabolism, although the evidence is inconsistent (Grau-Perez et al. 2017; Park et al. 2016; Rhee et al. 2013). In this study, we found that in addition to arsenic, other metals including copper, cobalt, molybdenum, lead, and zinc may also play a role. Most previous studies focused only on "priority toxic metals" while other potentially important metals were not investigated. Additionally, all previous studies have not addressed exposure to metal mixtures. Given the fact that people are co-exposed to multiple metals, and given the high degree of correlations between urinary metal concentrations in SWAN participants (Wang et al. 2019b), differences between our mixture analysis and previous studies might be attributed to complex correlation structures among metals. Simultaneously incorporating several metals as predictors in regression models is prone to over-fitting, leading to a poor model performance and variance inflation with a large number of predictors, especially when predictors are highly correlated (Tibshirani 1996). The statistical approach we used here (AENET) has been shown to overcome these issues (Zou and Zhang 2009) and offers the ability to identify which components of metal mixtures are potentially exerting adverse effects (Wang et al. 2018, 2019c). Our mixture analysis also accounted confounding due to co-exposure to other metal components as previous studies suggested metals may interfere with each other

metabolically (López Alonso et al. 2004). Furthermore, if individual metals have relatively small effects but exposure to metal mixtures influence the body's response to insulin and/or insulin secretion, the metal components that truly disrupt these physiological functions may not be adequately captured by the conventional single-pollutant approach. Our findings suggest the importance of considering metal mixtures, rather than individual metals with known toxicities, in evaluation of associations between metal exposures and health outcomes in future studies.

While underlying mechanisms are still not well understood, there is biological plausibility for a role for metals in the disturbance of insulin's action and insulin secretion. We observed that arsenic was adversely associated with HOMA-β in our study. Arsenic is a well-known toxicant that can induce oxidative stress through reactive oxygen species generation. Experimental studies suggest that, in pancreas, arsenic may increase amyloid formation and apoptotic death/damage of pancreatic β cells through the generation of oxidative stress (Lu et al. 2011; Mukherjee et al. 2006; Yen et al. 2007). Arsenic has also been shown to disrupt glucose-stimulated insulin secretion through induction of oxidative stress (Kirkley et al. 2018) and endoplasmic reticulum stress (Wu et al. 2018), and through interference with calcium-mediated signaling required for insulin secretory granule exocytosis (Díaz-Villaseñor et al. 2008). Additionally, arsenic has been suggested to substitute phosphate and to interact with sulfhydryl groups, which could impair the production of energy and interfere with the ATP-dependent insulin secretion of β-cells (Petrick et al. 2001).

We observed a positive association between urinary lead and HOMA-IR at baseline. Bone lead stores accrued from cumulative environmental exposures for decades are the major endogenous source of lead (Wang et al. 2019a). Urinary lead adjusted for urine dilution has been found to closely reflect lead mobilized from the bone (Tsaih et al. 1999, 2001; Wang et al.

2019a). Given the fact that midlife women may experience an increased bone turnover rate (Hernandez-Avila et al. 2000; Tsaih et al. 2001), the observed association could be attributed to a greater mobilization of lead from bone into the circulation. Lead is another well-known inducer of oxidative stress, which has been suggested to play a role in the pathogenesis of diseases including T2DM (Kim et al. 2015). Lead is also thought to disrupt a variety of intracellular signaling pathways by interfering with calcium homeostasis and calcium cellular uptake, and modulating activity of protein kinase C (Kim et al. 2015), all potential biological mechanisms through which they could be related to dysregulated glucose transportation and greater insulin resistance.

Urinary zinc concentration was adversely associated with both HOMA-IR and HOMA- β. Zinc is an essential nutrient that is necessary for biochemical pathways and required by thousands of proteins for catalytic functions (Jansen et al. 2009). Humans rely on a daily intake of dietary zinc to maintain health and prevent disease, and zinc leaves the body in urine and feces (Roohani et al. 2013). Urinary concentration of zinc reflects excretion of zinc in the urine (Roohani et al. 2013). Zinc intake has been associated with a lower risk of T2DM in women (32,33). The average intake levels in our participants were greater than the recommended dietary allowance, which is 8 mg/day for women (34). In our study, we found a positive association between urinary zinc and HOMA-IR, and an inverse association between urinary zinc and HOMA- β, after adjustment for zinc intake from both diets and supplements, suggesting that women with excess zinc excreted in urine may be at elevated risk of insulin resistance and β-cell dysfunction regardless of the amount of zinc intake. Mechanistic studies found that, in pancreatic β cells, zinc was necessary for insulin synthesis, storage and secretion, and has accounted for the conformational integrity of insulin in its hexameric crystalline form (Jansen et al. 2009).

Excessive urinary excretion of zinc was found to lead to a loss of zinc in β-cells, which accounted for a reduced insulin secretion (Jansen et al. 2009). Certain zinc complexes showed insulin-mimetic effects including reducing hyperglycemia and increasing lipogenesis in animal models (Jansen et al. 2009). Zinc has also been shown to improve glucose transportation in peripheral tissues by improving binding of insulin to its receptor through enhancing tyrosine kinase phosphorylation (Jansen et al. 2009). Additionally, zinc is a structural part of antioxidant enzymes such as superoxide dismutase that could protect insulin and β-cells from being attacked by free radicals (Jansen et al. 2009). Despite this evidence, hyperglycemia, on the other hand, was suggested to interfere the active transportation of zinc back to renal cells, leading to a loss of this mineral in the urine (Chausmer 1998). This raised the possibility that the observed association could also be explained by the increased urinary excretion of zinc in women who already had relatively high glucose levels at baseline. However, in the sensitivity analysis after excluding women who had relatively high glucose levels at baseline, the findings of associations between urinary zinc and HOMA measures did not change, though effect estimate for HOMA-β was attenuated, diminishing the likelihood that reverse causation bias drove the observed results. Our most recent study also reported that a higher urinary zinc excretion was associated with increased risk of T2DM over 16 years of follow-up in the SWAN. The results of current analysis suggest that an elevated urinary excretion of zinc may increase risk of T2DM possibly through its adverse effect on insulin resistance.

The evidence of underlying biological mechanisms linking other metal exposures to insulin resistance and β-cell dysfunction is limited. We found positive association of urinary copper concentration with HOMA-IR. Copper is also an essential element that is needed for multiple biological functions (ATSDR 2004c). However, long-term exposure to excess copper

through environmental contamination has also shown to induce oxidative damages (Gaetke and Chow 2003). In a study of diabetic mice, the treatment of a copper chelating agent was found to reduce insulin resistance and ameliorate glucose intolerance (Tanaka et al. 2009). We found molybdenum concentration was inversely associated with HOMA-IR. A potential beneficial effect of molybdenum on insulin sensitivity is supported by an study of mice which showed the molybdenum treatment improved glucose tolerance, replenished glycogen stores, and corrected lipogenic enzyme gene expression (Tanju Özcelikay et al. 1996), likely through its insulin-like actions (Fillat et al. 1992). We observed a positive association between urinary cobalt concentration and HOMA-β. Limited evidence suggested that cobalt may improve the insulin secretion profiles through its antioxidative effects (Vasudevan and McNeill 2007).

Our findings of associations between multiple metals and markers of insulin resistance and β-cell dysfunction may have important public health implications given the widespread exposure to environmental chemicals and increasing global burden of T2DM. The mixture analysis we used here enables us to identify those metals that impact the glucose homeostasis but have never been captured in previous studies and reflects the reality that no people are exposed to only a single metal. Our study also added reference on the fact that metal exposures could act as upstream risk factors of cardio-metabolic diseases which are potentially modifiable (Ding et al. 2018; Wang et al. 2017).

The primary strength of our study is its utilization of a large prospective cohort with repeated HOMA measures over 16 years follow-up. The prospective design also minimized the possibility of reverse causation. Furthermore, we used an advanced statistical method, AENET, for the first time, to investigate the association between exposure to metal mixtures and

longitudinal HOMA measures while accounting for statistical challenges such as complex correlations underlying metal mixtures and confounding due to co-pollutants.

Several limitations should be considered as well. First, metals considered in the current study have various half-lives. Urinary concentrations of metals with short half-lives such as arsenic mainly reflect recent exposures (ATSDR 2007a) while metals such as cadmium has half-lives of years to decades and urinary cadmium concentration provides an index of cumulative cadmium exposure in humans (Suwazono et al. 2009). Information on the temporal variability of urinary metals concentrations, especially for those with short half-lives, is needed in future epidemiologic studies. Second, in our study only total arsenic concentrations were measured; arsenic metabolism data were not available. One recent prospective study found that urinary monomethylarsonate concentration was associated with higher HOMA-IR when either inorganic arsenic or dimethylarsinate concentration decreased (Grau-Perez et al. 2017). Additional measurements of arsenic metabolism will be critical to providing a better understanding of arsenic exposures and associated health risks in our future studies. Third, metal-metal interactions were not considered in the mixture analysis when important metal components were selected. Exposure to metal mixtures with complex exposure profile may have additive, synergistic or antagonistic effects on the same adverse outcome (Wang et al. 2018). Given our sample size, adding the pairwise linear interaction terms in the AENET model might lead to problems including smoothing out the magnitude of exposures' effects, missing important variables, selection of spurious interaction effects and inflation of false positive results, particularly in presence of nonlinear interactions (Narisetty et al. 2019). Least absolute shrinkage and selection operator (LASSO) penalized linear mixed effects model is another shrinkage regression method designed for analyzing high-dimensional longitudinal data (Groll and Tutz

2014). However, with correlated variables as predictors, LASSO tends to randomly select only one out of these correlated variables and ignore the others (Friedman et al. 2010). More updated statistical interaction identification methods that can also be used in high-dimensional longitudinal data analysis are needed for future studies potentially interested in the interactions between pollutant mixture components, which also plays an important role in environmental research.

Conclusion

In this prospective cohort study with 16 years of follow-up, our mixture analysis demonstrated that arsenic, cobalt, copper, molybdenum, lead, and zinc as components of metal mixtures in urine were associated with HOMA-IR and/or HOMA-β. An increase in all metal concentrations was also adversely associated with HOMA-IR and HOMA-β over time. Our findings provide evidence that exposure to metal mixtures may be exerting effects on insulin resistance and β-cell dysfunctions, which might be mechanisms by which metal exposures may lead to elevated T2DM risks. More studies are warranted to elucidate other mechanisms underlying the link between exposure to metal mixtures and diabetes in humans.

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Table IV. 1. Descriptive characteristics at the time of metal measurements.

Characteristics[a]	**Summary statistics**
Age, median (IQR), year	49.6 (47.5, 51.7)
Race/ethnicity, n (%)	
White	645 (51.1)
Black	254 (20.1)
Chinese	166 (13.2)
Japanese	197 (15.6)
Study site, n (%)	
Michigan	218 (17.3)
Boston	195 (15.5)
Oakland	290 (23.0)
Los Angeles	346 (27.4)
Pittsburgh	213 (16.9)
BMI, median (IQR), kg/m^2	25.9 (22.5, 31.1)
Education, n (%)	
High school or less	214 (17.0)
Some College	405 (32.1)
College and above	643 (51.0)
Household income, n (%)	
Less than $19,999	74 (5.9)
$20,000 to 49,999	328 (26.0)
$50,000 to 99,999	528 (41.8)
$100,000 or more	332 (26.3)
Smoking status, n (%)	
Never	802 (63.6)
Former	338 (26.8)
Current	122 (9.7)
Alcohol drinking, n (%)	
Less than once per month	655 (51.9)
Once per month	298 (23.6)
Twice or more per month	309 (24.5)
Physical activity score, median (IQR),	7.9 (6.6, 9.0)
Menopausal status, n (%)	
Pre-menopausal	874 (69.3)
Post-menopausal	191 (15.1)
Unknown[b]	197 (15.6)
Hormone therapy, n (%)	260 (20.6)
Total energy intake, median (IQR), kcal/d	1685 (1336, 2183)
Dietary seafood intake, median (IQR), times/week	1.5 (0.8, 2.5)
Dietary rice intake, median (IQR), times/week	2.0 (1.0, 5.5)
Total zinc intake, median (IQR), mg/day	11.1 (7.6, 20.8)
Fasting glucose level, GM(GSD), mg/dL	86.8 (1.1)
Fasting insulin level, GM(GSD), uIU/mL	9.5 (1.6)
HOMA-IR, GM(GSD)	2.1 (1.7)
HOMA-β, GM(GSD)	154.5 (1.6)

[a] IQR: interquartile range; GM: geometric mean; GSD: geometric standard deviation.

[b] Menopausal status unknown due to hormone therapy or hysterectomy.

Table IV. 2. The associations of selected metals with baseline HOMA insulin resistance (HOMA-IR) and its annualized rate of change in adaptive elastic-net (AENET) models.

Baseline HOMA-IR	**Selected metals in AENET[a]**	**Percentage change in HOMA-IR[b] at baseline for 1-SD increase in log-transformed urinary metal concentration[c] (95% CI)**
	Copper	1.57% (-1.09%, 4.29%)
	Molybdenum	-3.25% (-5.45%, -1.00%)
	Lead	0.70% (-1.59%, 3.05%)
	Zinc	5.76% (3.05%, 8.55%)
Annualized rate of change in HOMA-IR (average rate of change = 1.51%, 95% CI: 1.41%, 1.61%)	**Selected metals in AENET**	**Percentage change in annualized rate of change in HOMA-IR for 1-SD increase in log-transformed urinary metal concentration (95% CI)**
	Zinc	0.06% (-0.03%, 0.15%)

[a] AENET models were adjusted for age, race/ethnicity, study site, education level, annual household income, body mass index, smoking, alcohol drinking, physical activity score, menopausal status, hormone therapy, dietary intake of seafood and rice, total zinc intake from diets and supplements, total energy intake, and urinary specific gravity.
[b] HOMA-IR was log-transformed.
[c] All urinary metal concentrations were log-transformed and standardized.

Table IV. 3. The associations of selected metals with baseline HOMA β-cell function (HOMA-β) and its annualized rate of change in adaptive elastic-net (AENET) models.

Baseline HOMA- β	**Selected metals in AENET[a]**	**Percentage change in HOMA-β[b] at baseline for 1-SD increase in log-transformed urinary metal concentration[c] (95% CI)**
	Arsenic	-1.59% (-3.63%, 0.50%)
	Cobalt	2.22% (-0.10%, 4.60%)
	Zinc	-2.66% (-5.07%, -0.30%)
Annualized rate of change in HOMA- β (average rate of change = -1.00%, 95% CI: -1.02%, -0.90%)	**Selected metals in AENET**	**Percentage change in annualized rate of change in HOMA-β for 1-SD increase in log-transformed urinary metal concentration (95% CI)**
	Arsenic	-0.02% (-0.05%, 0%)

[a] AENET models were adjusted for age, race/ethnicity, study site, education level, annual household income, body mass index, smoking, alcohol drinking, physical activity score, menopausal status, hormone therapy, dietary intake of seafood and rice, total zinc intake from diets and supplements, total energy intake, and urinary specific gravity.
[b] HOMA-β was log-transformed.
[c] All urinary metal concentrations were log-transformed and standardized.

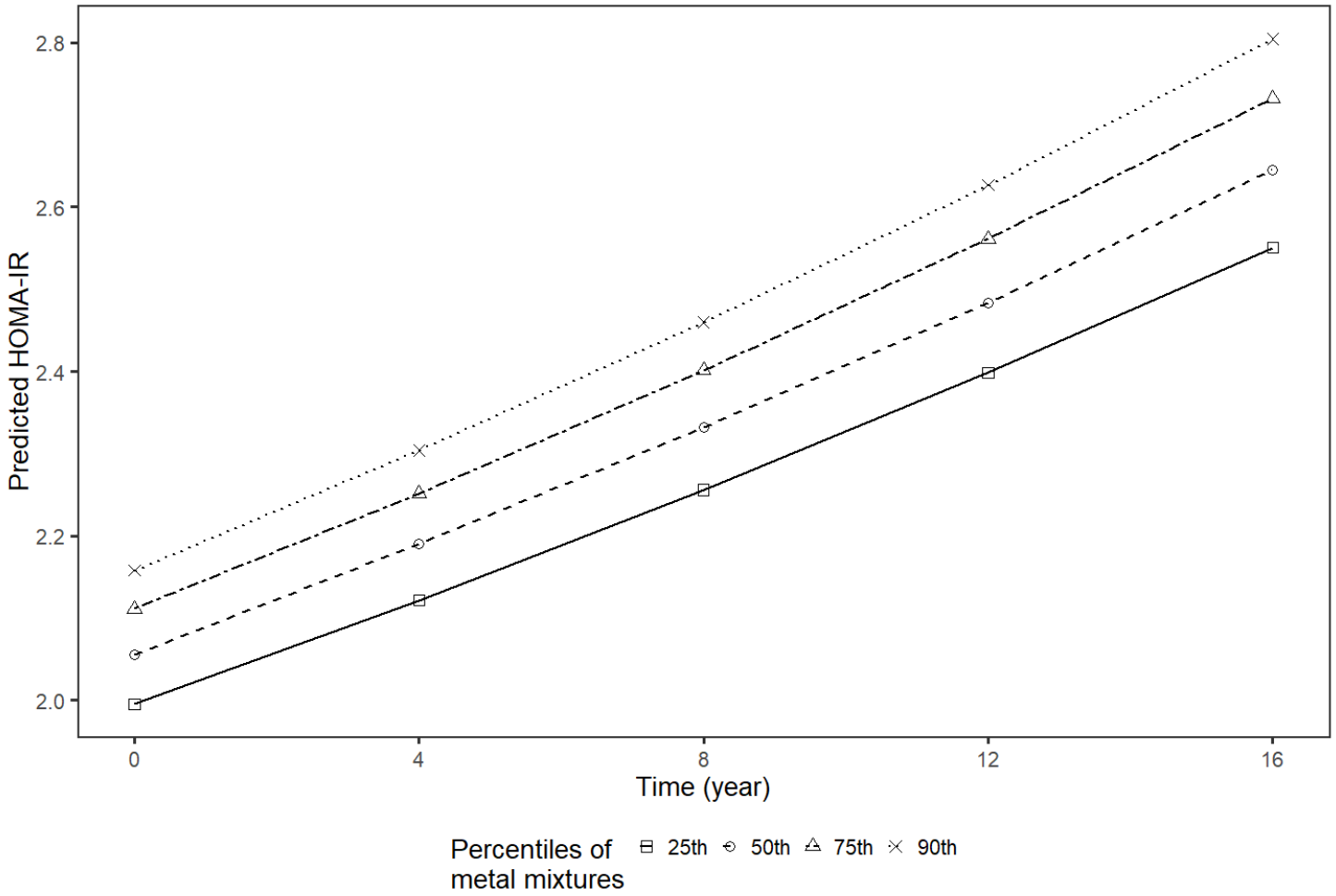


Figure IV. 1. Predicted HOMA-IR over time based on non-zero predictors in AENET models when all urinary metal concentrations are fixed at their 25th, 50th, 75th, and 90th percentile, respectively.
AENET models were adjusted for age, race/ethnicity, study site, education level, annual household income, body mass index, smoking, alcohol drinking, physical activity score, menopausal status, hormone therapy, dietary intake of seafood and rice, total zinc intake from diets and supplements, total energy intake, and urinary specific gravity.

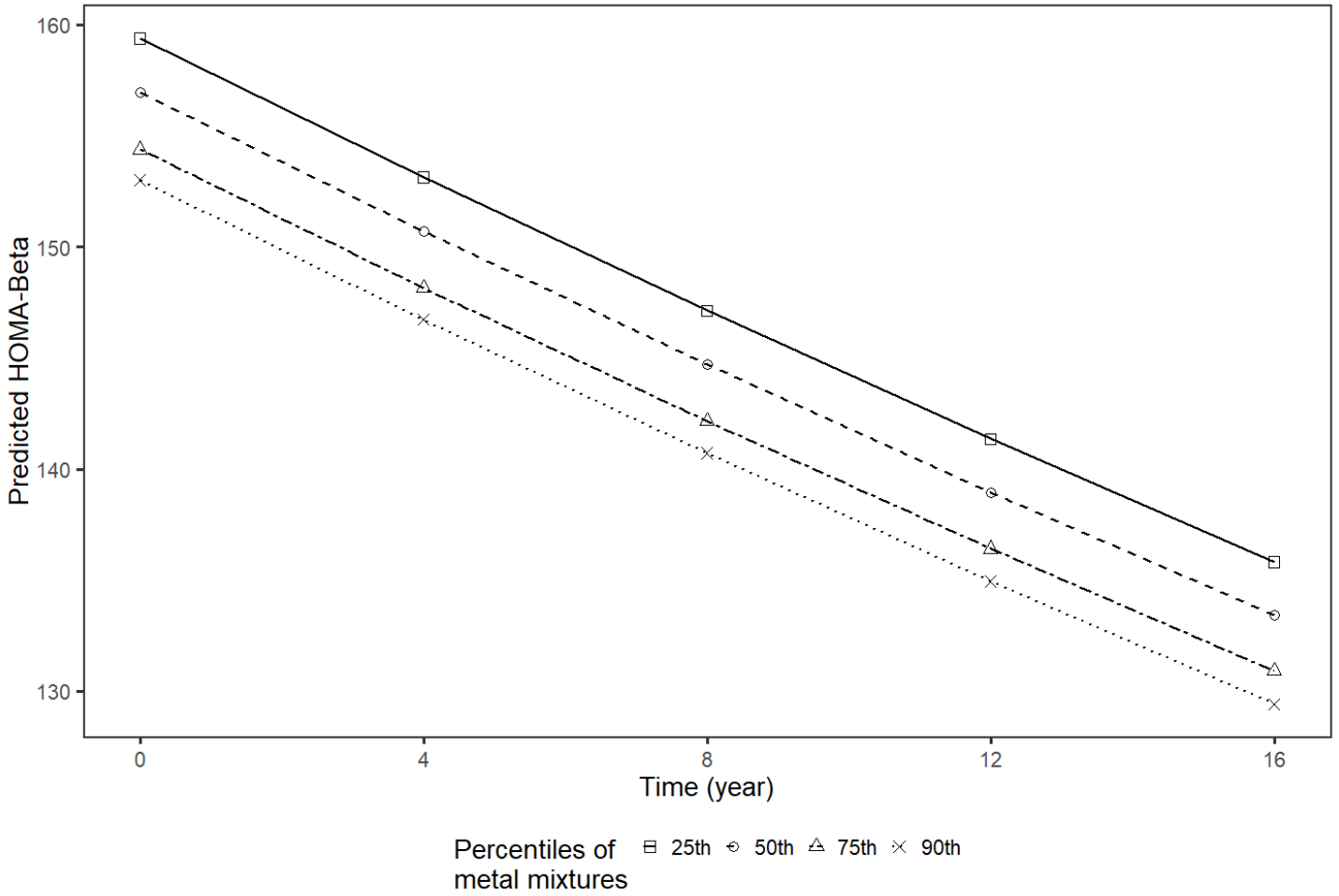


Figure IV. 2. Predicted HOMA-β over time based on non-zero predictors in AENET models when all urinary metal concentrations are fixed at their 25th, 50th, 75th, and 90th percentile, respectively.
AENET models were adjusted for age, race/ethnicity, study site, education level, annual household income, body mass index, smoking, alcohol drinking, physical activity score, menopausal status, hormone therapy, dietary intake of seafood and rice, total zinc intake from diets and supplements, total energy intake, and urinary specific gravity.

Supplemental Table IV. 1. Distribution of urinary metal concentrations in SWAN.

Metals (µg/L)	LOD[a]	% > LOD[b]	GM (GSD)[c]	Selected percentiles			
				25th	50th	75th	90th
Arsenic	0.3	100	17.43 (3.53)	7.00	15.23	40.66	95.55
Barium	0.1	99.5	1.71 (2.47)	0.99	1.77	2.95	4.86
Cadmium	0.06	94.6	0.41 (2.80)	0.23	0.44	0.81	1.43
Cobalt	0.05	99.3	0.61 (2.27)	0.38	0.62	0.96	1.70
Cesium	0.01	100	4.74 (1.99)	3.07	4.75	7.35	10.47
Copper	2.5	96.7	9.36 (2.03)	6.03	9.42	14.26	21.39
Mercury	0.05	99.8	1.21 (2.50)	0.67	1.23	2.37	3.68
Manganese	0.08	99.6	0.96 (2.20)	0.59	0.90	1.47	2.50
Molybdenum	0.3	100	42.18 (2.28)	25.19	44.20	72.69	114.78
Nickel	0.8	96.0	3.58 (2.09)	2.37	3.79	5.83	8.40
Lead	0.1	97.6	0.76 (2.34)	0.46	0.78	1.26	2.09
Antimony	0.04	78.9	0.08 (2.17)	0.05	0.07	0.13	0.21
Tin	0.1	96.9	0.97 (2.99)	0.49	0.93	1.77	3.52
Thallium	0.02	92.6	0.13 (2.56)	0.08	0.15	0.23	0.33
Zinc	2	100	272.44 (2.43)	162.81	300.43	507.32	777.14

[a] LOD: limit of detection.
[b] % > LOD: detection rate
[c] GM: geometric mean; GSD: geometric standard deviation.

Supplemental Table IV. 2. Selected non-zero beta coefficients of metals for baseline HOMA insulin resistance (HOMA-IR) and the annualized rate of change in adaptive elastic-net (AENET) models.

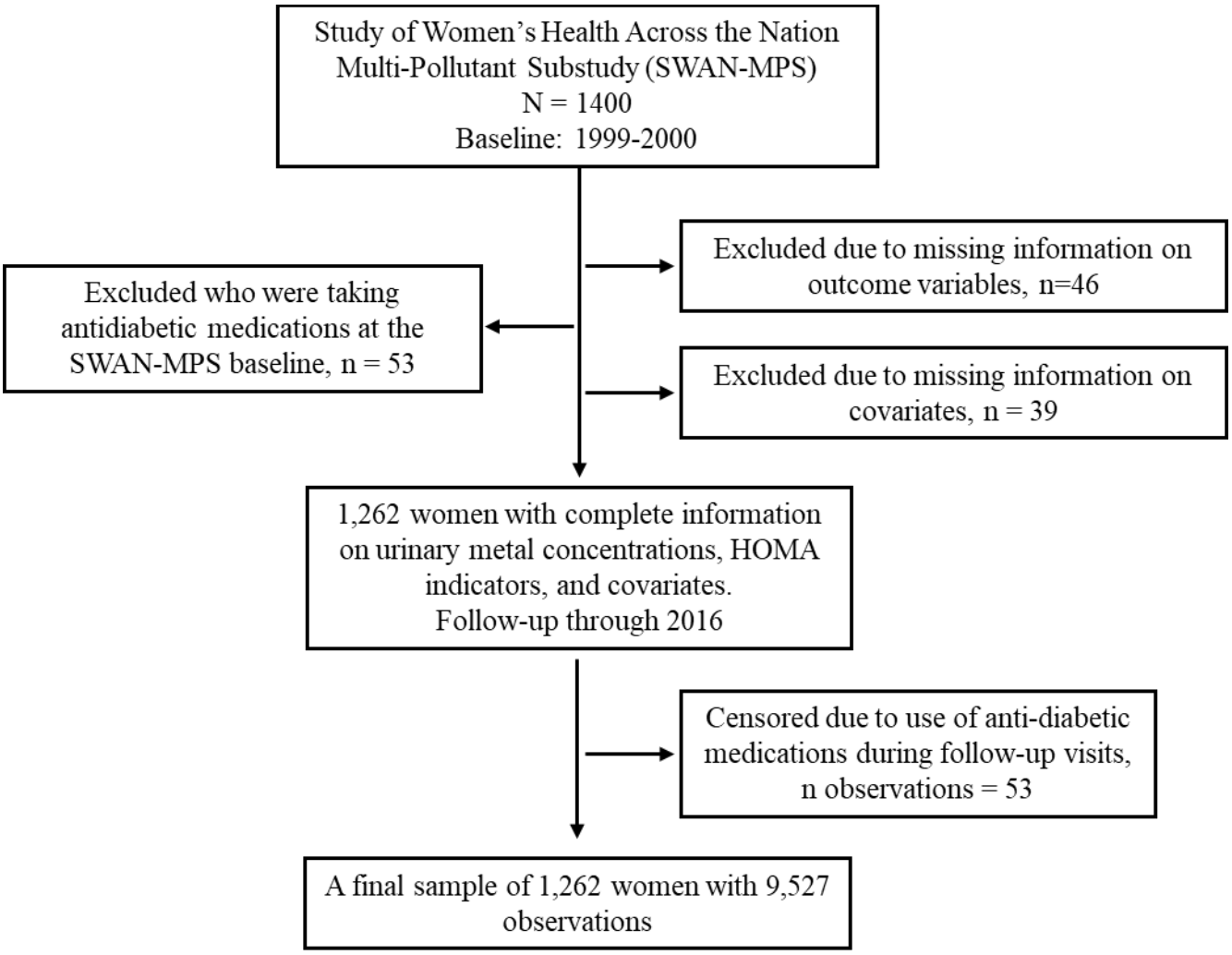
Baseline HOMA-IR[a]	**Selected metals in AENET[b]**	**β for 1-SD increase in log-transformed urinary metal concentration[c] (95% CI)**
	Copper	0.02 (-0.01, 0.04)
	Molybdenum	-0.03 (-0.06, -0.01)
	Lead	0.01 (-0.02, 0.03)
	Zinc	0.06 (0.03, 0.08)
Annualized rate of change in HOMA-IR	**Selected metals in AENET**	**β for 1-SD increase in log-transformed urinary metal concentration (95% CI)**
	Zinc	0.0006 (-0.0003, 0.0015)

[a] HOMA-IR was log-transformed.
[b] AENET models were adjusted for age, race/ethnicity, study site, education level, annual household income, body mass index, smoking, alcohol drinking, physical activity score, menopausal status, hormone therapy, dietary intake of seafood and rice, total zinc intake from diets and supplements, total energy intake, and urinary specific gravity.
[c] All urinary metal concentrations were log-transformed and standardized.

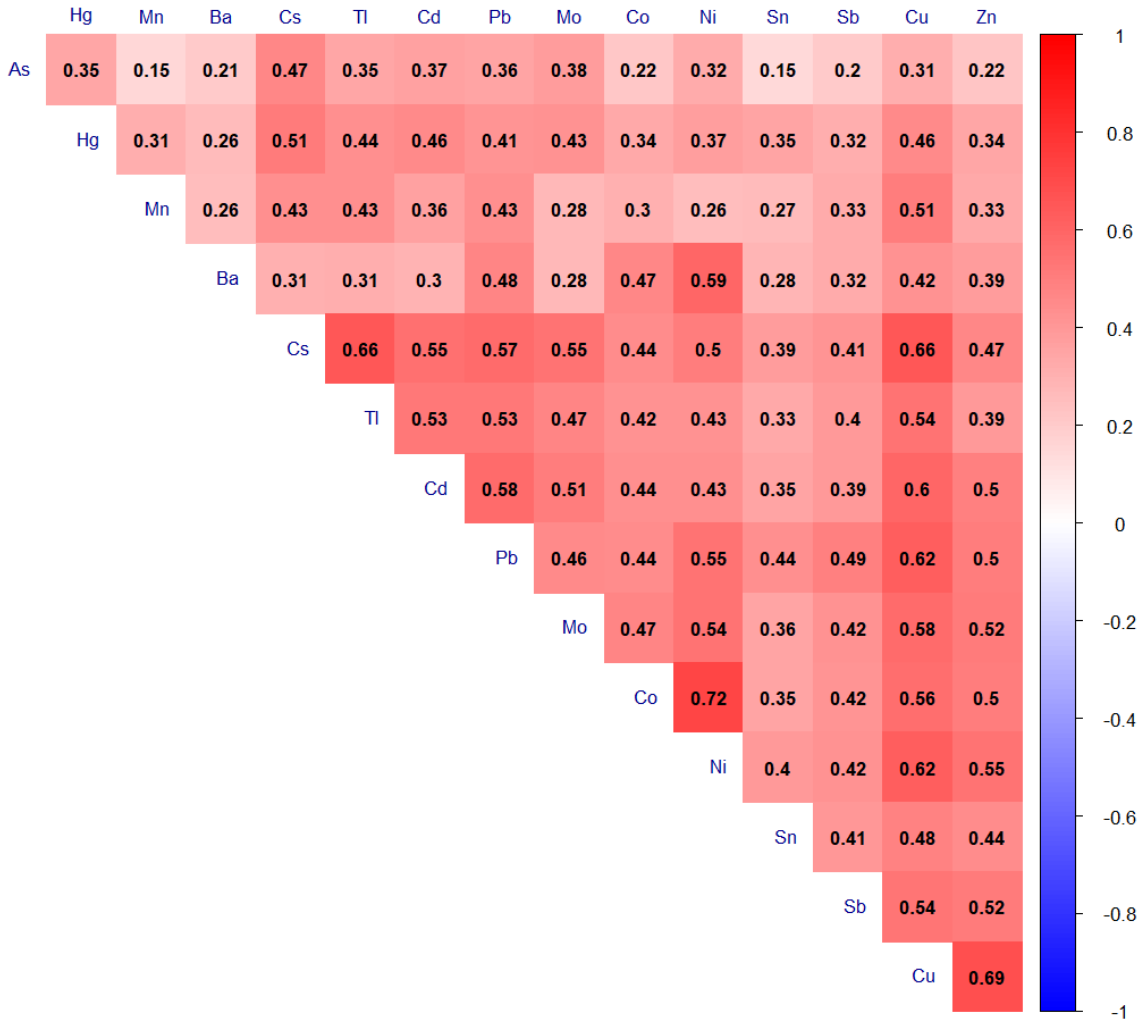
Supplemental Table IV. 3. Selected non-zero beta coefficients of metals for baseline HOMA β-cell function (HOMA- β) and the annualized rate of change in adaptive elastic-net (AENET) models.

Baseline HOMA- β[a]	**Selected metals in AENET[b]**	**β for 1-SD increase in log-transformed urinary metal concentration[c] (95% CI)**
	Arsenic	-0.02 (-0.04, 0.01)
	Cobalt	0.02 (-0.01, 0.04)
	Zinc	-0.03 (-0.05, -0.01)
Annualized rate of change in HOMA- β	**Selected metals in AENET**	**β for 1-SD increase in log-transformed urinary metal concentration (95% CI)**
	Arsenic	-0.0002 (-0.0005, 0)

[a] HOMA- β was log-transformed.
[b] AENET models were adjusted for age, race/ethnicity, study site, education level, annual household income, body mass index, smoking, alcohol drinking, physical activity score, menopausal status, hormone therapy, dietary intake of seafood and rice, total zinc intake from diets and supplements, total energy intake, and urinary specific gravity.
[c] All urinary metal concentrations were log-transformed and standardized.



Supplemental Figure IV. 1. Schematic diagram of analytic sample.



Supplemental Figure IV. 2. Spearman correlation matrix of metal concentrations.
As: arsenic, Ba: barium, Cd: cadmium, Co: cobalt, Cs: cesium, Cu: copper, Hg: mercury, Mn: manganese, Mo: molybdenum, Ni: nickel, Pb: lead, Sb: antimony, Sn: tin, Tl: thallium, Zn: zinc.

Chapter V. Conclusions

The etiology of type 2 diabetes mellitus is not fully understood and the roles of environmental exposures, specifically metals, in the pathogenesis of diabetes has received little attention by the medical community. Metals are widely dispersed in the environment, and the general population can be exposed to a myriad of metals through food, drinking water and ambient air throughout the lifetime. Metals such as arsenic, cadmium and lead, are well-known inducers of oxidative stress in a variety of tissues and cell types (Ercal et al. 2001). The accumulation of these metals in pancreatic islets is hypothesized to lead to impaired function and apoptotic death of β-cells via the induction of oxidative stress (Lu et al. 2011; Patra et al. 2011). On the other hand, essential metals such as zinc are necessary for insulin synthesis, storage and secretion in β-cells and have a preventative role in insulin resistance as certain zinc complexes showed insulin-like effects (Jansen et al. 2009). To date, however, only a few metals, most notably arsenic, have been studied with regard to diabetes risk and most studies have been conducted in predominantly cross-sectional settings. Thus little is known about the potential diabetogenic effects of a wide-range of metals. Further, no epidemiologic study has examined metal mixtures in relation to incidence of type 2 diabetes. This dissertation fills a critical gap in the literature by advancing our understanding of the potential role of multiple metals in the development of type 2 diabetes mellitus.

Summary of findings

This dissertation combined three studies that interrogated the distributions of urinary concentrations of multiple metals, the associations of metals with incidence of diabetes, and metal mixtures-related longitudinal changes in intermediate quantitative traits of diabetes in a cohort of midlife women using the data from the Study of Women's Health Across the Nation (SWAN).

Aim 1 evaluated concentrations of 21 metals in urine samples, identified overall exposure patterns, and examined demographic, socioeconomic, lifestyle, and dietary factors associated with both individual metals and metals exposure patterns. Significant race/ethnic differences were observed in the urinary concentrations of metals, i.e., significantly higher concentrations of arsenic, cadmium, copper, mercury, molybdenum, lead, and thallium in Asian populations including both Chinese and Japanese women, compared with other racial/ethnic groups. Seafood and rice intake were identified as important determinants of urinary arsenic, cesium, mercury, molybdenum and lead concentrations. This study also identified several other predictors of urinary metal concentrations: education level was inversely associated with cadmium and antimony concentrations but inversely associated with mercury concentration; cigarette smoking was positively associated concentrations of cadmium and lead; secondhand smoking also contributed to increased urinary lead concentration. A data-driven clustering approach—k-means clustering, was used to summarizing information of metal mixtures into distinct exposure patterns. Based on multiple restrict statistical criteria, two distinct overall exposure patterns- "high" vs. "low", were identified by k-means clustering, suggesting two distinct overall exposure patterns to metal mixtures. On average, women clustered to have the "high" overall exposure pattern had higher concentrations of all urinary metals compared to those clustered to the "low"

pattern. At the same time, those women in the "high" overall exposure pattern were more likely to be Asians, current smokers, and those with high intake of seafood and rice.

Aim 2 examined the associations between baseline urinary concentrations of metals and incidence of diabetes over 16 years of follow-up. After multivariable adjustment and controlling for multiple comparisons, high concentrations of toxic metals including arsenic and lead were positively associated with an increased risk of diabetes. An excessive urinary excretion of zinc, which is an essential metal, was also found to be significantly associated with elevated diabetes risk. In the mixture analysis, two exposure patterns of metal mixtures, i.e., "low" and "high" were identified, again, using the same k-means clustering. Women with high overall metal exposures had significantly higher incidence of diabetes compared to those with low exposure profiles.

Aim 3 further investigated associations between metal mixtures and longitudinal changes in insulin resistance and β-cell dysfunction, which are most important etiopathogenic mechanisms underlying the type 2 diabetes mellitus. Using the adaptive elastic-net (AENET), we identified copper, lead, and zinc as metals in urine that were associated with higher HOMA-IR at baseline, whereas molybdenum was associated with lower HOMA-IR at baseline. Urinary zinc was also associated with a faster rate of increase in HOMA-IR over time. For HOMA-β, arsenic and zinc were associated with lower levels at baseline, whereas cobalt was associated with higher levels at baseline. Arsenic was also associated with a faster rate of decline in HOMA-β over time, though the magnitude of the association was modest. An increase in all metal concentrations was predicted to have consistently higher HOMA-IR and lower HOMA-β across the 16 years of the study.

Integration of three specific aims

Across the three dissertation aims we observed evidence that people were exposed to many different metals. To improve our understanding of metal exposures, this dissertation began with describing distributions of a comprehensive list of 21 metals. Previous studies have reported racial/ethnical differences in lead exposure in the US (Hu et al. 1996; Theppeang et al. 2008). However, in Aim 1 several metals (a total of 13) were found to have concentrations that differed significantly between racial/ethnic groups. Additionally, both Chinese and Japanese women had higher concentrations of arsenic, cadmium, copper, mercury, molybdenum, lead, and thallium compared with other racial/ethnic groups. The most pronounced differences were for arsenic and cadmium. On average, concentrations of these two toxic metals in Asian women were about two times higher than those in white and black women. The racial/ethnic differences in metal concentrations persisted when the concentrations were compared between white and Chinese women within the Oakland site, and between white and Japanese women within the Los Angeles site, supporting the finding that Asian women may be experiencing higher exposures to many metals compared with other racial/ethnic groups, independent of geographic location of the SWAN recruitment. Some of the racial/ethnic differences may be attributed to diets, such as higher intakes of seafood and rice which was reported by Chinese and Japanese participants and was associated with higher concentrations of certain metals. These findings also suggested that race/ethnicity could act as an important confounder that should be considered in future research. Furthermore, our findings provide insight on exposure to multiple metals as mixtures. To the best of our knowledge, this is the first study to identify overall exposure patterns to metal mixtures in human populations. Understanding the exposure to metal mixtures is an important first step before evaluating their potential health effects (Aim 2 & Aim 3).

Aim 2 is the first study to examine the associations of exposure to multiple metals with incidence of diabetes in a well characterized prospective cohort study. In alignment with previous findings (Maull et al. 2012), we observed that urinary arsenic concentration was positively associated with incidence of diabetes. Given that the potential roles of environmental exposures to other metals in the development of diabetes were less well understood, this study provided valuable evidence about potential new diabetogenic risk factors of environmental origin. Urinary lead concentration was identified to be associated with increased diabetes risks. Additionally, urinary zinc concentration, which represents zinc loss in urine, was positively associated with diabetes risk. This association remained unchanged after further adjustment for zinc intake from diet and supplements. Because previous studies suggested that high glucose levels could interfere with the active transportation of zinc back to renal cells, therefore leading to a loss of this mineral in the urine. Thus the observed association between zinc and diabetes could be possibly explained by increased urinary excretion of zinc in women who already had relatively high glucose levels at baseline. Because of this, we conducted a sensitivity analysis excluding women with the highest fasting blood glucose values at baseline. Even after this sensitivity analysis, we still observed a positive association between urinary zinc and incident diabetes. Altogether, these findings suggest that an increased urinary zinc excretion may increase diabetes risk, independent of zinc intake. After its development, diabetes may, in turn, increase urinary zinc loss due to renal damages. This study also provided insight into how metal mixtures influence diabetes risk. Using the k-means clustering analysis, Aim 1 showed significant differences in sociodemographic, lifestyle, and dietary characteristics between women with different exposure profiles. Aim 2 further demonstrated a potential role for differences in exposure to metal mixtures in explaining health disparities as a significantly elevated risk of

diabetes was observed among women with high overall metal exposures. It should be acknowledged that there are uncertainties in cluster assignment in the k-means clustering. When these clusters (over exposure patterns) are used as predictor variables in the Cox regression in Aim 2, this uncertainty can be reproduced as measurement error in the predictor. Nondifferential misclassification of the exposure status commonly yields the regression parameters that are biased toward null, with accompanying variance estimates that are too small which inflates the type I error. Most recently Elliott et al. developed a joint modeling of the latent class (another clustering method) and the regression model using a fully Bayesian method to account for the uncertainty in the latent class analysis when it is treated as the predictor variable in regression analysis (Elliott et al. 2020). Future studies that use clustering methods as predictors of other health outcomes of interests should account for the uncertainties in cluster assignments. Additionally, it should be also acknowledged that the associations between the exposure to metal mixtures represented by k-means clusters and diabetes risk do not provide insight into which metals were responsible for these associations or allow for dose-response characterization. A more advanced statistical approach was used to address these issues in my Aim 3.

Aim 3 is the first study to evaluate the association of exposure to metal mixtures with longitudinal changes in insulin resistance and β-cell dysfunction. We used a two-stage modeling approach and employed a machine-learning based approach, AENET, which was proposed for analyzing high dimensional data while dealing with the collinearity problem (Zou and Zhang, 2009), to identify important metals associated with longitudinal changes in HOMA-IR and HOMA-β. AENET was used in this aim over the conventional ordinary least squares-based variable selection methods (forward selection, backward elimination, etc.) because the conventional methods could overfit in the presence of potentially high-dimensional predictors, or

when predictors are highly correlated (multicollinearity). Least absolute shrinkage and selection operator (LASSO) penalized linear mixed effects model is another shrinkage regression method designed for analyzing high-dimensional longitudinal data (Groll and Tutz 2014). However, when correlated variables or introduced as predictors, LASSO randomly selected only one out of these correlated metals and ignores the others, yielding potentially biased information. Aim 3 found that in addition to arsenic, for which a role in dysregulated glucose metabolism has been reported (Grau-Perez et al. 2017; Park et al. 2016; Rhee et al. 2013), other metals including copper, cobalt, molybdenum, lead, and zinc may also play a role. These differences are potentially due to the fact that most of the previous studies focused only on "priority toxic metals" while other potentially important metals were not investigated. Furthermore, if individual metals have relatively small effects but exposure to metal mixtures influences the body's response to insulin and/or insulin secretion, the metals that truly disrupt these physiological functions may not be adequately captured by the conventional single-pollutant approach. This study suggested the importance of considering metal mixtures, rather than individual metals with known toxicities, in evaluation of associations between metal exposures and health outcomes in future studies.

Future Research Questions

In combination with this dissertation's research findings, there are several additional research questions and recommendations for improvements in the design of future studies:

(1) **Arsenic speciation and metabolism:** This analysis found positive associations of urinary arsenic concentration with incidence of diabetes and longitudinal measures of HOMA-β. However, it should be acknowledged that in our study only total arsenic concentrations were

measured while arsenic speciation data were not available. Seafood intake is a major source of organic arsenic (Jones et al. 2016), which is generally considered to have low toxicity (Cullen and Reimer 1989). On the contrary, inorganic arsenic has been associated with adverse health outcomes such as cardiovascular disease, diabetes, and some cancers (Chen et al. 2013; Maull et al. 2012; Meliker et al. 2010; Steinmaus et al. 2014) and its major source are contaminated drinking water and rice intake (Gilbert-Diamond et al. 2011; Hughes et al. 2011). The toxicity of inorganic arsenic is also influenced by its metabolism. After absorption, inorganic arsenic is metabolized into mono- and di-methylated compounds (MMA and DMA) and the three arsenic forms are excreted in the urine (Aposhian and Aposhian 2006). An increased MMA% compared with DMA% in urine has been associated with several diseases including skin lesions, cardiovascular disease, skin cancer, and bladder cancer (Ruiz-Hernandez et al. 2015). In contrast, a lower MMA% compared with higher DMA% in urine has been related to diabetes risk and insulin resistance in a large prospective study (Grau-Perez et al. 2017; Kuo et al. 2015). In future studies, arsenic speciation and its metabolism data will be critical to providing a better understanding of arsenic exposures and their associations with different health outcomes.

(2) **Expanded coverage of environmental pollutants**: In our study, only associations between metal exposures and diabetes risk was evaluated. Recent biomonitoring studies suggest that there are a broader range of environmental pollutants, such as brominated flame retardants (Calafat et al. 2007), per- and polyfluoroalkyl substances (PFAS) (Calafat et al. 2007), and air pollution (Franklin et al. 2007), detected in most of the general US population, but their risks related to diabetes in humans are less well understood. The biomarkers of multiple environmental pollutants (PFASs, polychlorinated biphenyls, organochlorine pesticides, and polybrominated diphenyl ethers in serum; metals, phenols, phthalates, organophosphate

pesticides in urine) are available in the SWAN. Future studies using the prospective cohort design systematically evaluating associations between environmental pollutants and diabetes will contribute significantly to the understanding of modifiable type 2 diabetes risk factors with environmental origins and provide new insights into disease etiology and progression.

(3) **Expanded coverage of intermediate quantitative traits and mediation analysis:** The Aim 2 and Aim 3 of current dissertation provide evidence that exposure to metal mixtures may be exerting effects on insulin resistance and β-cell dysfunction, which might be mechanisms by which metal exposures may lead to elevated diabetes risks. However, the impacts of environmental exposures on other pathophysiological pathways, including dyslipidemia (Taskinen and Borén 2015), alterations in body composition (Gómez-Ambrosi et al. 2011; Hong et al. 2017), and inflammation (Hou et al. 2013; Masters et al. 2010), were not examined in the present dissertation and epidemiologic evidence is also lacking. For example, metals have been associated with body mass index, waist circumference, and total body fat (Wang et al. 2018). However, little is known about whether these metals influence diabetes risk through their effects on changes in obesity and body composition over the menopausal transition. Changes associated with the menopause transition, in particular loss of ovarian function and subsequent hormonal changes have been associated with an increased risk of diabetes (Ding et al. 2009). In addition to hormone changes, women going through the menopausal transition may also experience deleterious changes in fat mass and lean mass (Greendale et al. 2019). An increase in total body fat, preferentially of central or visceral adipose deposition may increase diabetes risk by decreasing tissue insulin sensitivity and glucose tolerance (Golay and Ybarra 2005). A decrease in skeletal muscle mass has also been associated with elevated diabetes risk, likely through a reduced capacity for glucose uptake from the blood (Hong et al. 2017). However, the

contribution of changes of obesity status or body composition due to metals exposure during the menopause transition to elevated diabetes risks still remains unclear. Metabolic outcomes including body composition and adiposity (measured by dual-energy X-ray absorptiometry), lipid profiles, and adipokines and inflammatory cytokines have been measured repeatedly in the SWAN. More studies are worth being conducted in the future (1) to evaluate whether changes in body composition, lipid profiles, inflammatory markers are associated with incidence of diabetes over the menopausal transition; and (2) to examine how exposures to multiple metals affect changes in these longitudinal traits. Finally, a mediation study would be warranted to examine causal mediation of the relationship between metal exposures and diabetes risk by different mechanisms pathways. A mediation analysis would help us better understand how metal exposures affect diabetes risk and inform potential interventions limiting the effects of exposure by intervening on intermediates.

(4) **Environmental risk score and risk prediction:** Type 2 diabetes mellitus is a major public health problem in the US as well as globally. Accurate assessment of diabetes risk is an essential step toward disease prevention and an important public health goal. Scoring algorithms such as the Cambridge Diabetes Risk Score, which uses general practice record data, for example age, sex, body mass index, history of antihypertensive or steroid medication, family history and smoking history, have been developed for diabetes risk assessment in the general population (Griffin et al. 2000). However, the accuracy of such algorithms has been questioned. Attempts to improve risk prediction algorithms have been made by incorporating additional risk factors, including novel biomarkers, nutrition measures, and genetic variations (Meigs et al. 2008; Sattar et al. 2008). However, the incremental information with regard to the risk prediction added by those risk markers beyond that of the conventional risk factors were mostly small or inconclusive

(Meigs et al. 2008; Sattar et al. 2008). This dissertation has provided foundational evidence that environmental exposures to metal may play a role in the pathogenesis of diabetes. Future studies might evaluate whether environmental factors could serve as a screening tool for predicting future diabetes, and hence play a part in its prevention.

In our recent analysis of National Health and Nutrition Examination Survey data, we examined whether blood markers of lead, cadmium, and mercury can improve prediction for cardiovascular (CVD) mortality (Wang et al. 2019c). We constructed an Environmental Risk Score (ERS) using machine learning algorithms as an integrated measures of CVD risk due to heavy metal exposures. We observed a significant improvement in risk prediction for CVD mortality when the ERS was incorporated into a model with established risk factors including age, gender, current smoking status, systolic blood pressure, total cholesterol level, high-density lipoprotein cholesterol level, diabetes, and body mass index (equivalent to the Framingham risk score or the Atherosclerotic CVD (ASCVD) risk estimator). By taking advantage of the rich longitudinal features and biomarkers of environmental pollutants in SWAN and using the same ERS approach these data could be replicated. Future studies are needed to examine whether the information on multiple environmental exposures which are potentially associated with incidence of diabetes improve the prediction for risk of diabetes in addition to the conventional risk factors/scores.

Public Health Implications

Environmental exposure to metals has been a prevalent and persistent public health problem. In recent years, the public health importance of metal exposures has attracted intense

interest due to large-scale lead exposure events, as in Flint, Michigan, with poisoning due to lead in the drinking water. In 2004, the U.S. Environmental Protection Agency (EPA) reported that more than 3% of drinking water distribution systems servicing more than 3300 people in the US exceeded the lead level of 15 μg/L defined by the Lead and Copper Rule (LCR) (Brown et al. 2012). Lead exposure may not be the only concern. Depending on the exposure levels, many different metals have been demonstrated to cause tissue damages and show adverse health impacts, including cardiovascular diseases, kidney diseases, metabolic diseases, neurocognitive outcomes, some cancers, and mortality (Jaishankar et al. 2014). Investigations of the observed impacts of low-level exposures to metals (e.g. 5 ug/dL for blood lead levels) are also vital because some metals may have no safe exposure threshold (Jaishankar et al. 2014).

Given the adverse health effects stemming from metal exposures, biomonitoring studies at the population level are important to provide a complete picture of the concentrations of multiple metals actually found in human bodies. Identification of the determinants of metal exposures is also critical because it can help us identify highly exposed subpopulations, their potential exposure sources and possible avenues for intervention. In our study, Asian women were found to experience the highest exposure to multiple toxic metals compared with other racial/ethnic groups. Seafood and rice intake, of which the highest consumption was reported by Asians, were found to be important exposure sources . Racial/ethnic-specific recommendations for reducing main sources of exposure such as dietary sources may be warranted to help mitigate the body burden of the toxic metals and subsequently health risks.

Following the Clean Water Act of 1972 and the Safe Drinking Water of 1974, EPA has regulated lead contamination in tap water. However, no toxic threshold has been identified in lead levels in humans because research suggests that no lead level is a “safe” level (Jaishankar et

al. 2014). The U.S. Food and Drug Administration (FDA) has not established regulatory limits for heavy metals in finished foodstuffs other than bottled water (FDA 2020). However, the Agency has provided guidance on lead levels in juice not exceeding 50 ppb and a maximum lead level of 100 ppb in candy (FDA 2020). To date, 31 states have adopted 97 policies on some toxic metals including arsenic, cadmium, lead or mercury in food and consumer products (http://www.saferstates.com/toxic-chemicals). However, are the current maximum contaminant levels and maximum contaminant level goals of metals low enough to protect people against their adverse health effects given a lack of updated epidemiologic findings? Recent years have witnessed an increase in our understanding of toxicological issues and bioavailability of these metals. This dissertation suggesting potential effects of metals on diabetes also continues to aid our ability to better focus on the toxicological impact of different metals on human health. It provides for sound, evidence-based legislation and will form an important component of any policy in this area.

Few studies have evaluated exposures to metals in midlife women, who are at increased risk of chronic diseases such as cardio-metabolic disorders, due to permanent changes in ovarian function (Davis et al. 2012b; Kim 2012; Polotsky and Polotsky 2010; Stuenkel 2017). Exposure to metals during this window of susceptibility may increase the risk of adverse health consequences associated with ovarian aging. For example, menopause has been suggested to play an important role in the mobilization of lead from bone into the circulation due to an increased bone turnover rate (Hernandez-Avila et al. 2000; Tsaih et al. 2001). Bone lead stores accrued from cumulative environmental exposures for decades are the major endogenous source of lead (Tsaih et al. 2001). Urinary lead adjusted for urine dilution has been found to closely reflect lead mobilized from the bone (Tsaih et al., 2001, 1999; Wang et al., 2019a). In Aim 2 and

Aim 3 of this dissertation, urinary lead was positively associated with HOMA-IR and risk of diabetes, which can be possibly attributed to a greater release of lead from bone stores during menopausal transition. This highlights evidence that reduction in metal exposure before midlife is critical, however, the current health reference guidelines only focuses on childhood exposure and exposure during pregnancy (CDC 2012a). As stated earlier in the list of future research questions, this dissertation also provides impetus for future investigators to find out whether metal exposures may induce unfavorable changes in hormone profiles, lipid metabolisms, adiposity and body composition, and inflammatory cytokines during the menopausal transition. Environmental Health Community should be aware of midlife as an important window of susceptibility and a reduction in exposure could reduce the disease burden in later life.

Diabetes is a significant public health issue with 34.1 million adults (13.0% of the population aged 20 years and older) in US estimated to have the disease (CDC 2020). Diabetes itself also constitutes a health risk as diabetes patients are at a higher risk of developing serious adverse health outcomes including cardiovascular disease, eye disease, neuropathy, renal failure, and mortality (DeFronzo et al. 2015). Therefore, a better understanding of the factors associated with the development of diabetes is of substantial public health importance. Most studies of diabetes to date have focused on the roles of unhealthy diet and lifestyle factors, and numerous clinical trials have definitively shown that the lifestyle can modify the development of diabetes (Franz et al. 2015). However, it should be acknowledged that diabetes has still been developed among many individuals who had made lifestyles changes including diet changes and weight losing (Franz et al. 2015). Given the dramatic increase in diabetes risks over the last two decades, there must be some factors beyond behavior and lifestyle contributing to the rise. Updating evidence suggests that in addition to unhealthy diet intake, diabetes is also primarily

attributed to obesity, tobacco smoking, and exposure to air pollution in the US (https://vizhub.healthdata.org/gbd-compare/). By evaluation of associations between environmental exposure to multiple metals and diabetes risk in a large prospective cohort study (Aim 2), our findings support the possibility that metals may also be important risk factors for diabetes.

Current diabetes intervention in clinical setting is primarily through treatments in an attempt to modify the elevated clinical indices. Our findings also highlight the importance of exploring novel approaches through interventions on underlying environmental toxicants in our human bodies. A recent prospective cohort study of middle-aged to elderly men reported that a 'prudent' dietary pattern, characterized by high intake of fruit, vegetables, legumes, tomatoes, poultry, and seafood, might reduce the risk of development of coronary heart disease in relation to bone lead, suggesting that benefits from dietary interventions on cardiovascular disease could be achieved by shifting to diets with a combination of natural antagonists to metals' toxicity (Ding et al. 2018; Wang et al. 2017). Toxic metal chelation has also been proposed recently as secondary prevention of atherosclerotic disease by mobilizing lead and cadmium from their chronic tissue storage compartments and facilitating their excretion from human bodies (Aneni et al. 2016; Lamas et al. 2016; Waters et al. 2001). The feasibility and effectiveness of these intervention approaches for reducing diabetes risk are worth investigating.

On the other hand, the findings that urinary zinc was adversely associated with diabetes outcomes in both Aim 2 and Aim 3 highlight zinc loss as a risk factor for diabetes. Previous cross-sectional studies have observed an association between reduced zinc status and diabetes and this association has been largely explained by loss of zinc through the kidneys due to diabetic nephropathy (Chausmer 1998). The current study leveraging a prospective cohort design

adds lines of evidence support a more direct causative role of zinc loss in the pathogenesis of diabetes. The most intuitive way to mitigate the risk of diabetes due to zinc loss is to increase zinc intake from food or supplements. Given the average intake levels in our participants have reached the recommended dietary allowance (RDA), which is 8 mg/day for women, our findings provide informative results for future investigators to further consider urinary zinc loss in evaluation of zinc supplementation for the prevention of diabetes, and to determine if the current RDA level is high enough to protect people from developing diabetes. Additionally, urinary zinc loss may be a factor as important as zinc intake that affects diabetes risks. Future studies should fill these gaps concerning determinants of urinary zinc loss and the feasibility and effectiveness of interventions for reducing zinc loss in diabetes prevention.

Overall Conclusions

This dissertation fills a critical gap in the literature by advancing our understanding of environmental exposures to metals and their potential roles in the development of type 2 diabetes mellitus. The associations characterized in this dissertation build a foundation for future mediation studies. These associations are also informative for future studies evaluating clinical values of metal biomarkers in diabetes risk assessment and prevention. By capturing exposure profiles to metal mixtures and examining their effects on diabetes and its intermediate traits, this dissertation also highlights the importance for quantifying the impact of exposure to pollutant mixtures for better understanding the relationship between environmental risk factors and diabetes risk. Each aim contains unique contributions to the literature. Aim 1 is the first study to evaluate exposure to multiple metals of environmental origins and identify overall exposure patterns in human populations. Aim 2 is the first to examine the associations of exposure to a

comprehensive suite of metals with incidence of diabetes in a prospective cohort study. Finally, Aim 3 is the first to apply mixture analysis approach to evaluate the association of exposure to metal mixtures with longitudinal measures of insulin resistance and β-cell dysfunction. Altogether, this dissertation advances our understanding of environmental metal exposures and provides novel points of view for the exploration of the pathogenesis of type 2 diabetes mellitus.

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