The Role of Dietary and Environmental Metals

in Cardiometabolic Health

BY

CATHERINE MARIE BULKA
B.A., Emory University, 2010
M.P.H., Emory University, 2012

THESIS

Submitted as partial fulfillment of the requirements
for the degree of Doctor of Philosophy in Public Health Sciences
in the Graduate College of the
University of Illinois at Chicago, 2018

Chicago, Illinois

Defense Committee:

Maria Argos, Chair and Advisor
Victoria W. Persky
Martha L. Daviglus, Medicine
Ramon A. Durazo-Arvizu, Loyola University
Daniela Sotres-Alvarez, University of North Carolina

This thesis is dedicated to my husband, Jacob Kilburn Kresovich, whose love and support have made me a better scientist, and more importantly a better person.

ACKNOWLEDGEMENTS

My research would not have been possible without the enthusiasm, knowledge, and generosity of my committee members—Drs. Maria Argos, Victoria Persky, Martha Daviglus, Ramon Durazo-Arvizu, and Daniela Sotres-Alvarez—and I am tremendously grateful for their guidance and support. In particular, Dr. Maria Argos has been an exceptional mentor to me. Her commitment to the field of environmental epidemiology and her dedication to conducting high-quality research have made a deep impression on me. I also wish to thank the staff and participants of the Hispanic Community Health Study/Study of Latinos, National Health and Nutrition Examination Survey, and Bangladesh Vitamin E and Selenium Trial.

CMB

TABLE OF CONTENTS

CHAPTER **PAGE**

TABLE OF CONTENTS (CONTINUED)

CHAPTER **PAGE**

LIST OF TABLES

LIST OF TABLES (CONTINUED)

LIST OF TABLES (CONTINUED)

LIST OF TABLES (CONTINUED)

LIST OF FIGURES

FIGURE **PAGE**

LIST OF FIGURES (CONTINUED)

FIGURE **PAGE**

LIST OF ABBREVIATIONS

As	Arsenic
BEST	Bangladesh Vitamin E and Selenium Trial
BMI	Body mass index
C	Celsius
Cd	Cadmium
CI	Confidence interval
Cu	Copper
dL	Deciliter
DRC	Dynamic reaction cell
GED	General Equivalency Diploma
GPAQ	Global Physical Activity Questionnaire
GPx	Glutathione peroxidase
HbA1c	Glycated hemoglobin
HCHS/SOL	Hispanic Community Health Study/Study of Latinos
HDL	High density lipoprotein
Hg	Mercury
HR	Hazard ratio
ICP	Inductively coupled plasma mass spectrometry
IQR	Interquartile range
IRR	Incidence rate ratio
L	Liter

LIST OF ABBREVIATIONS (CONTINUED)

LOD	Limit of detection
MT2A	Metallothionein 2A
MDDB	Master Drug Data Base
mL	Milliliter
mmHg	Millimeters of mercury
Mn	Manganese
MnSOD	Manganese superoxide dismutase
NCC	Nutrition Coordinating Center
NCEH	National Center for Environmental Health
NCI	National Cancer Institute
NIH	National Institutes of Health
NDSR	Nutrition Data Systems for Research
NHANES	National Health and Nutrition Examination Survey
Pb	Lead
PCA	Principal component analysis
PR	Prevalence ratio
PTP1B	Protein tyrosine phosphatase 1B
Q1	Quartile 1
Q4	Quartile 4
Se	Selenium
U.S.	United States

LIST OF ABBREVIATIONS (CONTINUED)

USDA	United States Department of Agriculture
Zn	Zinc
μg	Micrograms

SUMMARY

Metals are naturally present throughout the soil, air, water, and food supply, but can also occur as pollutants. This dissertation aimed to examine the influence of essential and toxic metal exposures on the development of metabolic syndrome, a constellation of cardiometabolic abnormalities. Metabolic syndrome and its components are associated with a considerable burden of morbidity and mortality, but the contribution of metals from environmental sources has not been comprehensively evaluated in spite of emerging animal and experimental data. Since exposures to metals are modifiable, the findings of this work could inform future strategies for preventing metabolic syndrome, and more distally diabetes and cardiovascular disease.

Using dietary data (including supplement use) collected by the Hispanic Community Health Study/Study of Latinos, we assessed long-term intakes of four metals that act as nutritionally essential minerals—copper, manganese, selenium, and zinc—with both prevalent and incident metabolic syndrome. We found that greater intakes of manganese and zinc from combined sources (food, beverages, and dietary supplements) were associated with a lower prevalence of metabolic syndrome, low HDL cholesterol, and abdominal obesity in diverse Hispanic and Latino adults. These associations, however, did not persist in preliminary prospective data. Instead, we observed greater total intakes of copper were associated with a reduced risk of developing high blood pressure, greater total manganese with reduced risks of high fasting glucose, greater total zinc with reduced risks of high blood pressure and high fasting glucose, and greater total selenium with an increased risk of dyslipidemia. It remains unclear whether these relationships are primarily driven by mineral intakes from foods and beverages or from supplementation. About 1 in 5 Hispanic/Latino adults use dietary supplements, and all individuals consume minerals daily through food and beverages. Thus, it is important to improve the current state of knowledge regarding the role of minerals as risk or protective factors in adverse cardiometabolic health.

In an analysis of data from the 2011-2014 National Health and Nutrition Examination Survey, we evaluated co-exposures to toxic (arsenic, cadmium, mercury, and lead) and essential (copper,

manganese, selenium, and zinc) metal exposures, as measured in urine, whole blood, and serum samples, with metabolic syndrome prevalence among the general U.S. adult population. We observed positive cross-sectional associations of arsenic-inorganic/elemental mercury and selenium-zinc patterns of exposure with metabolic syndrome while cadmium-lead co-exposures were inversely related. While arsenic has previously been shown to increase blood pressure, we identified novel associations with low HDL ("good") cholesterol and high triglycerides. The observed association of greater cadmium-lead exposures with a lower prevalence of low HDL cholesterol, high triglycerides, and abdominal obesity was also novel and intriguing. Increasing selenium-zinc exposures were specifically related to a greater likelihood of having high triglycerides, a finding consistent with our observation that selenium intakes from food, beverages, and supplements appeared to increase the risk of dyslipidemia among diverse Hispanics/Latinos in the Hispanic Community Health Study/Study of Latinos.

Finally, we analyzed whole blood concentrations of the essential metals manganese and zinc and the toxic metal lead in relation to longitudinal changes in blood pressure within a cohort of Bangladeshi adults enrolled in the Bangladesh Vitamin E and Selenium Trial. We found that individuals with manganese exposures within a specific range (8.2-12.4 μg/L) and selenium exposures above a certain threshold (>136 μg/L) had reductions in blood pressure over a 6-year period. Lead exposures, on the other hand, were monotonically associated with increases in systolic blood pressures per annum. Together these results suggest both essential and toxic metals may play an important role in cardiometabolic health at the population-level, but these relationships likely differ by exposure levels and geography.

I. INTRODUCTION

Globally and within the United States (U.S.), cardiovascular disease and type 2 diabetes are leading causes of morbidity and mortality. Risk factors for these diseases include high blood pressure, high glucose levels, abdominal obesity, low high-density lipoprotein (HDL) cholesterol levels, and high triglyceride levels, which tend to cluster together and are known collectively as metabolic syndrome. Despite advances in prevention and treatment of the individual components, metabolic syndrome is common and is currently estimated to affect over 1/3 of the adult U.S. population.(1) While physical inactivity, diet, and other health behaviors play a role, there are likely other factors involved.(2) To date, relatively little attention has been paid to environmental chemical exposures as non-traditional risk factors for cardiometabolic disease.

Recent data suggest that exposures to some metals and metalloids (hereafter referred to collectively as "metals"), especially to arsenic, cadmium, mercury, and lead, are associated with increased oxidative stress and inflammation, which may be related to a greater propensity for diabetes and cardiovascular disease development (Table I). As components of the earth's crust, these elements are ubiquitous in the environment. The general adult population is most commonly exposed through diet (food and drinking water), although cigarette smoking, air pollution, and occupation can also be sources. While some metals have no known physiologic purpose, others are essential micronutrients at trace amounts; however, both can be toxic depending on the dose. Essential metals are needed for lipid and carbohydrate metabolism, defense against oxidative stress, regulation of gene expression, and maintenance of protein structures. Conversely, even low-level exposures to non-essential metals have been linked with a broad scope of adverse health effects.

Given the abundance of metals in the environment and the high burden of cardiometabolic abnormalities, even weak to moderate causal associations could have important implications. From a public health perspective, building upon prior research on cardiometabolic effects of metals, in addition to quantifying population exposure levels and identifying sources of metal exposure are necessary steps

TABLE I.
METALS AND METALLOIDS ASSOCIATED WITH ADVERSE CARDIOMETABOLIC HEALTH

Element	Properties	Type
Arsenic (As)	Metalloid	Non-essential
Cadmium (Cd)	Metal	Non-essential
Copper (Cu)	Metal	Essential
Manganese (Mn)	Metal	Essential
Mercury (Hg)	Metal	Non-essential
Lead (Pb)	Metal	Non-essential
Selenium (Se)	Metalloid	Essential
Zinc (Zn)	Metal	Essential

towards prevention. Prior epidemiologic research on metals and chronic disease has mostly concentrated on the non-essential. *The focus of this work is on both non- and essential metals in relation to metabolic syndrome and its component cardiometabolic abnormalities since non-essential metals are established toxins and can interact with essential metals, which can be toxic depending on the level of exposure.*

A. Metals, Metabolic Syndrome, and Related Chronic Diseases

In the past few decades, evidence has been accumulating that long-term exposures to certain metals increase the risk for cardiovascular disease and type 2 diabetes. Coupled with the results of a recent randomized trial that found chelation therapy (which removes toxic metal stores from the human body) significantly lowers rates of cardiac events, the link between metal exposures and chronic disease is strengthening.(3) However, research on metals and metabolic syndrome, a clustering of cardiometabolic abnormalities including abdominal obesity, insulin resistance, hypertension, and dyslipidemia, is still sparse.(2) Collectively, these abnormalities affect more than 1 in 3 adults in the U.S., and confer a more than 2-fold increased risk of cardiovascular disease and 5-fold increased risk of diabetes.(4) Studying the role of metals, both essential and toxic, in metabolic syndrome could shed light on the underlying mechanisms through which exposures predispose individuals to subsequent chronic disease.

B. Essential Metals and Cardiometabolic Health

Animal and experimental human studies suggest biologic plausibility for cardiometabolic effects of metals. In animals fed micronutrient-deficient diets, inadequate intakes of copper, manganese, zinc, and selenium have resulted in altered lipid levels, elevated blood pressure, and reduced glucose tolerance.(5-13) There appear to be optimal ranges of intake, as excessive amounts also induce hypertension, hyperglycemia, and hypercholesterolemia in animals.(14-16) Human data echo some of these findings: copper depletion decreases glucose tolerance, manganese depletion decreases HDL cholesterol, and zinc depletion impairs glucose tolerance.(17-19) In contrast, selenium supplementation could increase the risk of diabetes.(20) *However, these experimental results may not accurately reflect real-world conditions.*

Epidemiologic studies of essential metals and cardiometabolic health are relatively rare. Observational studies of selenium suggest that excess exposures may increase the risk of diabetes and possibly hypercholesterolemia.(21, 22) For zinc, greater total intake has been found to be protective against developing type 2 diabetes, but may be positively associated with elevated triglycerides.(23, 24) A recent study identified greater dietary intake of copper (>1,100 mg/day, 22% above the recommended dietary allowance) as a risk factor for diabetes, but its prospective relationship with metabolic syndrome has not yet been evaluated.(25) Relations of dietary manganese and metabolic syndrome appear to be sex-dependent, with a positive association observed among women and an inverse association among men; however, these data were cross-sectional.(26) Biomarker studies suggest a positive role for copper in dyslipidemia and diabetes, and a U-shaped relationship for manganese in diabetes development, yet all were conducted in Asia where exposures could be very different than levels typically observed in U.S. populations.(27-29)

C. Toxic Metals and Cardiometabolic Health

Toxic metals are not required for normal physiologic functioning in humans. With respect to mechanisms related to metabolic syndrome, *in vivo* and *in vitro* studies have shown that arsenic alters vascular tone in blood vessels, impairs pancreatic β-cell functioning, and induces dyslipidemia; cadmium

accumulates in insulin-producing β-cells, impairing their function, and increases liver fatty acid synthesis; lead increases blood pressure; and mercury induces pancreatic cell dysfunction, increases blood pressure, and elevates triglyceride levels.(30-39) Mechanistic studies have also demonstrated low-level exposures to arsenic, mercury, and lead result in inflammation, a precursor to metabolic syndrome.(40-43)

Traditionally considered to have a threshold, or level below which exposures are not expected to cause harm, recent epidemiologic studies of toxic metals challenge this notion for type 2 diabetes, hypertension, and cardiovascular disease.(44-48) Few epidemiologic studies to date have evaluated frank metabolic syndrome,(49, 50) but some have associated metals with certain individual components. At moderate-to-high levels of exposure to arsenic, there is sufficient evidence for an association with diabetes and suggestive evidence for one with hypertension. At lower exposure levels more commonly encountered in the U.S., a few studies have observed positive associations, but the evidence is limited by a lack of prospective data.(51, 52) Positive associations between cadmium and lead exposures with elevated blood pressure, and mercury with incident diabetes have also been observed.(53-55) Associations of toxic metals with obesity and lipids have not been well characterized, although a recent study suggested that lead, cadmium, and mercury may interact synergistically to increase triglycerides.(49)

D. Metal Interactions

Once inside cells, concentrations of essential metals are generally maintained in homeostasis, while toxic metals lack such control.(56) Metal-binding proteins responsible for uptake and transport control this balance, but some of them lack specificity.(57) As such, they can be subject to molecular mimicry; that is, the protein cannot adequately differentiate between metals that are molecularly similar. Often, instances of molecular mimicry involve the replacement of an essential metal with a toxic one. *The repercussions of this are twofold: first, deficiencies in nutritionally essential metals can disrupt normal functioning; and second, even small amounts of toxic metal exposures can cause harm.*

E. Metals and Cardiometabolic Epidemiology: Methodological Challenges

In addition to being scant, the existing epidemiologic literature on metals and cardiometabolic conditions is limited by study design and methodology. First, the majority of studies have focused on toxic metals instead of nutritionally essential elements. Needed in small amounts for normal physiologic functioning, the essentiality of these metals does not negate their potential for toxicity. Supplements in particular may be a contributing factor, as their use can result in total intakes that exceed tolerable upper limits.(58) Second, metals are commonly analyzed separately, which could obscure how metals interact with one another. Metal exposures do not occur in isolation, thus studying single metal exposures may not adequately represent true associated health risks. Quantifying the joint biologic effects of exposure to multiple metals could have important implications if the joint toxicity exceeds individual effects (49) and/or if susceptibility to toxicity is modified by nutritional status.(59, 60) Third studies have generally concentrated on singular endpoints (most commonly blood pressure and diabetes, rather than obesity or dyslipidemia) instead of broader patterns of cardiometabolic abnormalities, which are often comorbid. Metabolic syndrome represents a clustering of factors that occur together more often than by chance alone would dictate, and its underlying causes are not entirely known. As the prevalence of metabolic syndrome increases, further etiologic research is warranted. Metals represent a ubiquitous, modifiable, and plausible exposure deserving of more rigorous epidemiologic study in the context of clustered cardiometabolic conditions. *This work can address gaps in current knowledge by quantifying exposures to essential and toxic metals within three diverse, population-based samples from the U.S. and Bangladesh, and by evaluating associations independently and jointly with metabolic syndrome and its components.*

F. Innovation

This work is innovative in the following ways: 1) it is among the first to investigate metal exposures as a risk factor for metabolic syndrome; 2) it examines both physiologically essential and toxic metals; 3) it quantifies intakes from dietary and supplement sources, allowing for direct implications of findings as nutritional interventions; 4) it further quantifies exposures using urine, whole blood, and serum

biomarkers which are less susceptible to measurement error; 5) it employs advanced statistical approaches, permitting analyses of multiple exposures, interactions, and dose-response relationships; and 6) it evaluates the proposed relations in three independent study samples, adding to the external validity of our findings. This study will be highly time- and cost-effective, because we will leverage the resources of three existing well-phenotyped study populations with previously collected biospecimens and ready-to-use sociodemographic, health behavior, dietary, supplement use, clinical, and biomarker data.

G. Specific Aims

1. Aim 1

Aim 1 is to characterize dietary and supplemental intakes of selected essential metals (Cu, Mn, Se, and Zn) among a large cohort of diverse Hispanics/Latinos in the U.S., and evaluate cross-sectional and prospective associations of dietary, supplemental, and total intake with metabolic syndrome. We hypothesize that essential metal intake outside of optimal ranges will be positively associated with metabolic syndrome and its components.

2. Aim 2

Aim 2 is to characterize biomarkers (urine, blood, and serum) of exposure to selected essential and non-essential metals (As, Cd, Cu, Pb, Mn, Hg, Se, and Zn) among a representative population of adults from the U.S., and evaluate cross-sectional and prospective associations with metabolic syndrome. We hypothesize that we will identify levels of biomarkers of exposure to metals that are positively associated with the prevalence of metabolic syndrome and its components; associations will likely be U-shaped for essential metals and linear for non-essential metals.

3. Aim 3

Aim 3 is to characterize blood levels of selected essential and non-essential metals (Pb, Mn, and Se) among a cohort of Bangladeshi adults, and evaluate associations with prospective blood pressure trajectories. We anticipate that baseline blood concentrations of Pb will be linearly associated with

increases in blood pressure over time, while mid-range concentrations of Mn and Se (compared to lower and upper extremes) will be associated with longitudinal decreases in blood pressure.

II. INTAKES OF COPPER, MANGANESE, SELENIUM, AND ZINC WITH METABOLIC SYNDROME: RESULTS FROM THE HISPANIC COMMUNITY HEALTH STUDY/ STUDY OF LATINOS (HCHS/SOL)

A. Background

Essential trace minerals are required for various physiological processes such as lipid and carbohydrate metabolism, maintenance of protein structures, regulation of gene expression, and defense against oxidative stress.(61, 62) Experimental studies and randomized trials suggest certain trace minerals, including copper, manganese, selenium, and zinc exert cardiometabolic health effects. For example, depletion of copper and zinc have each been shown to impair glucose tolerance, while depletion of manganese decreases HDL cholesterol levels.(12, 13, 17) In contrast, randomized trials of supplementation suggest both beneficial and adverse effects depending on the specific mineral. Zinc supplements have been linked with decreases in blood glucose concentrations whereas selenium supplements may increase the risk of diabetes.(20, 63, 64) However, the findings of the aforementioned experiments and trials may not be reflective of dietary or supplement use patterns under real-world conditions, nor how these patterns might increase or reduce the risk for adverse cardiometabolic health.

Despite biologic plausibility, few prospective epidemiologic studies of essential trace mineral intakes and cardiometabolic conditions have been conducted. A recent Japanese observational study suggested greater dietary intakes of copper were positively associated with type 2 diabetes risk, but the relationships with the risk of other cardiometabolic conditions, including metabolic syndrome, remain unknown.(65) The same Japanese study observed an inverse association of dietary zinc with incident diabetes, corroborating a finding previously observed in an analysis of women enrolled in the Nurses' Health Study.(23, 65) Dietary and supplemental manganese intakes have not been evaluated prospectively with metabolic syndrome nor its component conditions, although an analysis of plasma manganese concentrations (which may reflect non-dietary environmental exposures) suggested a U-shaped dose-response curve with the odds of diabetes development.(27) Lastly, longitudinal

epidemiologic studies of dietary and supplemental selenium have been inconsistent with regards to glycemic control while associations with hypertension, abdominal obesity, and lipid profiles are understudied.(66, 67)

Given the high prevalence of metabolic syndrome (which affects about one-third of adults in the United States) and its importance in the development of subsequent cardiovascular disease, high-quality observational studies of potentially causal factors are warranted.(1) We therefore evaluated cross-sectional and prospective associations of dietary and supplemental intakes of copper, manganese, selenium, and zinc with metabolic syndrome and its component conditions within a multi-center community-based cohort of diverse Hispanic and Latino adults across the United States.

B. Methods

1. Study population

The Hispanic Community Health Study/Study of Latinos (HCHS/SOL) is a landmark prospective cohort of 16,415 Hispanic/Latino participants, aged 18 to 74 years at enrollment (2008-2011). Recruitment was population-based and utilized two-stage probability sampling of households within census blocks across four field centers (Bronx, NY; Chicago, IL; Miami, FL; and San Diego, CA). Details of sampling methods used have been published elsewhere.(68, 69) Written informed consent was provided by all study participants. All field centers, the coordinating center, central laboratory, and reading center obtained approval from their institutional review boards.

2. Dietary and Dietary Supplement Assessment

Detailed dietary information was gathered through two 24-hour recalls; the first was conducted in-person at the time of the baseline interview and the second via telephone approximately 30 (range: 3-335) days later. Trained interviewers used Nutrition Data System for Research (NDSR) Version 11 software, developed at the University of Minnesota Nutrition Coordinating Center (NCC), to conducted interviews in the participant's preferred language (English or Spanish).(70) Immediately following the

first dietary recall, participants underwent a supplement interview in which they were queried about all dietary supplements taken in the past 30 days, including the full product name and frequency of use, using the Dietary Supplement Assessment Module in NDSR.(71) A multiple-pass approach was utilized to ensure data accuracy and completeness.(72) The University of Minnesota NCC Food and Nutrient Database served as the source of food composition information in the NDSR.(73) This database includes over 18,000 foods including 8,000 brand-name products, and many Hispanic and Latino foods. Ingredient choices and preparation methods provide more than 160,000 food variants. Values for 165 nutrients are generated from the database, with the USDA Nutrient Data Laboratory serving as the primary source of this information.(74) Of the 16,415 participants enrolled in the study, ninety nine percent completed the first 24-hour recall (n=16,285), and ninety four percent completed the second (n=15,424).

3. Metabolic Syndrome Assessment

Participants underwent a standardized clinical examination at enrollment that included anthropometric and laboratory measurements performed by trained research technicians. Participants were asked to fast for 8 hours, abstain from smoking for 12 hours, and refrain from vigorous physical activity the morning of the clinical examination. Participants were instructed to bring all medications taken in the past month (prescription and nonprescription) with them to the enrollment examination. Medications were scanned using Universal Product Code barcodes where available. Otherwise, medications were recorded using centralized manual coding. Medications were then inventoried and classified using a Master Drug Data Base (Medispan MDDB©). Waist circumference was measured at the uppermost lateral border of the right ilium using measuring tape and recorded to the nearest 0.1 centimeter. Participants were asked to sit for 5 minutes prior to taking three systolic and diastolic blood pressure measurements each at 1 minute intervals using an automated sphygmomanometer (Omron model HEM-907 XL, Omron Healthcare Inc., Bannockburn, IL). The average of the three readings were calculated and subsequently used in all analyses. Fasting blood samples were collected and shipped to the HCHS/SOL Central Laboratory at the University of Minnesota for processing. There, high-density

lipoprotein cholesterol (HDL) was measured using a direct magnesium/dextran sulfate method (Roche Diagnostics, Indianapolis, IN). Serum triglycerides were measured via a Roche Modular P chemistry analyzer using a glycerol blanking enzymatic method. Fasting glucose was measured using a hexokinase enzymatic method (Roche Diagnostics).

Metabolic syndrome was defined as the presence of at least 3 of following 5 conditions, based on the harmonized definition: abdominal obesity (waist circumference of ≥88 cm for women, ≥102 cm for men), high triglyceride levels (≥150 mg/dL or current use of medication to treat high triglycerides), low HDL cholesterol levels (<50 mg/dL for women, <40 mg/dL for men, or current use of medication to treat low HDL cholesterol), high blood pressure (systolic blood pressure ≥130 mmHg, diastolic blood pressure ≥85 mmHg, or current use of medication to treat high blood pressure) or high fasting blood glucose levels (≥100 mg/dL or current use of medication to treat hyperglycemia).(4) Fibrates and nicotinic acids (prescribed or supplemental) satisfied criteria for treatment of both high triglyceride and low HDL cholesterol levels.

At the follow-up examination (2014-2017), identical procedures were used to measure waist circumference, blood pressure, HDL cholesterol, triglycerides, and fasting glucose. Data regarding medication use from this visit have not yet been coded and classified, and thus we relied on self-reported information from a medication questionnaire regarding usage in the past 4 weeks. Individuals who reported taking medications to treat high blood pressure or diabetes satisfied the respective criteria for high blood pressure or high fasting blood glucose. Of note, participants were not specifically asked about fibrates or nicotinic acid medication use; an affirmative response to "were there any medications you took in the last four weeks for high blood cholesterol?" was instead considered treatment for the dyslipidemia components.

4. <u>Sociodemographic and Lifestyle Characteristics</u>

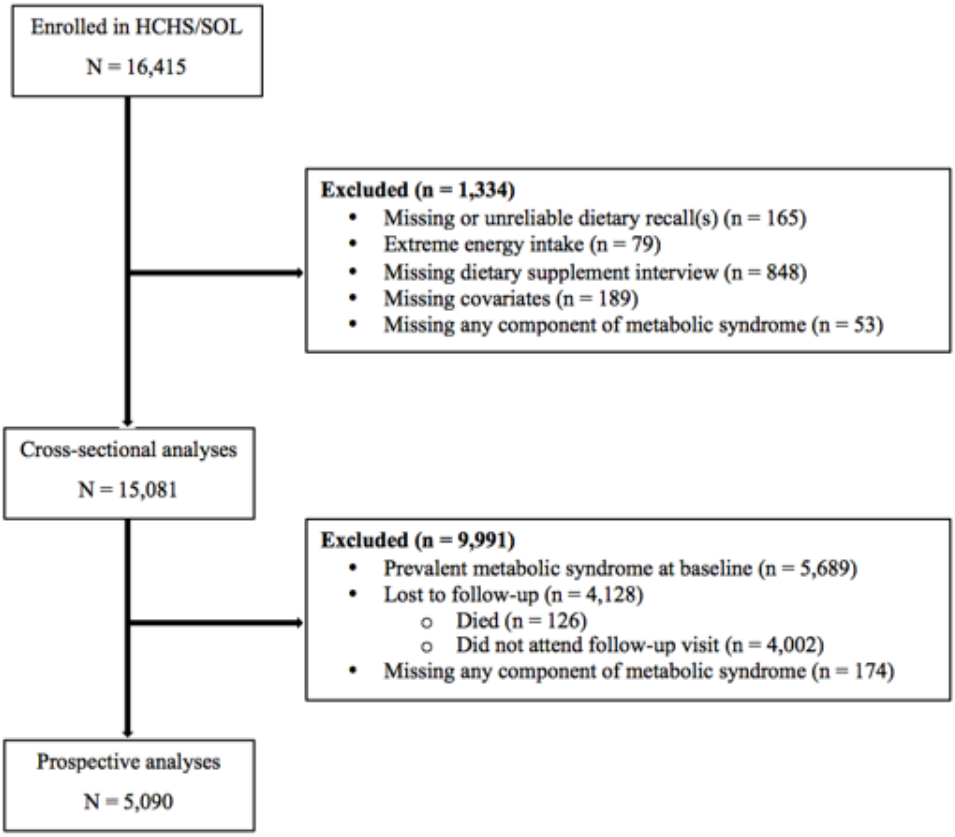
Sociodemographic and lifestyle characteristic data were obtained from the interviewer-administered enrollment questionnaire. Hispanic/Latino background groups were categorized as Central

American, Cuban, Dominican, Mexican, Puerto Rican, South American, and more than one heritage/other heritage. Years of residence within the mainland United States (excluding territories) was used as a proxy for acculturation; the variable was parameterized with the following categories: born in mainland, <5, 5-9, 10-14, 15-19, or ≥20 years. Educational attainment was categorized as less than a high school diploma or GED, high school diploma or GED, or college or vocational schooling. Current health insurance coverage was assessed as coverage through an employer, individual plan, Medicaid/Medicare, military, Indian Health Services, or other coverage. Alcohol intake was categorized as no current use, low/moderate (<7 drinks per week for females, <14 drinks per week for males), or heavy (≥7 drinks per week for females or ≥14 drinks per week for males). Cigarette smoking status was classified as never, former, or current. A modified Global Physical Activity Questionnaire (GPAQ), originally developed by the World Health Organization was used to collect information on physical activity across three domains (work, recreation, and transport) in a typical week.(75) Total physical activity across these domains was categorized as inactive (no activity beyond baseline activities of daily living), low (> 0 to <75 min/week of vigorous-intensity activity; > 0 to <150 min/week of moderate-intensity activity; or an equivalent combination of vigorous- and moderate-intensity activity), medium (≥75 to 150 min/week of vigorous-intensity activity; ≥150 to 300 min/week of moderate-intensity activity; or an equivalent combination of vigorous- and moderate-intensity activity), or high (>150 min/week of vigorous-intensity activity; >300 min/week of moderate-intensity activity; or an equivalent combination of both.(76)

5. <u>Statistical Analyses</u>

Of the original 16,415 HCHS/SOL participants at baseline, we excluded those who did not complete at least one reliable dietary recall (n=165), reported extreme values of energy intake less than the 1st or greater than the 99th sequence-gender specific percentiles (n=79), did not complete the 30-day dietary supplement use interview (n=848), had missing data on relevant covariates (n=189), or were missing data on any component condition of metabolic syndrome at baseline (n=53) resulting in a sample size of 15,081 individuals for cross-sectional analyses (Figure 1). For prospective analyses, we further

Figure 1. Inclusion/exclusion criteria flow diagram for HCHS/SOL



excluded individuals with metabolic syndrome prevalent at baseline (n=5,689), those lost to follow-up (n=126 who died between the baseline examination and follow-up visit), or those missing data on any component condition of metabolic syndrome (n=174) at the follow-up visit for an analytic sample size of 5,090 individuals. As of December 2016, 4,002 individuals have not yet completed their follow-up examination.

Dietary data from 24-hour recalls do not represent usual, or long-term average daily, intakes because individuals do not eat the same foods or drink the same beverages every day.(77) Thus, we used methodologies and corresponding SAS macros (version 2.1) developed by the National Cancer Institute (NCI) to derive usual intakes of copper, manganese, selenium, zinc, and energy.(78, 79) The NCI methods involved 3 steps: 1.) a non-linear mixed effects model for repeated measures of intake data adjusted for covariates (MIXTRAN macro); 2.) estimation of percentiles of usual intakes generalizable to the target population (DISTRIB macro); and 3.) prediction of usual intake for each individual (INDIVINT methodologies and corresponding SAS macros (version 2.1) developed by the National Cancer Institute (NCI) to derive usual intakes of copper, manganese, selenium, zinc, and energy.(78, 79) The NCI methods involved 3 steps: 1.) a non-linear mixed effects model for repeated measures of intake data adjusted for covariates (MIXTRAN macro); 2.) estimation of percentiles of usual intakes generalizable to the target population (DISTRIB macro); and 3.) prediction of usual intake for each individual (INDIVINT macro). The second and third steps (DISTRIB and INDIVINT) rely on the covariate parameters from the first step (MIXTRAN). The NCI method was similarly used to predict usual intakes of several food groups in servings per day, including vegetables, fruits, grains, whole grains, meat, red/processed meat, nuts and legumes, and dairy. For individuals with zero intakes of these food groups on recall days (<6%), one-half of the minimum observed non-zero value was substituted.(80)

5a. <u>MIXTRAN Macro</u>

For each mineral of interest and for energy, participants were first stratified into two groups: individuals who reported using supplement(s) that contained the respective nutrient in the

last 30 days and those that did not thereby allowing each group to have different intake distributions, as research suggests supplement users consume more minerals from food sources than non-users.(81, 82) An "add, then shrink" approach was used, meaning we summed the intakes from food/beverages reported during the recalls with the average daily supplemental intake per recall day to obtain total intakes for supplement users.(77) Hence, the MIXTRAN macro, which fits non-linear mixed effects models using a Box-Cox transformation, was run twice per nutrient amongst supplement users in order to obtain parameter estimates adjusted for covariates: once modeling intakes from food/beverages alone ("dietary intakes"); and again modeling intakes from food/beverages and supplements ("total intakes"). For non-users, MIXTRAN was implemented only one time per nutrient as their total intakes were set to equal their dietary intakes. All MIXTRAN runs included the following covariates: age (categorized as 18-24, 25-34, 35-44, 45-54, 55-64, 65-74 years), gender (male or female), Hispanic/Latino background (Central American, Cuban, Dominican, Mexican, Puerto Rican, South American, or mixed/other), field center (Chicago, Bronx, Miami, or San Diego), weekend (Friday-Sunday), self-reported intake amount (more, same, or less than usual amount), and sequence (first recall conducted in person, second recall conducted most via phone). Dietary intake models performed among supplement users additionally included the average supplemental intake amount of the respective nutrient from the prior 30 days as a continuous covariate. Food group intakes were modeled using a similar approach. HCHS/SOL sampling weights for the baseline or follow-up visit were incorporated into the models corresponding to derivations for use in either cross-sectional or prospective analyses, as appropriate. These sampling weights are the product of a "base weight" (reciprocal of the probability of selection) with adjustments for non-response, trimming of extreme values, and calibration to the 2010 U.S. Census according to age, sex and Hispanic/Latino background.(68)

5b. DISTRIB and INDIVINT Macros

The DISTRIB macro was subsequently run using the parameter estimates and linear predictor values obtained from MIXTRAN. Monte Carlo simulation of the person-specific random effects was performed to empirically estimate distributions of usual dietary and total intakes for both the cross-sectional and prospective analytic samples. The INDIVINT macro then performed adaptive Gaussian quadrature to predict usual intake for each individual. The resulting values were categorized into quartiles based on the 25th, 50th, and 75th percentiles generated by DISTRIB. More information regarding the NCI methodology and SAS macros to facilitate the modeling and Monte Carlo-based estimation of usual intakes can be found elsewhere.(83)

5c. Descriptive Analyses

All descriptive analyses and regression models accounted for cluster sampling and stratification in the sample selection, and were conducted using SAS version 9.3 (Cary, NC) or Stata version 14.1 (College Station, TX). Pearson correlation coefficients were calculated to estimate linear correlations between baseline total intakes of copper, manganese, selenium, and zinc, in addition to correlations of these minerals with food groups. Usual total and dietary intakes of the selected minerals were reported as medians with interquartile ranges (i.e., 25th to 75th percentiles) overall and by baseline sociodemographic and lifestyle characteristics. The proportion of supplement users was also evaluated overall and by baseline characteristics.

5d. Cross-Sectional Analyses

Cross-sectional associations of usual total intake (categorized into quartiles), usual dietary intake (quartiles), and supplement use (users versus non-users) for each mineral with metabolic syndrome and its component conditions were evaluated using separate Poisson regression models to estimate prevalence ratios and their 95% confidence intervals. P-values for linear trends were estimated by modeling quartiles of usual intakes as ordinal variables.

Bootstrapped variances were estimated using 100 bootstrap replicate sampling weights.(84). In addition to unadjusted models, two sequentially adjusted models were fit: 1) adjusted for predicted usual total energy intake, age, gender, and Hispanic/Latino background; and 2) further adjusted for field center, educational attainment, cigarette smoking, alcohol intake, physical activity, and years residing in the U.S. Models of usual mineral intakes from dietary sources included the use of supplements containing copper, manganese, selenium, or zinc as an additional covariate; models of supplementation status additionally adjusted for usual dietary intakes of the corresponding mineral. These covariates were selected *a priori* based on the existing epidemiologic literature and included factors known to be associated with intakes of the selected minerals or known risk factors for metabolic syndrome.(85-88) We performed subgroup analyses by age group (18-24, 25-34, 35-44, 45-54, 55-64, and 65+ years), gender, Hispanic/Latino background, and supplementation status to assess effect modification on the multiplicative scale.

To assess joint associations of the selected minerals with metabolic syndrome, we dichotomized usual total and dietary intakes at the median to characterize intakes as low or high. We then categorized combinations of intakes as follows: low intake of all, high intake of any one, high intake of any two, high intake of any three, or high intake of all four minerals. For supplements, we classified use as having any supplemental intake of copper, manganese, selenium, or zinc. Indicator variables were used to estimate associations with prevalent metabolic syndrome and components using models identical to those previously described for single-mineral associations.

5e. <u>Prospective Analyses</u>

Prospective associations with incident metabolic syndrome and its components were also evaluated with Poisson regression, with adjustment for the same baseline covariates included in the cross-sectional models. Linear trends were again assessed by parameterizing quartiles of usual intakes as ordinal variables. Subgroup analyses were performed to assess multiplicative effect

medication by age, gender, Hispanic/Latino background, and supplement use. These models were weighted using interim sampling weights (released with data as of December 2016) that account for non-response at the follow-up visit. Log-transformed person-years at risk (calculated as the number of days between the baseline examination and the follow-up visit, divided by 365.25) was included as an offset term in order to obtain incidence rate ratios. For prospective models of individual metabolic syndrome components (high blood pressure, abdominal obesity, high triglycerides, low HDL cholesterol, or high fasting glucose), we only included participants who were free of both the corresponding condition and metabolic syndrome at baseline. Associations of combined intakes and supplemental use of the selected minerals with risk of metabolic syndrome and individual components were evaluated as described previously.

C. Results

1. Descriptive Statistics

Metabolic syndrome was prevalent in 32.3%, with abdominal obesity the most common component affecting 54.8% and high triglyceride levels the least common component affecting 28.9% (Table II). Supplement use, male gender, higher socioeconomic status (assessed using household income and educational attainment), and physical activity were positively associated with both dietary and total intakes of copper, manganese, selenium, and zinc at baseline (Tables III-IV). Total intakes of copper and manganese were lowest amongst younger individuals (18-24 years) while selenium and zinc were lowest amongst the elderly (65+ years). For all minerals, a Cuban background was positively associated with higher dietary and total intakes whereas a Dominican background was significantly associated with lower dietary and total intakes. Geographically, individuals from Miami had the highest usual intakes from both dietary and total sources, with individuals from the Bronx consistently having the lowest. Individuals residing in the mainland U.S. for fewer than 5 years had the highest dietary and total intakes, with a longer duration associated with lower intakes. Not drinking alcohol and never smoking were inversely

TABLE II.
PREVALENCE OF METABOLIC SYNDROME AND COMPONENTS AT BASELINE (2008-2011), HCHS/SOL (N=15,081)

Condition	Prevalence (95% CI)
Metabolic syndrome	32.3 (31.1-33.6)
High blood pressure	31.5 (30.1-32.9)
High triglycerides	28.9 (27.8-30.1)
Low HDL	43.2 (42.0-44.5)
High fasting glucose	31.0 (29.8-32.2)
Abdominal obesity	54.8 (53.4-56.3)

TABLE III.
USUAL DIETARY AND TOTAL INTAKES OF SELECTED MINERALS OVERALL AND BY SUPPLEMENT USE STATUS AT BASELINE (N=15,081)[a]

			Supplement Use	
Mineral		**Overall**	**Non-User**	**User**
Copper, mg/day	**N (%)**	15,081 (100.0)	12,166 (82.2)	2,915 (17.8)
	Dietary	1.23 (0.98-1.54)	1.20 (0.96-1.51)	1.36 (1.08-1.71)
	Total	1.30 (1.01-1.72)	1.20 (0.96-1.51)	2.47 (1.78-3.42)
Manganese, mg/day	**N (%)**	15,081 (100.0)	12,181 (82.4)	2,900 (17.6)
	Dietary	2.98 (2.23-3.98)	2.90 (2.18-3.84)	3.46 (2.53-4.73)
	Total	3.12 (2.30-4.34)	2.90 (2.18-3.84)	5.32 (3.65-7.70)
Selenium, µg/day	**N (%)**	15,081 (100.0)	12,349 (83.3)	2,732 (16.7)
	Dietary	114.2 (91.2-141.7)	113.0 (90.4-140.3)	119.9 (95.5-149.1)
	Total	118.0 (93.3-149.3)	113.0 (90.4-140.3)	157.1 (119.7-204.7)
Zinc, mg/day	**N (%)**	15,081 (100.0)	11,841 (80.2)	3,240 (19.8)
	Dietary	10.91 (8.62-13.68)	10.79 (8.55-13.51)	11.42 (8.96-14.38)
	Total	11.73 (9.02-15.66)	10.79 (8.55-13.51)	21.62 (15.66-29.25)

[a] Data are presented as median (25th-75th percentile) estimated using NCI method.(77)

TABLE IV.

USUAL DIETARY AND TOTAL INTAKES BY BASELINE CHARACTERISTICS (N=15,081)[a]

Characteristic	N (%)	Copper (mg/day)		Manganese (mg/day)		Selenium (µg/day)		Zinc (mg/day)	
		Dietary	Total	Dietary	Total	Dietary	Total	Dietary	Total
Overall	15,081 (100.0)	1.23 (0.98-1.54)	1.30 (1.01-1.72)	2.98 (2.23-3.98)	3.12 (2.30-4.34)	114.2 (91.2-141.7)	118.0 (93.3-149.3)	10.91 (8.62-13.68)	11.73 (9.02-15.66)
Age, years									
18-24	1,532 (16.8)	1.16 (0.92-1.46)	1.19 (0.94-1.53)	2.74 (2.06-3.65)	2.80 (2.09-3.79)	118.4 (95.2-145.7)	120.1 (96.0-149.0)	11.37 (9.00-14.18)	11.72 (9.18-14.97)
25-34	1,942 (22.0)	1.25 (0.99-1.57)	1.30 (1.01-1.71)	2.93 (2.19-3.92)	3.02 (2.23-4.17)	119.3 (95.7-147.4)	122.0 (96.8-153.7)	11.40 (9.03-14.28)	12.01 (9.28-15.96)
35-44	2,760 (21.4)	1.28 (1.02-1.61)	1.34 (1.04-1.77)	3.12 (2.34-4.16)	3.27 (2.41-4.50)	118.9 (95.4-147.4)	122.1 (96.9-154.5)	11.23 (8.90-14.03)	11.96 (9.22-15.93)
45-54	4,505 (18.8)	1.25 (1.00-1.57)	1.34 (1.03-1.80)	3.09 (2.31-4.13)	3.27 (2.40-4.62)	113.2 (90.8-140.4)	118.0 (92.9-150.5)	10.76 (8.53-13.46)	11.68 (8.95-15.80)
55-64	3,149 (12.6)	1.21 (0.96-1.51)	1.31 (1.00-1.79)	3.01 (2.25-4.00)	3.22 (2.37-4.51)	104.3 (83.7-129.1)	109.5 (86.3-138.9)	10.13 (8.03-12.67)	11.22 (8.54-15.39)
65+	1,193 (8.5)	1.18 (0.94-1.48)	1.33 (1.02-1.85)	3.00 (2.24-4.00)	3.28 (2.38-4.66)	98.8 (79.3-122.9)	106.2 (83.6-135.0)	9.61 (7.64-12.09)	11.24 (8.49-16.08)
Gender									
Female	9,046 (52.2)	1.08 (0.87-1.32)	1.13 (0.90-1.46)	2.66 (2.01-3.50)	2.77 (2.07-3.79)	96.7 (79.8-115.9)	99.4 (81.4-121.3)	9.31 (7.56-11.36)	9.94 (7.88-12.98)
Male	6,035 (47.8)	1.42 (1.15-1.75)	1.50 (1.19-1.95)	3.39 (2.57-4.47)	3.56 (2.64-4.88)	136.7 (114.4-162.1)	141.6 (117.1-171.9)	12.97 (10.65-15.65)	13.74 (11.05-17.52)
Hispanic/Latino background									
Central American	1,606 (7.3)	1.23 (0.99-1.54)	1.29 (1.01-1.71)	2.95 (2.21-3.93)	3.10 (2.28-4.29)	112.5 (89.7-139.3)	116.6 (91.6-147.2)	10.61 (8.43-13.27)	11.43 (8.81-15.28)
Cuban	2,237 (20.6)	1.37 (1.11-1.69)	1.43 (1.14-1.83)	3.36 (2.55-4.42)	3.49 (2.62-4.71)	127.2 (102.3-156.1)	130.5 (104.2-162.2)	11.83 (9.49-14.65)	12.49 (9.83-16.15)
Dominican	1,268 (9.3)	1.01 (0.82-1.25)	1.06 (0.84-1.38)	2.59 (1.96-3.42)	2.70 (2.01-3.67)	97.0 (77.8-120.00)	100.0 (79.6-125.6)	8.46 (6.76-10.57)	9.06 (7.06-12.00)
Mexican	6,152 (38.3)	1.29 (1.04-1.59)	1.35 (1.07-1.77)	2.98 (2.25-3.95)	3.11 (2.31-4.29)	113.4 (91.6-139.5)	117.1 (93.4-147.5)	11.56 (9.30-14.30)	12.38 (9.67-16.38)
Puerto Rican	2,332 (15.1)	1.02 (0.81-1.29)	1.09 (0.84-1.51)	2.60 (1.95-3.49)	2.76 (2.02-3.92)	108.7 (86.2-135.7)	113.3 (88.8-144.4)	9.79 (7.72-12.33)	10.73 (8.18-14.67)
South American	1,018 (5.1)	1.31 (1.05-1.62)	1.37 (1.08-1.79)	3.62 (2.74-4.80)	3.79 (2.81-5.18)	118.9 (95.8-146.6)	122.3 (97.3-153.5)	10.93 (8.72-13.58)	11.92 (9.22-15.76)
More than one/other	468 (4.2)	1.21 (0.96-1.53)	1.29 (0.99-1.75)	2.93 (2.19-3.95)	3.09 (2.25-4.37)	118.2 (95.3-147.2)	123.3 (97.9-156.1)	10.75 (8.55-13.51)	11.57 (8.90-15.78)
Center									
Bronx	3,386 (26.5)	0.99 (0.80-1.23)	1.04 (0.82-1.37)	2.50 (1.89-3.31)	2.62 (1.95-3.61)	101.2 (80.9-125.6)	104.4 (82.7-131.9)	9.01 (7.17-11.27)	9.66 (7.48-12.93)
Chicago	3,992 (16.6)	1.31 (1.06-1.62)	1.37 (1.08-1.77)	2.97 (2.25-3.92)	3.10 (2.31-4.23)	113.4 (91.3-139.5)	117.1 (93.3-146.2)	11.52 (9.24-14.22)	12.26 (9.65-15.84)
Miami	3,872 (30.3)	1.36 (1.10-1.68)	1.42 (1.12-1.84)	3.38 (2.56-4.47)	3.52 (2.63-4.79)	125.3 (100.7-154.2)	128.9 (102.7-160.9)	11.71 (9.38-14.54)	12.49 (9.79-16.31)
San Diego	3,831 (26.7)	1.29 (1.04-1.59)	1.36 (1.08-1.81)	3.07 (2.33-4.07)	3.22 (2.39-4.49)	116.0 (93.8-142.9)	120.6 (96.1-152.5)	11.62 (9.37-14.33)	12.58 (9.80-16.86)

TABLE IV. (CONTINUED)

USUAL DIETARY AND TOTAL INTAKES BY BASELINE CHARACTERISTICS (N=15,081) [a]

Characteristic	N (%)	Copper (mg/day)		Manganese (mg/day)		Selenium (µg/day)		Zinc (mg/day)	
		Dietary	Total	Dietary	Total	Dietary	Total	Dietary	Total
Annual household income									
Missing	1,252 (8.8)	1.18 (0.93-1.48)	1.39 (1.07-1.89)	2.87 (2.14-3.83)	2.98 (2.20-4.07)	110.0 (87.4-137.4)	113.1 (89.2-142.3)	10.35 (8.20-13.02)	10.97 (8.54-14.34)
≤$10,000	2,127 (13.0)	1.16 (0.92-1.47)	1.23 (0.96-1.60)	2.86 (2.13-3.84)	3.00 (2.19-4.17)	106.9 (85.3-133.9)	110.7 (87.2-141.2)	10.16 (7.98-12.89)	11.01 (8.36-14.92)
$10,001-20,000	4,535 (28.9)	1.21 (0.96-1.52)	1.23 (0.94-1.64)	2.95 (2.21-3.94)	3.07 (2.26-4.23)	112.5 (90.1-139.6)	115.7 (91.7-146.0)	10.72 (8.50-13.45)	11.37 (8.81-15.05)
$20,001-40,000	3,580 (23.3)	1.26 (1.00-1.58)	1.27 (0.99-1.68)	3.03 (2.27-4.04)	3.16 (2.33-4.38)	116.2 (93.2-143.8)	119.9 (95.2-151.2)	11.17 (8.86-13.94)	11.98 (9.27-15.86)
$40,001-75,000	1,845 (13.0)	1.27 (1.01-1.58)	1.32 (1.03-1.75)	3.05 (2.29-4.08)	3.23 (2.37-4.52)	119.0 (95.8-146.6)	123.9 (98.6-155.9)	11.44 (9.10-14.23)	12.53 (9.64-16.85)
>$75,000	1,742 (12.9)	1.29 (1.02-1.60)	1.35 (1.05-1.81)	3.11 (2.33-4.15)	3.31 (2.42-4.68)	119.3 (95.5-147.7)	125.1 (98.7-158.6)	11.47 (9.07-14.28)	12.63 (9.68-17.20)
Education									
Less than high school	5,690 (31.7)	1.20 (0.95-1.51)	1.26 (0.98-1.66)	2.90 (2.18-3.85)	3.02 (2.23-4.14)	109.7 (87.6-136.3)	113.0 (89.5-142.5)	10.57 (8.32-13.28)	11.29 (8.69-14.89)
High school or equivalent	3,877 (28.5)	1.24 (0.99-1.56)	1.30 (1.01-1.71)	2.99 (2.24-4.00)	3.11 (2.29-4.29)	117.3 (93.7-145.1)	120.6 (95.4-151.6)	11.21 (8.87-14.02)	11.87 (9.20-15.60)
Greater than high school	5,514 (39.8)	1.24 (0.99-1.56)	1.33 (1.03-1.80)	3.05 (2.27-4.08)	3.22 (2.35-4.53)	115.6 (92.5-143.5)	120.3 (95.0-153.1)	10.97 (8.70-13.75)	12.00 (9.19-16.40)
Years lived in mainland U.S.									
Born in mainland U.S.	2,580 (22.5)	1.17 (0.93-1.49)	1.23 (0.95-1.66)	2.81 (2.10-3.78)	2.94 (2.16-4.11)	116.8 (93.7-144.2)	120.4 (95.4-152.2)	11.07 (8.73-13.87)	11.84 (9.06-15.96)
Less than 5	1,681 (14.0)	1.29 (1.03-1.60)	1.33 (1.06-1.72)	3.12 (2.35-4.15)	3.22 (2.39-4.37)	120.5 (96.5-149.0)	123.2 (98.0-153.8)	11.40 (9.09-14.17)	11.93 (9.36-15.38)
5-9	1,920 (14.4)	1.27 (1.02-1.59)	1.32 (1.04-1.71)	3.07 (2.31-4.09)	3.18 (2.36-4.34)	117.0 (94.0-144.7)	119.8 (95.3-150.1)	11.21 (8.93-14.00)	11.79 (9.19-15.36)
10-14	1,785 (11.9)	1.27 (1.01-1.58)	1.31 (1.03-2.03)	3.05 (2.28-4.06)	3.15 (2.33-4.33)	115.7 (92.5-143.3)	118.5 (93.8-149.2)	11.11 (8.80-13.88)	11.75 (9.11-15.38)
15-19	1,544 (10.4)	1.24 (0.98-1.55)	1.30 (1.01-1.72)	2.99 (2.23-4.00)	3.13 (2.30-4.34)	112.3 (89.6-140.0)	115.9 (91.2-147.6)	10.77 (8.50-13.55)	11.55 (8.84-15.48)
20 or more	5,571 (26.8)	1.20 (0.95-1.51)	1.31 (1.00-1.80)	2.97 (2.23-3.96)	3.19 (2.33-4.51)	107.4 (85.8-133.5)	112.9 (89.0-144.1)	10.34 (8.15-13.02)	11.55 (8.75-16.05)

TABLE IV. (CONTINUED)

USUAL DIETARY AND TOTAL INTAKES BY BASELINE CHARACTERISTICS (N=15,081) [a]

Characteristic	N (%)	Copper (mg/day)		Manganese (mg/day)		Selenium (µg/day)		Zinc (mg/day)	
		Dietary	Total	Dietary	Total	Dietary	Total	Dietary	Total
Alcohol use level									
None	7,923 (43.4)	1.19 (0.95-1.49)	1.25 (0.98-1.66)	2.91 (2.18-3.88)	3.05 (2.25-4.22)	108.3 (86.9-134.4)	111.9 (88.9-141.6)	10.38 (8.26-13.03)	11.20 (8.66-15.02)
Low/Moderate	6,397 (45.5)	1.27 (1.00-1.59)	1.34 (1.03-1.78)	3.05 (2.27-4.08)	3.19 (2.34-4.45)	119.1 (95.4-146.9)	123.3 (97.6-155.3)	11.35 (8.98-14.17)	12.18 (9.38-16.21)
Heavy	761 (6.1)	1.31 (1.04-1.64)	1.37 (1.06-1.76)	3.12 (2.32-4.14)	3.23 (2.38-4.40)	126.1 (101.2-153.3)	129.6 (103.2-159.1)	12.00 (9.48-14.78)	12.61 (9.83-16.08)
Cigarette smoking									
Never	9,181 (51.5)	1.20 (0.96-1.50)	1.27 (0.99-1.69)	2.91 (2.19-3.89)	3.05 (2.25-4.24)	110.7 (88.7-137.7)	114.5 (90.8-145.3)	10.61 (8.41-13.31)	11.43 (8.81-15.33)
Former	2,982 (17.2)	1.30 (1.03-1.63)	1.40 (1.08-1.86)	3.17 (2.37-4.23)	3.35 (2.46-4.69)	118.5 (94.8-146.0)	123.5 (97.5-155.2)	11.40 (9.00-14.17)	12.44 (9.55-16.58)
Current	2,918 (21.4)	1.26 (0.99-1.59)	1.31 (1.01-1.72)	3.04 (2.26-4.06)	3.14 (2.31-4.33)	120.8 (96.7-148.5)	124.1 (98.3-154.8)	11.42 (9.00-14.27)	12.05 (9.30-15.78)
Physical activity level									
Inactive	3,418 (20.7)	1.21 (0.97-1.51)	1.26 (0.99-1.65)	2.97 (2.22-3.95)	3.08 (2.28-4.23)	109.3 (88.0-135.5)	112.6 (89.6-141.3)	10.46 (8.33-13.07)	11.09 (8.65-14.56)
Low	2,050 (12.5)	1.17 (0.93-1.47)	1.24 (0.96-1.66)	2.87 (2.15-3.84)	3.01 (2.21-4.19)	106.1 (85.4-131.7)	109.7 (87.2-139.4)	10.21 (8.10-12.83)	11.04 (8.46-15.04)
Medium	1,694 (10.7)	1.19 (0.94-1.59)	1.25 (0.97-1.65)	2.90 (2.17-3.89)	3.02 (2.22-4.19)	108.6 (86.7-135.7)	111.9 (88.4-141.9)	10.37 (8.21-13.09)	11.12 (8.58-14.90)
High	7,919 (56.1)	1.26 (1.00-1.58)	1.34 (1.03-1.78)	3.03 (2.27-4.05)	3.19 (2.34-4.44)	119.0 (95.2-146.9)	123.4 (97.6-155.2)	11.35 (8.97-14.18)	12.25 (9.44-16.31)

[a] Data are presented as median (25th-75th percentile).

associated with dietary and total intakes of all four minerals, while low/moderate alcohol use and formerly smoking were independently positively related to higher intakes.

Sociodemographic characteristics of supplement use are provided in Table V. Older age, San Diego residence, higher socioeconomic status, U.S. nativity or 20+ years in the U.S., never or former cigarette smoking, and physical activity were significantly associated with using supplements containing copper, manganese, selenium, and zinc. Male gender was associated with using copper, manganese, and selenium supplements but no gender differences were observed for zinc supplementation. In general, Dominican and Cuban background were associated with non-use, while Mexican, Puerto Rican, South American, and more than one/other backgrounds were more likely to use supplements. Among individuals who reported using supplements containing copper, manganese, selenium, or zinc during the dietary supplement interview, 81% were using all four, most likely in the form of multi-vitamins/multi-minerals (Figure 2).

Figure 2. Unweighted frequencies of supplemental use of the selected minerals in HCHS/SOL

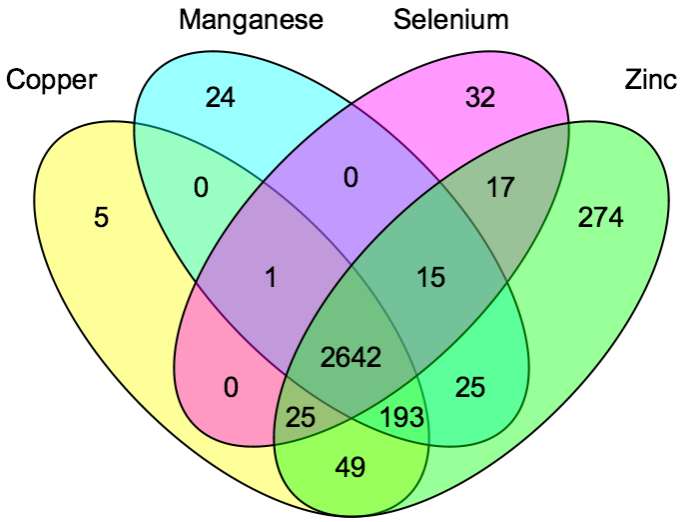


TABLE V.

BASELINE CHARACTERISTICS ACCORDING TO SUPPLEMENT USE (N=15,081)

	Copper Supplements		Manganese Supplements		Selenium Supplements		Zinc Supplements	
Characteristic	**Non-User**	**User**	**Non-User**	**User**	**Non-User**	**User**	**Non-User**	**User**
Frequency, unweighted	12,166	2,915	12,181	2,900	12,349	2,732	11,841	3,240
Age (years), %								
18-24	18.5	9.3	18.5	9.0	18.6	8.1	18.6	9.5
25-34	22.6	19.0	22.6	19.0	22.4	19.6	22.4	20.2
35-44	21.4	21.0	21.4	21.0	21.4	21.1	21.2	21.8
45-54	18.1	21.7	18.1	21.9	18.2	21.8	18.2	21.0
55-64	11.9	16.0	11.9	16.0	11.9	16.2	11.9	15.3
65+	7.5	13.0	7.5	13.0	7.5	13.2	7.6	12.1
Gender, %								
Female	53.1	48.5	53.1	48.2	53.2	47.3	52.3	51.9
Male	46.9	51.5	46.9	51.8	46.8	52.7	47.7	48.1
Hispanic/Latino background, %								
Dominican	9.6	7.8	9.7	7.5	9.6	7.7	9.7	7.7
Central American	7.4	7.3	7.3	7.4	7.3	7.7	7.4	7.3
Cuban	21.4	17.0	21.4	16.8	21.2	17.7	21.7	16.4
Mexican	37.7	40.9	37.7	40.9	38.2	38.7	37.4	42.0
Puerto Rican	14.8	16.7	14.8	16.8	14.7	17.4	14.9	16.0
South American	5.0	5.4	5.0	5.5	5.0	5.5	4.9	5.9
More than one/Other	4.1	4.9	4.0	5.0	4.0	5.2	4.1	4.7
Center, %								
Bronx	26.9	24.7	26.9	24.5	26.7	25.5	27.1	24.0
Chicago	16.8	15.4	16.8	15.5	16.8	15.3	16.9	15.4
Miami	30.8	27.7	30.8	27.7	30.6	28.7	31.0	27.3
San Diego	25.5	32.3	25.5	32.3	25.9	30.5	25.0	33.3
Annual household income, %								
Missing	9.4	6.3	9.4	6.4	9.4	6.2	9.5	6.1
≤$10,000	13.3	12.0	13.2	12.3	13.2	12.2	13.1	12.7
$10,001-20,000	29.8	25.1	29.7	25.2	29.7	25.1	30.0	24.6
$20,001-40,000	23.2	23.3	23.3	22.9	23.3	23.0	23.3	23.1
$40,001-75,000	12.5	15.7	12.5	15.6	12.5	15.9	12.2	16.3
>$75,000	11.9	17.6	11.8	17.7	11.9	17.6	11.8	17.2
Education, %								
Less than high school	32.8	26.8	32.7	27.0	32.8	26.4	33.0	26.6
High school or equivalent	29.4	24.4	29.4	24.4	29.4	24.4	29.6	24.3
Greater than high school	37.8	48.8	37.9	48.6	37.8	49.3	37.4	49.1

TABLE V. (CONTINUED)

BASELINE CHARACTERISTICS ACCORDING TO SUPPLEMENT USE (N=15,081)[a]

	Copper Supplements		Manganese Supplements		Selenium Supplements		Zinc Supplements	
Characteristic	**Non-User**	**User**	**Non-User**	**User**	**Non-User**	**User**	**Non-User**	**User**
Years lived in mainland U.S., %								
Born in mainland U.S.	22.4	23.3	22.4	23.1	22.4	23.4	22.2	24.1
Less than 5	14.9	9.7	14.9	9.7	14.9	9.6	15.1	9.7
5-9	15.1	11.0	15.1	11.1	15.1	10.8	15.2	11.0
10-14	12.3	10.0	12.4	9.8	12.4	9.6	12.4	10.0
15-19	10.4	10.3	10.4	10.3	10.4	10.2	10.4	10.2
20 or more	24.9	35.8	24.8	36.0	24.9	36.4	24.8	35.0
Alcohol use, %								
No current use	48.6	47.4	48.6	47.2	48.7	46.6	48.5	47.7
Low/Moderate	45.0	47.9	45.0	48.1	44.9	48.7	45.0	47.6
Heavy	6.4	4.6	6.4	4.7	6.4	4.8	6.5	4.7
Cigarette smoking, %								
Never	61.2	62.4	61.3	62	61.4	61.7	61.2	62.5
Former	16.6	20.0	16.5	20.4	16.5	20.3	16.5	19.9
Current	22.2	17.5	22.2	17.5	22.1	18.0	22.3	17.6
Physical activity level, %								
Inactive	21.5	17.0	21.4	17.1	21.5	16.7	21.6	16.8
Low	12.5	12.4	12.5	12.3	12.4	12.5	12.3	13.1
Medium	11.0	9.4	11.0	9.4	11.0	9.4	11.0	9.8
High	55.0.	61.1	55.0	61.2	55.1	61.5	55.1	60.3

[a] Data are presented as weighted proportions.

The strongest linear correlation between usual total mineral intakes was for selenium and zinc (Pearson correlation coefficient = 0.62, Table VI). Analyses of food groups revealed usual total copper was most correlated with nuts and legumes, manganese with vegetables and nuts and legumes, selenium with meat (red or processed meats in particular), and zinc with dairy intakes (Table VII). The distributions of intake at baseline were mostly within the range between the estimated average requirements (EAR), or adequate intake (AI) for manganese, and tolerable upper intake levels (UL) determined by the Institute of Medicine (Table VIII).(61, 62)

2. Cross-Sectional Associations

Stratified analyses by age group, gender, Hispanic/Latino background, and supplement use did not reveal heterogeneity in point estimates, thus only pooled results are presented. In multivariable models, an inverse and linear trend was observed between usual total manganese intake and metabolic syndrome (Table IX), although only intakes in the highest quartile (≥4.34 mg/day) were associated with a significantly lower prevalence after adjustment for confounders (PR=0.84, 95% CI: 0.75-0.94, p_{trend} = 0.003). A similar pattern was observed for usual total zinc intakes ($PR_{Q4\ vs.\ Q1}$ =0.89, 95% CI: 0.81-0.98, p_{trend} = 0.007). The strongest inverse relationships for total manganese and zinc intakes with metabolic syndrome components were for low HDL cholesterol and abdominal obesity (Table X). Associations for total copper and selenium intakes with prevalent metabolic syndrome were largely null (Table IX).

TABLE VI.
PEARSON CORRELATION COEFFICIENTS BETWEEN USUAL TOTAL INTAKES AT BASELINE (N=15,081)

Mineral	Copper	Manganese	Selenium	Zinc
Copper	1.00	-	-	-
Manganese	0.33	1.00	-	-
Selenium	0.45	0.43	1.00	-
Zinc	0.50	0.38	0.62	1.00

TABLE VII.
PEARSON CORRELATION COEFFICIENTS BETWEEN FOOD GROUPS AND USUAL TOTAL INTAKES AT BASELINE (N=15,081)

Food group	Copper	Manganese	Selenium	Zinc
Vegetables	0.20	0.12	0.36	0.23
Whole fruits	0.11	0.06	-0.02	0.08
Grains	0.13	0.11	0.43	0.19
Whole grains	0.10	0.05	0.10	0.15
Meat	0.12	0.07	0.47	0.22
Red/processed meat	0.13	0.09	0.57	0.25
Nuts and legumes	0.21	0.12	0.38	0.22
Dairy	0.09	0.07	0.21	0.27

When evaluating intakes from food and beverages alone, the previously identified inverse association of manganese intake (from all sources) with metabolic syndrome was nullified (Table XI). However, individuals with dietary manganese in the third quartile (1.30-1.72 mg/day) had a slightly lower prevalence of abdominal obesity (Table XII, PR=0.93, 95% CI: 0.88-0.98). Estimates for dietary zinc also differed from those observed with total zinc intakes and were reversed in direction. Individuals with dietary zinc intakes above the 75th percentile (≥13.69 mg/day) were 21% (95% CI: 4-41%) more likely to have metabolic syndrome (Table XI). In particular, greater dietary zinc was marginally associated with prevalent high triglycerides and significantly associated with high fasting glucose (Table XII); these associations did not differ by zinc supplementation use (data not shown). In addition, a positive association of selenium intake from dietary sources was observed such that individuals with intakes above the median of 114.2 μg/day had a 15-32% (Table XI, $p_{trend} = 0.002$) higher likelihood of having metabolic syndrome, specifically a 23-42% higher likelihood of having high fasting glucose levels (Table XII, $p_{trend} < 0.001$) that did not differ by selenium supplementation (data not shown). With regards to cross-sectional associations with supplemental use, nearly all point estimates were significantly below the null for metabolic syndrome and each individual component (Tables XIII-XIV). The results from assessing

TABLE VIII.

DIETARY REFERENCE INTAKES AND ASSESSMENT BASED ON USUAL TOTAL INTAKES AT BASELINE (N=15,081)

Mineral	**EAR/AI**[a]	**% < EAR/AI**	**UL**	**% > UL**
Copper	18 years: 0.685 mg/day ≥19 years: 0.7 mg/day	4.70	18 years: 8 mg/day ≥19 years: 10 mg/day	0.03
Manganese	Females, 18 years: 1.6 mg/day Females, ≥19 year: 1.8 mg/day Males, 18 years: 2.2 mg/day Males, ≥19 year: 2.3 mg/day	15.68	18 years: 9 mg/day ≥19 years: 11 mg/day	1.67
Selenium	≥18 years: 45 μg/day	0.27	≥18 years: 400 μg/day	0.09
Zinc	Females, 18 years: 7.3 mg/day Females, ≥19 year: 6.8 mg/day Males, 18 years: 8.1 mg/day Males, ≥19 year: 9.4 mg/day	12.63	18 years: 34 mg/day ≥19 years: 40 mg/day	1.57

[a] All minerals have an estimated average requirement (EAR) except for manganese which only has an adequate intake (AI).

TABLE IX.
PREVALENCE RATIOS (95% CI) FOR METABOLIC SYNDROME ACCORDING TO QUARTILES OF USUAL TOTAL INTAKE (N=15,081)

Usual total mineral intake	Quartile 1	Quartile 2	Quartile 3	Quartile 4	p_{trend}
Copper (mg/day)	0.25-1.00	1.01-1.29	1.30-1.72	1.72-24.18	
Prevalent cases, unweighted	1,313	1,841	1,474	1,061	
Crude model	1.00 (ref.)	0.96 (0.89-1.03)	1.01 (0.94-1.09)	0.98 (0.89-1.07)	0.956
Adjusted model 1[a]	1.00 (ref.)	0.97 (0.89-1.06)	1.03 (0.93-1.13)	0.91 (0.82-1.01)	0.091
Adjusted model 2 [b]	1.00 (ref.)	0.97 (0.89-1.06)	1.03 (0.93-1.13)	0.94 (0.84-1.04)	0.303
Manganese (mg/day)	0.27-2.29	2.30-3.12	3.13-4.33	4.34-81.57	
Prevalent cases, unweighted	1,123	1,865	1,708	993	
Crude model	1.00 (ref.)	1.01 (0.93-1.08)	1.02 (0.93-1.12)	0.96 (0.87-1.05)	0.487
Adjusted model 1[a]	1.00 (ref.)	0.98 (0.90-1.05)	0.93 (0.84-1.04)	0.80 (0.72-0.90)	<0.001
Adjusted model 2[b]	1.00 (ref.)	0.98 (0.91-1.06)	0.96 (0.86-1.07)	0.84 (0.75-0.94)	0.003
Selenium (µg/day)	18.1-93.2	93.3-118.0	118.1-149.2	149.3-684.0	
Prevalent cases, unweighted	1,853	1,778	1,312	746	
Crude model	1.00 (ref.)	0.84 (0.77-0.92)	0.83 (0.77-0.89)	0.73 (0.67-0.80)	<0.001
Adjusted model 1[a]	1.00 (ref.)	0.94 (0.86-1.03)	0.97 (0.88-1.07)	0.89 (0.78-1.01)	0.127
Adjusted model 2[b]	1.00 (ref.)	0.94 (0.86-1.04)	0.98 (0.89-1.08)	0.90 (0.79-1.03)	0.220
Zinc (mg/day)	1.39-9.02	9.03-11.73	111.74-15.66	15.67-138.59	
Prevalent cases, unweighted	1,656	1,763	1,250	1,020	
Crude model	1.00 (ref.)	0.93 (0.86-1.02)	0.87 (0.80-0.94)	0.85 (0.77-0.93)	<0.001
Adjusted model 1[a]	1.00 (ref.)	1.00 (0.91-1.09)	1.02 (0.92-1.14)	0.87 (0.79-0.95)	0.001
Adjusted model 2[b]	1.00 (ref.)	1.01 (0.92-1.10)	1.02 (0.92-1.14)	0.89 (0.81-0.98)	0.007

[a] Adjusted for total energy intake (usual kilocalories per day from food/beverages and supplements, continuous), age (continuous), gender (male or female), and Hispanic/Latino background (Central American, Cuban, Dominican Mexican, Puerto Rican, South American, or more than one/other).
[b] Same as Adjusted model 1, with the addition of study center (Bronx, Chicago, Miami, or San Diego), education (less than high school, high school or equivalent, or greater than high school), years lived in the mainland U.S. (born in mainland, <5, 5-9, 10-14, 15-19, or ≥20), alcohol use level (no current use, low/moderate, or high), cigarette smoking (never, former, or current), and physical activity level (inactive, low, medium, or high).

TABLE X.

ADJUSTED PREVALENCE RATIOS (95% CI) FOR METABOLIC SYNDROME COMPONENTS ACCORDING TO QUARTILES OF USUAL TOTAL INTAKE (N=15,081)[a]

Usual total mineral intake	High Blood Pressure	High Triglycerides	Low HDL	High Fasting Glucose	Abdominal Obesity
Prevalent cases, unweighted	5,663	4,836	6,531	5,359	9,173
Copper (mg/day)					
Quartile 1	1.00 (ref.)	1.00 (ref.)	1.00 (ref.)	1.00 (ref.)	1.00 (ref.)
Quartile 2	0.97 (0.89-1.05)	1.03 (0.93-1.15)	0.98 (0.93-1.05)	1.02 (0.93-1.12)	0.99 (0.94-1.05)
Quartile 3	0.97 (0.88-1.07)	1.08 (0.96-1.20)	0.92 (0.84-1.01)	1.03 (0.94-1.14)	0.97 (0.92-1.04)
Quartile 4	1.00 (0.91-1.10)	1.10 (0.97-1.26)	0.86 (0.78-0.95)	0.98 (0.87-1.09)	0.87 (0.81-0.93)
P_{trend}	0.724	0.114	<0.001	0.487	<0.001
Manganese (mg/day)					
Quartile 1	1.00 (ref.)	1.00 (ref.)	1.00 (ref.)	1.00 (ref.)	1.00 (ref.)
Quartile 2	1.02 (0.95-1.10)	0.97 (0.86-1.09)	0.99 (0.93-1.05)	1.04 (0.96-1.12)	0.95 (0.91-0.99)
Quartile 3	1.03 (0.95-1.12)	0.98 (0.88-1.10)	0.91 (0.84-0.98)	1.00 (0.90-1.10)	0.93 (0.87-0.98)
Quartile 4	0.97 (0.88-1.07)	1.02 (0.88-1.18)	0.85 (0.78-0.93)	0.92 (0.82-1.04)	0.84 (0.79-0.90)
P_{trend}	0.477	0.584	<0.001	0.074	<0.001
Selenium (µg/day)					
Quartile 1	1.00 (ref.)	1.00 (ref.)	1.00 (ref.)	1.00 (ref.)	1.00 (ref.)
Quartile 2	0.95 (0.89-1.02)	0.96 (0.87-1.07)	0.89 (0.83-0.96)	0.98 (0.90-1.08)	0.99 (0.95-1.04)
Quartile 3	0.96 (0.88-1.05)	0.93 (0.82-1.07)	0.90 (0.83-0.98)	1.08 (0.98-1.19)	0.97 (0.92-1.03)
Quartile 4	0.95 (0.85-1.05)	0.89 (0.74-1.07)	0.80 (0.70-0.91)	1.09 (0.97-1.22)	0.93 (0.85-1.01)
P_{trend}	0.369	0.206	0.002	0.050	0.097
Zinc (mg/day)					
Quartile 1	1.00 (ref.)	1.00 (ref.)	1.00 (ref.)	1.00 (ref.)	1.00 (ref.)
Quartile 2	0.94 (0.87-1.02)	1.04 (0.93-1.16)	1.01 (0.95-1.08)	1.04 (0.96-1.12)	0.98 (0.93-1.02)
Quartile 3	1.01 (0.91-1.13)	1.00 (0.88-1.13)	0.88 (0.811-0.95)	0.99 (0.90-1.08)	0.99 (0.93-1.06)
Quartile 4	0.96 (0.89-1.04)	0.96 (0.84-1.11)	0.87 (0.79-0.95)	0.92 (0.85-1.01)	0.90 (0.85-0.95)
P_{trend}	0.728	0.334	<0.001	0.010	0.002

[a] Adjusted for total energy intake (usual kilocalories per day from food/beverages and supplements, continuous), age (continuous), gender (male or female), Hispanic/Latino background (Central American, Cuban, Dominican , Mexican, Puerto Rican, South American, or more than one/other), study center (Bronx, Chicago, Miami, or San Diego), education (less than high school, high school or equivalent, or greater than high school), years lived in the mainland U.S. (born in mainland, <5, 5-9, 10-14, 15-19, or ≥20), alcohol use level (no current use, low/moderate, or high), cigarette smoking (never, former, or current), and physical activity level (inactive, low, medium, or high).

TABLE XI.
PREVALENCE RATIOS (95% CI) FOR METABOLIC SYNDROME ACCORDING TO QUARTILES OF USUAL DIETARY INTAKE ONLY (N=15,081)

Usual dietary mineral intake	Quartile 1	Quartile 2	Quartile 3	Quartile 4	p_{trend}
Copper (mg/day)	0.25-0.97	0.98-1.22	1.23-1.54	1.55-6.41	
Prevalent cases, unweighted	1,290	1,915	1,666	818	
Crude model	1.00 (ref.)	0.93 (0.87-0.99)	0.98 (0.92-1.06)	0.86 (0.79-0.94)	0.016
Adjusted model 1[a]	1.00 (ref.)	0.95 (0.88-1.03)	1.06 (0.96-1.17)	1.02 (0.88-1.18)	0.287
Adjusted model 2[b]	1.00 (ref.)	0.95 (0.87-1.03)	1.05 (0.95-1.17)	1.01 (0.88-1.17)	0.343
Manganese (mg/day)	0.27-1.00	1.01-1.29	1.30-1.72	1.73-33.62	
Prevalent cases, unweighted	1,124	1,933	1,810	822	
Crude model	1.00 (ref.)	0.99 (0.91-1.07)	0.95 (0.87-1.03)	0.94 (0.85-1.03)	0.098
Adjusted model 1[a]	1.00 (ref.)	0.96 (0.88-1.06)	0.90 (0.81-0.99)	0.91 (0.78-1.06)	0.104
Adjusted model 2[b]	1.00 (ref.)	0.96 (0.88-1.04)	0.91 (0.82-1.01)	0.93 (0.79-1.08)	0.200
Selenium (µg/day)	18.1-91.2	91.3-114.1	114.2-141.7	141.8-381.1	
Prevalent cases, unweighted	1,878	1,874	1,258	679	
Crude model	1.00 (ref.)	0.80 (0.74-0.87)	0.76 (0.70-0.82)	0.72 (0.67-0.78)	<0.001
Adjusted model 1[a]	1.00 (ref.)	1.00 (0.92-1.10)	1.16 (1.01-1.32)	1.34 (1.13-1.59)	0.002
Adjusted model 2[b]	1.00 (ref.)	1.00 (0.91-1.09)	1.15 (1.01 -1.31)	1.32 (1.11-1.56)	0.002
Zinc (mg/day)	2.00-8.62	8.62-10.90	10.91-13.68	13.69-68.50	
Prevalent cases, unweighted	1,668	1,860	1,421	740	
Crude model	1.00 (ref.)	0.86 (0.80-0.92)	0.84 (0.78-0.90)	0.75 (0.69-0.81)	<0.001
Adjusted model 1[a]	1.00 (ref.)	1.00 (0.92-1.09)	1.13 (1.01 -1.26)	1.24 (1.07-1.44)	0.002
Adjusted model 2[b]	1.00 (ref.)	1.00 (0.92-1.09)	1.11 (0.99-1.23)	1.21 (1.04-1.41)	0.008

[a] Adjusted for supplement use (i.e. use of any supplement containing copper, manganese, selenium or zinc), total energy intake (usual kilocalories per day from food/beverages and supplements, continuous), age (continuous), gender (male or female), and Hispanic/Latino background (Central American, Cuban, Dominican , Mexican, Puerto Rican, South American, or more than one/other).
[b] Same as Adjusted model 1, with the addition of study center (Bronx, Chicago, Miami, or San Diego), education (less than high school, high school or equivalent, or greater than high school), years lived in the mainland U.S. (born in mainland, <5, 5-9, 10-14, 15-19, or ≥20), alcohol use level (no current use, low/moderate, or high), cigarette smoking (never, former, or current), and physical activity level (inactive, low, medium, or high).

TABLE XII.

ADJUSTED PREVALENCE RATIOS (95% CI) FOR METABOLIC SYNDROME COMPONENTS ACCORDING TO QUARTILES OF USUAL DIETARY INTAKE ONLY (N=15,081)[a]

Usual dietary mineral intake	High Blood Pressure	High Triglycerides	Low HDL	High Fasting Glucose	Abdominal Obesity
Prevalent cases, unweighted	5,663	4,836	6,531	5,359	9,173
Copper (mg/day)					
Quartile 1	1.00 (ref.)	1.00 (ref.)	1.00 (ref.)	1.00 (ref.)	1.00 (ref.)
Quartile 2	0.93 (0.86-1.02)	1.04 (0.93-1.16)	0.99 (0.93-1.05)	1.02 (0.94-1.11)	0.98 (0.93-1.03)
Quartile 3	0.92 (0.83-1.03)	1.11 (0.98-1.25)	1.02 (0.94-1.12)	1.07 (0.95-1.19)	1.00 (0.94-1.06)
Quartile 4	0.90 (0.78-1.03)	1.20 (1.03-1.40)	1.00 (0.90-1.11)	1.11 (0.96-1.27)	0.97 (0.88-1.08)
P_{trend}	0.162	0.010	0.719	0.145	0.810
Manganese (mg/day)					
Quartile 1	1.00 (ref.)	1.00 (ref.)	1.00 (ref.)	1.00 (ref.)	1.00 (ref.)
Quartile 2	1.03 (0.96-1.12)	1.00 (0.89-1.12)	0.99 (0.92-1.05)	1.02 (0.94-1.12)	0.96 (0.92-1.01)
Quartile 3	1.05 (0.96-1.15)	0.95 (0.84-1.06)	0.96 (0.89-1.04)	0.98 (0.89-1.08)	0.93 (0.88-0.98)
Quartile 4	1.00 (0.88-1.13)	1.06 (0.90-1.25)	1.01 (0.91-1.12)	1.01 (0.87-1.16)	0.94 (0.87-1.02)
P_{trend}	0.878	0.705	0.764	0.767	0.044
Selenium (µg/day)					
Quartile 1	1.00 (ref.)	1.00 (ref.)	1.00 (ref.)	1.00 (ref.)	1.00 (ref.)
Quartile 2	0.95 (0.89-1.01)	0.96 (0.86-1.07)	0.92 (0.86-0.98)	1.05 (0.96-1.15)	1.03 (0.98-1.07)
Quartile 3	1.02 (0.92-1.14)	1.00 (0.87-1.14)	0.94 (0.86-1.02)	1.23 (1.09-1.39)	1.06 (0.98-1.15)
Quartile 4	1.13 (0.98-1.29)	1.09 (0.91-1.32)	0.89 (0.77-1.03)	1.46 (1.24-1.72)	1.12 (0.978-1.28)
P_{trend}	0.116	0.352	0.113	<0.001	0.089
Zinc (mg/day)					
Quartile 1	1.00 (ref.)	1.00 (ref.)	1.00 (ref.)	1.00 (ref.)	1.00 (ref.)
Quartile 2	0.92 (0.85-0.99)	1.05 (0.94-1.18)	1.03 (0.97-1.10)	1.00 (0.93-1.09)	0.99 (0.94-1.03)
Quartile 3	0.93 (0.83-1.05)	1.10 (0.96-1.27)	0.96 (0.88-1.04)	1.04 (0.93-1.16)	1.03 (0.97-1.10)
Quartile 4	0.93 (0.79-1.08)	1.20 (0.98-1.45)	0.98 (0.87-1.10)	1.20 (1.03-1.39)	1.05 (0.96-1.15)
P_{trend}	0.390	0.067	0.388	0.023	0.215

[a] Adjusted for supplement use (i.e., use of any supplement containing copper, manganese, selenium or zinc), total energy intake (usual kilocalories per day from food/beverages and supplements, continuous), age (continuous), gender (male or female), Hispanic/Latino background (Central American, Cuban, Dominican , Mexican, Puerto Rican, South American, or more than one/other), study center (Bronx, Chicago, Miami, or San Diego), education (less than high school, high school or equivalent, or greater than high school), years lived in the mainland U.S. (born in mainland, <5, 5-9, 10-14, 15-19, or ≥20), alcohol use level (no current use, low/moderate, or high), cigarette smoking (never, former, or current), and physical activity level (inactive, low, medium, or high).

TABLE XIII.
PREVALENCE RATIOS (95% CI) FOR METABOLIC SYNDROME BY SUPPLEMENT USE (N=15,081)

Supplement use	Non-User	User
Copper		
Prevalent cases, unweighted	4,657	1,032
Crude model	1.00 (ref.)	0.96 (0.88-1.06)
Adjusted model 1[a]	1.00 (ref.)	0.82 (0.75-0.88)
Adjusted model 2[b]	1.00 (ref.)	0.84 (0.77-0.91)
Manganese		
Prevalent cases, unweighted	4,654	1,035
Crude model	1.00 (ref.)	0.98 (0.90-1.07)
Adjusted model 1[a]	1.00 (ref.)	0.83 (0.77-0.90)
Adjusted model 2[b]	1.00 (ref.)	0.85 (0.79-0.92)
Selenium		
Prevalent cases, unweighted	4,720	969
Crude model	1.00 (ref.)	0.97 (0.88-1.06)
Adjusted model 1[a]	1.00 (ref.)	0.80 (0.74-0.86)
Adjusted model 2[b]	1.00 (ref.)	0.82 (0.76-0.89)
Zinc		
Prevalent cases, unweighted	4,563	1,126
Crude model	1.00 (ref.)	0.94 (0.86-1.03)
Adjusted model 1[a]	1.00 (ref.)	0.81 (0.75-0.87)
Adjusted model 2[b]	1.00 (ref.)	0.83 (0.77-0.89)

[a] Adjusted for usual dietary intakes of the respective mineral counterpart (copper [mg/day], manganese [mg/day], selenium [µg/day], or zinc [mg/day], continuous), total energy intake (usual kilocalories per day from food/beverages and supplements, continuous), age (continuous), gender (male or female), and Hispanic/Latino background (Central American, Cuban, Dominican, Mexican, Puerto Rican, South American, or more than one/other).
[b] Same as Adjusted model 1, with the addition of study center (Bronx, Chicago, Miami, or San Diego), education (less than high school, high school or equivalent, or greater than high school), years lived in the mainland U.S. (born in mainland, <5, 5-9, 10-14, 15-19, or ≥20), alcohol use level (no current use, low/moderate, or high), cigarette smoking (never, former, or current), and physical activity level (inactive, low, medium, or high).

TABLE XIV.

ADJUSTED PREVALENCE RATIOS (95% CI) FOR METABOLIC SYNDROME COMPONENTS BY SUPPLEMENT USE (N=15,081)[a]

Supplement use	High Blood Pressure	High Triglycerides	Low HDL	High Fasting Glucose	Abdominal Obesity
Prevalent cases, unweighted	5,663	4,836	6,531	5,359	9,173
Copper					
Non-user	1.00 (ref.)	1.00 (ref.)	1.00 (ref.)	1.00 (ref.)	1.00 (ref.)
User	0.92 (0.87-0.99)	0.99 (0.91-1.09)	0.87 (0.81-0.94)	0.87 (0.81-0.94)	0.88 (0.83-0.92)
Manganese					
Non-user	1.00 (ref.)	1.00 (ref.)	1.00 (ref.)	1.00 (ref.)	1.00 (ref.)
User	0.93 (0.88-0.99)	1.01 (0.92-1.12)	0.87 (0.81-0.94)	0.89 (0.83-0.95)	0.88 (0.84-0.93)
Selenium					
Non-user	1.00 (ref.)	1.00 (ref.)	1.00 (ref.)	1.00 (ref.)	1.00 (ref.)
User	0.92 (0.86-0.99)	0.99 (0.90-1.08)	0.88 (0.81-0.95)	0.85 (0.78-0.92)	0.86 (0.82-0.90)
Zinc					
Non-user	1.00 (ref.)	1.00 (ref.)	1.00 (ref.)	1.00 (ref.)	1.00 (ref.)
User	0.94 (0.88-0.99)	0.97 (0.89-1.06)	0.88 (0.82-0.94)	0.85 (0.80-0.91)	0.88 (0.84-0.92)

[a] Adjusted for usual dietary intakes of the respective mineral counterpart (copper [mg/day], manganese [mg/day], selenium [µg/day], or zinc [mg/day], continuous), total energy intake (usual kilocalories per day from food/beverages and supplements, continuous), age (continuous), gender (male or female), and Hispanic/Latino background (Central American, Cuban, Dominican, Mexican, Puerto Rican, South American, or more than one/other), study center (Bronx, Chicago, Miami, or San Diego), education (less than high school, high school or equivalent, or greater than high school), years lived in the mainland U.S. (born in mainland, <5, 5-9, 10-14, 15-19, or ≥20), alcohol use level (no current use, low/moderate, or high), cigarette smoking (never, former, or current), and physical activity level (inactive, low, medium, or high).

combined intakes of the selected minerals suggested higher total intakes were linearly associated with a lower prevalence of low HDL cholesterol and abdominal obesity ($p_{trend} < 0.001$ and $p_{trend} = 0.006$, respectively, Table XV). Point estimates for joint dietary intakes were positively and linearly related to prevalent metabolic syndrome (Table XV, $p_{trend} = 0.007$), and specifically with high fasting glucose (Table XV, $p_{trend} = 0.010$) likely due to the previously described associations of dietary selenium intake, whereas those for use of any supplements were consistently below the null (Table XV).

3. Prospective Associations

The median follow-up time was 5.8 years (range: 4.1-8.5). The incidence rate of metabolic syndrome was 35.4 cases per 1,000 person-years, with abdominal obesity being the most common component condition (Table XVI). In these preliminary analyses, usual total zinc intakes above the median (11.84 mg/day) were associated with a 24-32% lower risk of developing metabolic syndrome after adjusting for energy intake, sociodemographic, and lifestyle factors ($p_{trend} = 0.067$; Table XVII; Figure 5, Appendix A). The specific metabolic syndrome components inversely associated with total zinc intakes included high blood pressure ($p_{trend} = 0.002$) and high fasting glucose levels ($p_{trend} = 0.027$; Table XVIII; Figure 5, Appendix A). We additionally observed linear negative associations for total copper intakes with the risk of high blood pressure ($p_{trend} = 0.012$) and for total manganese intakes with the risk of high fasting glucose ($p_{trend} = 0.012$; Table XVIII; Figure 5, Appendix A). Total selenium intakes in the highest quartile (≥149.2 μg/day) were associated with a 70% greater risk of high triglycerides (95% CI: 4-278%;Table XVIII; Figure 5, Appendix A) and were marginally associated with a greater risk of low HDL cholesterol (IRR=1.56, 95% CI: 0.95-2.55; Table XVIII; Figure 5, Appendix A). To explore if the heightened risks of dyslipidemia were due to individuals consuming adequate amounts of selenium from dietary sources coupled with supplementation, we tested interaction terms between quartiles of usual dietary selenium intake and supplemental intake in incident high triglycerides and low HDL models. Neither term was statistically significant (data not shown). Further, we did not observe any effect modification by age, gender, or Hispanic/Latino backgrounds.

TABLE XV.

PREVALENCE RATIOS (95% CI) FOR METABOLIC SYNDROME AND COMPONENTS ACCORDING TO COMBINED INTAKES OF COPPER, MANGANESE, SELENIUM, AND ZINC (N=15,081)

	Metabolic Syndrome	High Blood Pressure	High Triglycerides	Low HDL	High Fasting Glucose	Abdominal Obesity
Usual total intake[a]						
Low intake of all 4	1.00 (ref.)	1.00 (ref.)	1.00 (ref.)	1.00 (ref.)	1.00 (ref.)	1.00 (ref.)
High intake of any 1	0.98 (0.89-1.08)	1.01 (0.92-1.10)	1.02 (0.92-1.13)	0.94 (0.87-1.02)	1.04 (0.94-1.14)	0.98 (0.93-1.03)
High intake of any 2	0.92 (0.83-1.02)	0.96 (0.87-1.05)	0.99 (0.88-1.11)	0.96 (0.88-1.04)	1.00 (0.90-1.11)	0.93 (0.87-1.00)
High intake of any 3	0.96 (0.89-1.05)	1.08 (0.99-1.17)	0.96 (0.86-1.08)	0.84 (0.77-0.91)	1.02 (0.93-1.11)	0.95 (0.89-1.00)
High intake of all 4	0.95 (0.87-1.03)	0.99 (0.91-1.08)	1.01 (0.90-1.14)	0.85 (0.78-0.92)	0.97 (0.89-1.05)	0.91 (0.85-0.96)
P_{trend}	0.219	0.718	0.929	<0.001	0.432	<0.001
Usual dietary intake[b]						
Low intake of all 4	1.00 (ref.)	1.00 (ref.)	1.00 (ref.)	1.00 (ref.)	1.00 (ref.)	1.00 (ref.)
High intake of any 1	0.98 (0.90-1.08)	1.00 (0.92-1.10)	1.04 (0.94-1.16)	0.97 (0.91-1.04)	1.03 (0.94-1.13)	0.97 (0.93-1.02)
High intake of any 2	0.97 (0.88-1.08)	0.96 (0.88-1.06)	1.04 (0.94-1.15)	0.95 (0.89-1.02)	1.02 (0.91-1.13)	0.97 (0.91-1.03)
High intake of any 3	1.07 (0.96-1.19)	1.01 (0.91-1.14)	1.04 (0.94-1.15)	0.96 (0.88-1.05)	1.04 (0.93-1.16)	1.01 (0.94-1.08)
High intake of all 4	1.18 (1.05-1.32)	1.05 (0.93-1.19)	1.08 (0.96-1.21)	0.98 (0.89-1.08)	1.13 (0.99-1.28)	1.02 (0.94-1.12)
P_{trend}	0.007	0.515	0.242	0.558	0.010	0.645
Supplement use[c]						
Non-user	1.00 (ref.)	1.00 (ref.)	1.00 (ref.)	1.00 (ref.)	1.00 (ref.)	1.00 (ref.)
Use of any	0.83 (0.77-0.90)	0.93 (0.87-0.98)	0.97 (0.88-1.06)	0.89 (0.83-0.95)	0.85 (0.79-0.91)	0.89 (0.85-0.94)

[a] Adjusted for total energy intake (usual kilocalories per day from food/beverages and supplements, continuous), age (continuous), gender (male or female), Hispanic/Latino background (Central American, Cuban, Dominican , Mexican, Puerto Rican, South American, or more than one/other), center (Bronx, Chicago, Miami, or San Diego), education (less than high school, high school or equivalent, or greater than high school), years lived in the mainland U.S. (born in mainland, <5, 5-9, 10-14, 15-19, or ≥20), alcohol use level (no current use, low/moderate, or high), cigarette smoking (never, former, or current), and physical activity level (inactive, low, medium, or high).

[b] Adjusted for the variables listed above ([a]), with additional adjustment for use of any supplement containing copper, manganese, selenium or zinc.

[c] Adjusted for the variables listed above ([a]), with additional adjustment for usual dietary intakes of copper (mg/day, continuous), manganese (mg/day, continuous), selenium (µg/day, continuous), and zinc (mg/day, continuous); supplement use refers to any dietary supplements containing copper, manganese, selenium, or zinc.

TABLE XVI.
INCIDENCE RATES FOR METABOLIC SYNDROME AND COMPONENTS

Condition	Participants[a]	Incidence Rate[b]
Metabolic syndrome	5,090	35.4
High blood pressure	3,961	31.3
High triglycerides	4,428	21.3
Low HDL	3,807	25.2
High fasting glucose	4,295	41.3
Abdominal obesity	2,652	42.4

[a] All events are incident, thus the number of analyzed participants varies by the individual component condition.

[b] Rates are expressed per 1,000 person-years at risk.

TABLE XVII.
INCIDENCE RATE RATIOS (95% CI) FOR METABOLIC SYNDROME ACCORDING TO QUARTILES OF USUAL TOTAL INTAKE (N=5,090)

Usual total mineral intake	Quartile 1	Quartile 2	Quartile 3	Quartile 4	P_{trend}
Copper (mg/day)	0.28-1.01	1.02-1.31	1.32-1.76	1.77-18.02	
Incident cases, unweighted	246	428	306	253	
Crude model	1.00 (ref.)	1.01 (0.81-1.25)	0.98 (0.79-1.21)	1.00 (0.78-1.28)	0.894
Adjusted model 1[a]	1.00 (ref.)	0.95 (0.75-1.21)	0.96 (0.74-1.25)	0.87 (0.66-1.16)	0.350
Adjusted model 2[b]	1.00 (ref.)	0.96 (0.75-1.22)	0.95 (0.72-1.24)	0.89 (0.66-1.19)	0.415
Manganese (mg/day)	0.43-2.34	2.35-3.18	3.19-4.42	4.43-61.97	
Incident cases, unweighted	231	422	333	247	
Crude model	1.00 (ref.)	1.01 (0.83-1.24)	0.90 (0.73-1.11)	1.01 (0.78-1.30)	0.727
Adjusted model 1[a]	1.00 (ref.)	0.92 (0.74-1.13)	0.79 (0.61-1.03)	0.80 (0.59-1.08)	0.120
Adjusted model 2[b]	1.00 (ref.)	0.92 (0.74-1.14)	0.82 (0.63-1.07)	0.84 (0.62-1.14)	0.230
Selenium (µg/day)	22.2-93.2	93.3-117.4	117.5-149.1	149.2-654.9	
Incident cases, unweighted	358	405	263	207	
Crude model	1.00 (ref.)	0.80 (0.66-0.96)	0.72 (0.58-0.89)	0.84 (0.68-1.04)	0.065
Adjusted model 1[a]	1.00 (ref.)	0.90 (0.73-1.10)	0.87 (0.67-1.13)	1.10 (0.82-1.48)	0.717
Adjusted model 2[b]	1.00 (ref.)	0.92 (0.75-1.13)	0.91 (0.70-1.18)	1.20 (0.89-1.60)	0.390
Zinc (mg/day)	1.55-9.02	9.03-11.84	11.85-16.13	16.14-135.78	
Incident cases, unweighted	335	381	261	256	
Crude model	1.00 (ref.)	0.81 (0.67-0.97)	0.69 (0.56-0.85)	0.80 (0.64-1.01)	0.025
Adjusted model 1[a]	1.00 (ref.)	0.81 (0.66-0.99)	0.69 (0.52-0.92)	0.74 (0.57-0.97)	0.046
Adjusted model 2[b]	1.00 (ref.)	0.81 (0.66-1.00)	0.68 (0.51-0.91)	0.76 (0.57-0.99)	0.067

[a] Adjusted for total energy intake (usual kilocalories per day from food/beverages and supplements, continuous), age (continuous), gender (male or female), and Hispanic/Latino background (Central American, Cuban, Dominican, Mexican, Puerto Rican, South American, or more than one/other).

[b] Same as Adjusted model 1, with the addition of study center (Bronx, Chicago, Miami, or San Diego), education (less than high school, high school or equivalent, or greater than high school), years lived in the mainland U.S. (born in mainland, <5, 5-9, 10-14, 15-19, or ≥20), alcohol use level (no current use, low/moderate, or high), cigarette smoking (never, former, or current), and physical activity level (inactive, low, medium, or high).

TABLE XVIII.

ADJUSTED INCIDENCE RATE RATIOS (95% CI) FOR METABOLIC SYNDROME COMPONENTS ACCORDING TO QUARTILES OF USUAL TOTAL INTAKE[a]

Usual total mineral intake	High Blood Pressure	High Triglycerides	Low HDL	High Fasting Glucose	Abdominal Obesity
Participants, unweighted[b]	3,961	4,428	3,807	4,295	2,652
Incident cases, unweighted	885	531	552	1,185	708
Copper (mg/day)					
Quartile 1	1.00 (ref.)	1.00 (ref.)	1.00 (ref.)	1.00 (ref.)	1.00 (ref.)
Quartile 2	0.83 (0.66-1.05)	1.31 (0.92-1.86)	1.28 (0.92-1.77)	0.97 (0.74-1.26)	0.84 (0.64-1.09)
Quartile 3	0.77 (0.59-1.01)	1.37 (0.89-2.11)	1.19 (0.78-1.80)	0.97 (0.74-1.27)	0.96 (0.70-1.32)
Quartile 4	0.68 (0.50-0.91)	1.51 (0.96-2.36)	1.25 (0.84-1.86)	0.79 (0.60-1.05)	0.77 (0.57-1.06)
P_{trend}	0.012	0.120	0.473	0.057	0.197
Manganese (mg/day)					
Quartile 1	1.00 (ref.)	1.00 (ref.)	1.00 (ref.)	1.00 (ref.)	1.00 (ref.)
Quartile 2	0.93 (0.74-1.16)	1.35 (0.98-1.87)	0.97 (0.69-1.36)	0.96 (0.73-1.26)	1.08 (0.84-1.39)
Quartile 3	0.99 (0.77-1.28)	1.22 (0.84-1.78)	0.96 (0.68-1.36)	0.75 (0.56-1.01)	0.87 (0.65-1.17)
Quartile 4	0.80 (0.59-1.08)	1.30 (0.80-2.13)	1.15 (0.76-1.73)	0.73 (0.53-1.01)	0.77 (0.54-1.11)
P_{trend}	0.175	0.528	0.471	0.012	0.059
Selenium (µg/day)					
Quartile 1	1.00 (ref.)	1.00 (ref.)	1.00 (ref.)	1.00 (ref.)	1.00 (ref.)
Quartile 2	0.92 (0.73-1.16)	1.15 (0.80-1.63)	1.36 (0.94-1.96)	0.86 (0.67-1.11)	1.08 (0.84-1.38)
Quartile 3	0.91 (0.69-1.18)	1.50 (0.96-2.33)	1.52 (0.97-2.38)	0.93 (0.70-1.23)	0.94 (0.68-1.32)
Quartile 4	0.82 (0.56-1.20)	1.70 (1.04-2.78)	1.56 (0.95-2.55)	0.87 (0.70-1.23)	1.08 (0.75-1.55)
P_{trend}	0.323	0.029	0.072	0.583	0.905
Zinc (mg/day)					
Quartile 1	1.00 (ref.)	1.00 (ref.)	1.00 (ref.)	1.00 (ref.)	1.00 (ref.)
Quartile 2	0.79 (0.65-0.95)	0.96 (0.68-1.35)	1.17 (0.86-1.60)	0.88 (0.70-1.11)	1.06 (0.84-1.35)
Quartile 3	0.68 (0.53-0.87)	1.09 (0.70-1.70)	0.89 (0.60-1.32)	0.95 (0.74-1.24)	1.02 (0.74-1.39)
Quartile 4	0.63 (0.48-0.83)	1.04 (0.67-1.61)	1.01 (0.72-1.41)	0.74 (0.56-0.96)	0.89 (0.66-1.20)
P_{trend}	0.002	0.772	0.682	0.027	0.302

[a] Adjusted for total energy intake (usual kilocalories per day from food/beverages and supplements, continuous), age (continuous), gender (male or female), Hispanic/Latino background (Central American, Cuban, Dominican, Mexican, Puerto Rican, South American, or more than one/other), study center (Bronx, Chicago, Miami, or San Diego), education (less than high school, high school or equivalent, or greater than high school), years lived in the mainland U.S. (born in mainland, <5, 5-9, 10-14, 15-19, or ≥20), alcohol use level (no current use, low/moderate, or high), cigarette smoking (never, former, or current), and physical activity level (inactive, low, medium, or high).

[b] All events are incident, thus the number of participants analyzed varies with the individual component condition.

Mineral intakes from food and beverages alone were not associated with significant differences in metabolic syndrome risk (Table XIX; Figure 6, Appendix A), although dietary copper intakes in quartiles 3 and 4 were associated with a 32-36% lower risk of high blood pressure (p_{trend} = 0.026; Table XX) but also a 64-69% higher risk of high triglycerides (p_{trend} = 0.046, Table XX). Incidence rate ratios for supplement use status were suggestive of protective associations, but the confidence intervals were wide and all overlapped the null value (Tables XXI-XXII; Figure 7, Appendix A). No obvious patterns were seen when assessing intakes or supplement use jointly (Table XXIII).

D. Discussion

We observed inverse cross-sectional relationships of total manganese and zinc intakes with prevalent metabolic syndrome, low HDL cholesterol, and abdominal obesity; these associations appeared to be driven by supplementation rather than intakes from dietary sources. Prospective analyses suggested intakes of these minerals may confer protective effects, but the reductions in risk were observed for contrasting conditions (manganese with high fasting glucose, zinc with high blood pressure and high fasting glucose). We further observed greater copper intakes were associated with a lower risk of high blood pressure. Comparing intakes from food and beverages versus supplementation did not provide clear answers as to whether either source was more important for lowering risks over time, likely due to limited statistical power of the preliminary data. Taken together, the results may suggest individuals who are obese and/or have altered lipid profiles could be modifying their diets or initiating supplement use in response to a diagnosis or in an attempt to lose weight. While these lifestyle changes may not reduce the likelihood of becoming abdominally obese or developing dyslipidemia, increasing intakes of copper, manganese, and zinc could be effective strategies for the prevention of hypertension and diabetes.

Our findings regarding zinc intake are consistent with a growing body of epidemiologic literature suggesting the mineral exerts antidiabetic effects.(23, 65, 89) In contrast, the observed protective association of greater zinc intake against high blood pressure is novel. Only a handful of small cross-

TABLE XIX.

INCIDENCE RATE RATIOS (95% CI) FOR METABOLIC SYNDROME ACCORDING TO QUARTILES OF USUAL DIETARY INTAKE ONLY (N=5,090)

Usual dietary mineral intake	Quartile 1	Quartile 2	Quartile 3	Quartile 4	P_{trend}
Copper (mg/day)	0.28-0.98	0.99-1.23	1.24-1.55	1.56-6.32	
Incident cases, unweighted	234	428	380	201	
Crude model	1.00 (ref.)	1.05 (0.84-1.31)	1.05 (0.85-1.29)	0.94 (0.73-1.22)	0.641
Adjusted model 1[a]	1.00 (ref.)	1.04 (0.82-1.32)	1.13 (0.88-1.47)	1.12 (0.78-1.61)	0.420
Adjusted model 2[b]	1.00 (ref.)	1.04 (0.82-1.33)	1.11 (0.85-1.44)	1.06 (0.73-1.55)	0.646
Manganese (mg/day)	0.42-2.26	2.27-3.02	3.03-4.02	4.03-26.50	
Incident cases, unweighted	220	441	386	186	
Crude model	1.00 (ref.)	1.06 (0.86-1.31)	0.85 (0.69-1.04)	1.02 (0.78-1.33)	0.483
Adjusted model 1[a]	1.00 (ref.)	0.96 (0.77-1.21)	0.77 (0.59-1.01)	0.92 (0.63-1.35)	0.347
Adjusted model 2[b]	1.00 (ref.)	0.97 (0.77-1.22)	0.79 (0.60-1.03)	0.95 (0.65-1.39)	0.450
Selenium (µg/day)	23.0-90.9	91.0-112.9	113.0-140.3	140.4-401.3	
Incident cases, unweighted	356	432	280	168	
Crude model	1.00 (ref.)	0.79 (0.65-0.95)	0.75 (0.61-0.93)	0.72 (0.57-0.91)	0.007
Adjusted model 1[a]	1.00 (ref.)	0.93 (0.74-1.16)	1.08 (0.79-1.47))	1.23 (0.78-1.93)	0.445
Adjusted model 2[b]	1.00 (ref.)	0.94 (0.75-1.17)	1.11 (0.81-1.50)	1.29 (0.83-2.00)	0.336
Zinc (mg/day)	2.00-8.57	8.58-10.87	10.88-13.71	13.72-74.83	
Incident cases, unweighted	324	405	328	176	
Crude model	1.00 (ref.)	0.75 (0.62-0.91)	0.72 (0.59-0.87)	0.68 (0.53-0.86)	0.002
Adjusted model 1[a]	1.00 (ref.)	0.78 (0.62-0.97)	0.79 (0.59-1.06)	0.84 (0.54-1.30)	0.334
Adjusted model 2[b]	1.00 (ref.)	0.78 (0.62-0.98)	0.78 (0.58-1.05)	0.82 (0.53-1.28)	0.294

[a] Adjusted for supplement use (i.e., use of any supplement containing copper, manganese, selenium or zinc), total energy intake (usual kilocalories per day from food/beverages and supplements, continuous), age (continuous), gender (male or female), and Hispanic/Latino background (Central American, Cuban, Dominican, Mexican, Puerto Rican, South American, or more than one/other).

[b] Same as Adjusted model 1, with the addition of study center (Bronx, Chicago, Miami, or San Diego), education (less than high school, high school or equivalent, or greater than high school), years lived in the mainland U.S. (born in mainland, <5, 5-9, 10-14, 15-19, or ≥20), alcohol use level (no current use, low/moderate, or high), cigarette smoking (never, former, or current), and physical activity level (inactive, low, medium, or high).

TABLE XX.

ADJUSTED INCIDENCE RATE RATIOS (95% CI) FOR METABOLIC SYNDROME COMPONENTS ACCORDING TO QUARTILES OF USUAL DIETARY INTAKE ONLY[a]

Usual dietary mineral intake	High Blood Pressure	High Triglycerides	Low HDL	High Fasting Glucose	Abdominal Obesity
Participants, unweighted[b]	3,076	3,897	3,255	3,110	1,944
Incident cases, unweighted	885	531	552	1,185	708
Copper (mg/day)					
Quartile 1	1.00 (ref.)	1.00 (ref.)	1.00 (ref.)	1.00 (ref.)	1.00 (ref.)
Quartile 2	0.79 (0.62-0.99)	1.37 (0.94-2.00)	1.28 (0.89-1. 85)	0.98 (0.75-1.29)	1.01 (0.79-1.30)
Quartile 3	0.68 (0.51-0.91)	1.69 (1.11-2.57)	1.59 (1.00-2.51)	1.07 (0.79-1.43)	1.19 (0.85-1.66)
Quartile 4	0.64 (0.43-0.95)	1.64 (0.97-2.79)	1.15 (0.61-2.14)	0.96 (0.57-1.30)	1.19 (0.77-1.85)
P_{trend}	0.026	0.046	0.484	0.625	0.314
Manganese (mg/day)					
Quartile 1	1.00 (ref.)	1.00 (ref.)	1.00 (ref.)	1.00 (ref.)	1.00 (ref.)
Quartile 2	0.83 (0.65-1.06)	1.32 (0.94-1.85)	1.14 (0.81-1.60)	0.94 (0.71-1.25)	1.19 (0.92-1.55)
Quartile 3	1.02 (0.79-1.31)	1.21 (0.81-1.80)	1.04 (0.70-1.53)	0.73 (0.54-0.99)	0.90 (0.64-1.28)
Quartile 4	0.88 (0.61-1.26)	1.27 (0.75-2.16)	0.93 (0.54-1.58)	0.85 (0.58-1.23)	1.07 (0.70-1.64)
P_{trend}	0.983	0.579	0.638	0.156	0.685
Selenium (µg/day)					
Quartile 1	1.00 (ref.)	1.00 (ref.)	1.00 (ref.)	1.00 (ref.)	1.00 (ref.)
Quartile 2	1.06 (0.83-1.35)	1.14 (0.78-1.65)	1.26 (0.89-1.78)	0.94 (0.73-1.21)	1.06 (0.82-1.36)
Quartile 3	1.17 (0.85-1.61)	1.48 (0.85-2.59)	1.53 (0.91-2.59)	1.12 (0.81-1.56)	1.12 (0.75-1.69)
Quartile 4	1.25 (0.74-2.09)	1.59 (0.76-3.33)	1.29 (0.64-2.60)	1.17 (0.73-1.86)	1.52 (0.89-2.59)
P_{trend}	0.347	0.181	0.325	0.451	0.202
Zinc (mg/day)					
Quartile 1	1.00 (ref.)	1.00 (ref.)	1.00 (ref.)	1.00 (ref.)	1.00 (ref.)
Quartile 2	0.95 (0.75-1.19)	0.98 (0.69-1.39)	1.13 (0.79-1.63)	0.79 (0.62-0.99)	1.01 (0.79-1.30)
Quartile 3	0.98 (0.71-1.35)	1.09 (0.71-1.68)	1.20 (0.78-1.85)	0.79 (0.59-1.07)	0.85 (0.58-1.23)
Quartile 4	1.14 (0.72-1.81)	1.16 (0.63-2.14)	0.71 (0.37-1.38)	0.81 (0.52-1.26)	0.73 (0.43-1.23)
P_{trend}	0.699	0.588	0.486	0.407	0.176

[a] Adjusted for supplement use (i.e., use of any supplement containing copper, manganese, selenium or zinc), total energy intake (usual kilocalories per day from food/beverages and supplements, continuous), age (continuous), gender (male or female), Hispanic/Latino background (Central American, Cuban, Dominican, Mexican, Puerto Rican, South American, or more than one/other), study center (Bronx, Chicago, Miami, or San Diego), education (less than high school, high school or equivalent, or greater than high school), years lived in the mainland U.S. (born in mainland, <5, 5-9, 10-14, 15-19, or ≥20), alcohol use level (no current use, low/moderate, or high), cigarette smoking (never, former, or current), and physical activity level (inactive, low, medium, or high).

[b] All events are incident, thus the number of participants analyzed varies with the individual component condition.

TABLE XXI.
INCIDENCE RATE RATIOS (95% CI) FOR METABOLIC SYNDROME
BY SUPPLEMENT USE (N=5,090)

Supplement use	Non-User	User
Copper		
Incident cases, unweighted	975	258
Crude model	1.00 (ref.)	0.94 (0.77-1.14)
Adjusted model 1[a]	1.00 (ref.)	0.79 (0.65-0.96)
Adjusted model 2[b]	1.00 (ref.)	0.83 (0.68-1.01)
Manganese		
Incident cases, unweighted	975	258
Crude model	1.00 (ref.)	0.97 (0.80-1.18)
Adjusted model 1[a]	1.00 (ref.)	0.85 (0.70-1.05)
Adjusted model 2[b]	1.00 (ref.)	0.89 (0.73-1.08)
Selenium		
Incident cases, unweighted	995	238
Crude model	1.00 (ref.)	0.95 (0.77-1.16)
Adjusted model 1[a]	1.00 (ref.)	0.79 (0.64-0.97)
Adjusted model 2[b]	1.00 (ref.)	0.82 (0.66-1.01)
Zinc		
Incident cases, unweighted	947	286
Crude model	1.00 (ref.)	0.91 (0.75-1.11)
Adjusted model 1[a]	1.00 (ref.)	0.80 (0.66-0.97)
Adjusted model 2[b]	1.00 (ref.)	0.83 (0.69-1.02)

[a] Adjusted for usual dietary intakes of the respective mineral counterpart (copper [mg/day], manganese [mg/day], selenium [µg/day], or zinc [mg/day], continuous), total energy intake (usual kilocalories per day from food/beverages and supplements, continuous), age (continuous), gender (male or female), and Hispanic/Latino background (Central American, Cuban, Dominican, Mexican, Puerto Rican, South American, or more than one/other).

[b] Same as Adjusted model 1, with the addition of study center (Bronx, Chicago, Miami, or San Diego), education (less than high school, high school or equivalent, or greater than high school), years lived in the mainland U.S. (born in mainland, <5, 5-9, 10-14, 15-19, or ≥20), alcohol use level (no current use, low/moderate, or high), cigarette smoking (never, former, or current), and physical activity level (inactive, low, medium, or high).

TABLE XXII.

ADJUSTED INCIDENCE RATE RATIOS (95% CI) FOR METABOLIC SYNDROME COMPONENTS BY SUPPLEMENT USE[a]

Supplement use	High Blood Pressure	High Triglycerides	Low HDL	High Fasting Glucose	Abdominal Obesity
Participants, unweighted[b]	3,961	4,428	3,807	4,295	2,652
Incident cases, unweighted	885	531	552	1,185	708
Copper					
Non-user	1.00 (ref.)	1.00 (ref.)	1.00 (ref.)	1.00 (ref.)	1.00 (ref.)
User	0.87 (0.71-1.07)	0.91 (0.67-1.24)	1.01 (0.78-1.33)	0.88 (0.73-1.05)	0.89 (0.71-1.10)
Manganese					
Non-user	1.00 (ref.)	1.00 (ref.)	1.00 (ref.)	1.00 (ref.)	1.00 (ref.)
User	0.87 (0.70-1.09)	0.93 (0.67-1.27)	1.02 (0.78-1.35)	0.84 (0.70-1.00)	0.91 (0.74-1.12)
Selenium					
Non-user	1.00 (ref.)	1.00 (ref.)	1.00 (ref.)	1.00 (ref.)	1.00 (ref.)
User	0.83 (0.67-1.04)	0.87 (0.62-1.21)	0.93 (0.70-1.24)	0.83 (0.69-0.99)	0.88 (0.71-1.10)
Zinc					
Non-user	1.00 (ref.)	1.00 (ref.)	1.00 (ref.)	1.00 (ref.)	1.00 (ref.)
User	0.88 (0.71-1.07)	0.93 (0.69-1.25)	0.96 (0.74-1.24)	0.87 (0.73-1.04)	0.91 (0.74-1.12)

[a] All events are incident, thus the number of analyzed participants varies by the individual component condition.

[b] Adjusted for usual dietary intakes of the respective mineral counterpart (copper [mg/day], manganese [mg/day], selenium [µg/day], or zinc [mg/day], continuous), total energy intake (usual kilocalories per day from food/beverages and supplements, continuous), age (continuous), gender (male or female), Hispanic/Latino background (Central American, Cuban, Dominican , Mexican, Puerto Rican, South American, or more than one/other), study center (Bronx, Chicago, Miami, or San Diego), education (less than high school, high school or equivalent, or greater than high school), years lived in the mainland U.S. (born in mainland, <5, 5-9, 10-14, 15-19, or ≥20), alcohol use level (no current use, low/moderate, or high), cigarette smoking (never, former, or current), and physical activity level (inactive, low, medium, or high).

TABLE XXIII.

INCIDENCE RATE RATIOS (95% CI) FOR METABOLIC SYNDROME AND COMPONENTS ACCORDING TO COMBINED INTAKES OF COPPER, MANGANESE, SELENIUM, AND ZINC

	Metabolic Syndrome[a]	High Blood Pressure[a]	High Triglycerides[a]	Low HDL[a]	High Fasting Glucose[a]	Abdominal Obesity[a]
Usual total intake[b]						
Low intake of all 4	1.00 (ref.)	1.00 (ref.)	1.00 (ref.)	1.00 (ref.)	1.00 (ref.)	1.00 (ref.)
High intake of any 1	0.85 (0.67-1.09)	1.07 (0.84-1.38)	0.85 (0.59-1.24)	1.19 (0.84-1.68)	0.72 (0.55-0.95)	0.92 (0.68-1.24)
High intake of any 2	0.93 (0.71-1.23)	0.92 (0.67-1.27)	1.17 (0.75-1.83)	0.92 (0.58-1.47)	0.75 (0.55-1.02)	0.87 (0.59-1.28)
High intake of any 3	1.03 (0.79-1.33)	0.93 (0.71-1.22)	1.56 (1.00-2.44)	1.24 (0.84-1.83)	0.94 (0.73-1.21)	1.13 (0.83-1.54)
High intake of all 4	0.80 (0.63-1.01)	0.81 (0.63-1.04)	1.08 (0.71-1.63)	0.98 (0.69-1.38)	0.75 (0.57-0.99)	0.76 (0.56-1.04)
P_{trend}	0.213	0.065	0.320	0.992	0.157	0.224
Usual dietary intake[c]						
Low intake of all 4	1.00 (ref.)	1.00 (ref.)	1.00 (ref.)	1.00 (ref.)	1.00 (ref.)	1.00 (ref.)
High intake of any 1	0.94 (0.74-1.18)	1.06 (0.84-1.34)	0.96 (0.65-1.43)	1.04 (0.71-1.50)	0.72 (0.55-0.96)	0.79 (0.58-1.07)
High intake of any 2	0.80 (0.64-1.01)	1.00 (0.77-1.29)	0.92 (0.60-1.41)	1.17 (0.80-1.71)	0.63 (0.47-0.85)	0.79 (0.57-1.11)
High intake of any 3	1.04 (0.78-1.39)	0.86 (0.60-1.24)	1.50 (0.96-2.37)	1.46 (0.92-2.31)	0.94 (0.70-1.27)	1.03 (0.71-1.47)
High intake of all 4	0.95 (0.69-1.32)	1.24 (0.88-1.74)	1.24 (0.71-2.17)	1.03 (0.61-1.75)	0.91 (0.62-1.34)	0.76 (0.47-1.23)
P_{trend}	0.812	0.632	0.235	0.438	0.661	0.548
Supplement use[d]						
Non-user	1.00 (ref.)	1.00 (ref.)	1.00 (ref.)	1.00 (ref.)	1.00 (ref.)	1.00 (ref.)
Use of any	0.82 (0.67-1.00)	0.90 (0.73-1.11)	0.87 (0.64-1.18)	0.96 (0.74-1.26)	0.87 (0.73-1.03)	0.90 (0.73-1.11)

[a] All events are incident, thus the analytic sample size corresponds to 5,090 for metabolic syndrome, 3,961 for high blood pressure, 4,428 for high triglycerides, 3,807 for low HDL, 4,295 for high fasting glucose, and 2,652 for abdominal obesity.

[b] Adjusted for total energy intake (usual kilocalories per day from food/beverages and supplements, continuous), age (continuous), gender (male or female), Hispanic/Latino background (Central American, Cuban, Dominican , Mexican, Puerto Rican, South American, or more than one/other), center (Bronx, Chicago, Miami, or San Diego), education (less than high school, high school or equivalent, or greater than high school), years lived in the mainland U.S. (born in mainland, <5, 5-9, 10-14, 15-19, or ≥20), alcohol use level (no current use, low/moderate, or high), cigarette smoking (never, former, or current), and physical activity level (inactive, low, medium, or high).

[c] Adjusted for the variables listed above (a), with additional adjustment for use of any supplement containing copper, manganese, selenium or zinc.

[d] Adjusted for the variables listed above (a), with additional adjustment for usual dietary intakes of copper (mg/day, continuous), manganese (mg/day, continuous), selenium (μg/day, continuous), and zinc (mg/day, continuous); supplement use refers to any dietary supplements containing copper, manganese, selenium, or zinc.

sectional and case-control design studies have evaluated the relationship of zinc and blood pressure to date, with each producing inconsistent results.(24, 90-93) Prior studies of manganese have suggested both low and high levels of exposure are positively associated with diabetes.(94-96) However, these studies have assessed manganese biomarkers, which could reflect excessive environmental exposures (e.g., from contaminated drinking water) in addition to intakes from dietary and supplemental sources. Our results suggesting greater intakes reduce the risk of developing high glucose levels might therefore only be capturing the lower portion of the dose-response curve, and could thus be considered in line with the existing research. Manganese, copper, and zinc each serve as cofactors for superoxide dismutases (SOD), a major class of antioxidant enzymes.(97) Copper and zinc are required for two isoforms of SOD – SOD1 and SOD3 –while manganese is required for SOD2 (also known as manganese superoxide dismutase, or MnSOD). In experimental studies, supplementation of these minerals has resulted in increased SOD activity.(98-100) Thus, it is plausible that greater intakes of these minerals could aid in the defense against reactive oxygen species (ROS), which have been implicated as triggers of insulin resistance and vascular injury.(101, 102)

In the current analysis, greater dietary intakes of selenium were significantly associated with an increased risk of developing high triglycerides and marginally associated with an increased risk of low HDL cholesterol. To our knowledge, no studies to date have prospectively evaluated selenium exposure – neither as dietary intakes nor using biomarkers – with lipid profiles among Americans, who are generally selenium-replete.(103) Cross-sectional data from multiple cycles of the National Health and Nutrition Examination Survey (NHANES) have been mixed. Overall, higher selenium status, as measured in serum, has been consistently linked to higher levels of triglycerides although the relationship may be non-linear (U-shaped).(104-106) Associations with HDL (or "good" cholesterol), on the other hand, have been reported as both null and positive in direct contrast to the inverse association we observed.(104-106) Selenium is a key component in numerous selenoproteins, including glutathione peroxidases (GPx) which are widely recognized for their antioxidant properties.(107) Evidence for biologic mechanisms underlying the role of selenium intake on lipogenesis or lipid metabolism are lacking, but an *in vivo* model has shown

that while selenium intake indeed influences expression and activity of GPx, this process decreases glutathionylation of protein tyrosine phosphatase 1B (PTP1B), a recognized therapeutic target for metabolic syndrome.(108, 109) Glutathionylated PTP1B is catalytically inactive, hence, this lowered inhibition of the enzyme results in increased PTP1B activity in the liver and stimulation of triglyceride synthesis.(108)

Despite the large sample size and prospective design of the present analysis, our study has some limitations worth noting. Treatment with antihypertensives, antidiabetes, fibrates, or nicotinic acids was assessed using documented (scanned) medications at baseline, but only self-reported at the follow-up visit. Some individuals may have therefore been misclassified for incident metabolic syndrome and individual component conditions, although we expect this would be non-differential with regards to dietary and supplemental mineral intakes. In addition medication data were complemented with clinical parameters measured in-person by trained technicians, thus the degree of misclassification is presumed to be minimal. The use of the National Cancer Institute method to derive usual dietary intakes allows accounting for the measurement error from self-reported data. Nevertheless, the two 24-hour recalls and dietary supplement use interview were performed only at baseline, so we could not account for longitudinal changes in intakes. Further, nutrient composition values may not accurately reflect true intakes of the minerals, particularly for selenium given its wide geographic variation in soil and subsequently in the food supply.(110) Such inaccuracies, however, are unlikely to be related to metabolic syndrome risk. Lastly, although we adjusted for multiple potential sociodemographic and lifestyle confounders, we cannot rule out the possibility of residual confounding in this observational study.

In summary, we found evidence that higher total intakes of copper, manganese, and zinc are associated with a lower risk of metabolic syndrome and component cardiometabolic conditions whereas greater total selenium intakes could be a risk factor for dyslipidemia in U.S. Hispanic/Latino adults. These findings are preliminary, and will be updated once complete follow-up data are made available. Future nutritional epidemiologic studies in other populations should also consider the role of minerals in metabolic syndrome, as the results could have serious public health implications. If our findings are

confirmed, optimizing intakes of copper, manganese, selenium, and zinc through dietary or supplementation interventions could be promising strategies for reducing the vast burden of cardiometabolic disease.

III. MULTIPLE METAL EXPOSURES AND METABOLIC SYNDROME: A CROSS-SECTIONAL ANALYSIS OF THE NATIONAL HEALTH AND NUTRITION EXAMINATION SURVEY 2011-2014

A. Background

Metabolic syndrome is a constellation of cardiometabolic conditions (high blood pressure, dyslipidemia, high glucose, and abdominal obesity) that now affects 1 in 3 adults within the U.S.(1) As a recognized risk factor for type 2 diabetes and cardiovascular disease, there is a need to better understand the drivers of the syndrome so that targeted preventive efforts can be undertaken.(4) While excessive macronutrient intakes and physical inactivity have been identified as major contributors, the high burden of metabolic syndrome remains not fully explained.(2) Recent studies suggest that environmental exposures, in particular to metals and metalloids (hereafter referred to collectively as "metals"), could play an important role in cardiometabolic health.(111)

To date, few epidemiologic studies have been conducted regarding metal exposures and metabolic syndrome. However, studies of some of the individual component conditions provide suggestive evidence for toxic metal exposures. For example, positive associations of arsenic, cadmium, and lead have each been observed with high blood pressure, whereas mercury exposure has been linked to incident diabetes.(52, 55, 112, 113) Data regarding nutritionally essential metals are sparse, but suggest both low and high levels of exposure to copper, manganese, selenium, and zinc (for which the predominant exposure pathways are dietary or supplemental mineral intakes) correspond with altered cardiometabolic phenotypes.(24, 28, 29, 65, 94, 104, 114-116) Specifically, greater dietary zinc intakes have been inversely related to diabetes risk, but have also been associated with elevated triglycerides.(24, 65, 114) In contrast, copper and selenium have both been shown to be positively associated with diabetes and dyslipidemia.(28, 29, 104, 116) Finally, a positive relationship has been observed for manganese with blood pressure, but a U-shaped relationship has been seen with diabetes.(94, 115)

Toxic metals have no known biological roles in humans, while the essential ones are required for normal physiologic functioning.(61, 117) However, some of the metal-binding proteins responsible for the uptake and transport of essential metals—ultimately controlling their homeostasis—can lack specificity.(57) These metallothioneins can thus be subjected to molecular mimicry, such that a nutritionally essential metal can be replaced by a toxic one.(118) Given the possibility for deficiencies or excesses of essential metals to disrupt biological processes and for non-essential metals to exert toxic effects, we sought to investigate the cross-sectional associations of multiple metal exposures with metabolic syndrome and its individual component conditions in U.S. adults.

B. Methods

1. Study Population

The National Health and Nutrition Examination Survey (NHANES) employs stratified, multi-stage probability sampling of the non-institutionalized civilian resident population of the U.S. to assess health and nutritional status through interviews and physical examinations.(119) In the 2011-2012 and 2013-2014 cycles, a total of 19,931 individuals were selected to participate from 60 different locations.(119) A random one-third subsample was selected for urinary and serum metal assays, a random one-half subsample was selected for blood metal assays during the 2013-2014 cycle (all were eligible for blood metal assays during the 2011-2012 cycle), and participants randomly assigned for examination during the morning session had glucose levels measured after an overnight fast. These subsamples only partially overlapped.(120, 121) We restricted our analyses to non-pregnant and non-lactating individuals 20 years of age or older (n=11,145). We excluded individuals who were missing measurements of the selected metal biomarkers (n=7,879), and those who fasted fewer than 8 hours (n=1,793), were missing glucose measurements (n=1), triglyceride measurements (n=11), blood pressure readings (n=54), waist circumference measurements (n=39), and missing covariate information (n=280). Thus, our final analytic sample was 1,088 participants. Compared to the study sample, excluded individuals were similar in age,

but were slightly more likely to be male and non-Hispanic white ($p < 0.05$, data not shown). Written, informed consent was provided by all participants.

TABLE XXIV.
METAL BIOMARKERS AVAILABLE IN NHANES 2011-14[a]

Element	Urine	Serum	Whole blood
Arsenic	Recent exposure		
Cadmium	Body burden		Recent exposure
Copper		Unknown	
Manganese	Less sensitive		Recent exposure
Mercury	Inorganic/elemental		Methylmercury
Lead	Unreliable		Recent exposure
Selenium		Recent exposure	Body burden
Zinc		Unknown	

[a] Selected biomarkers are highlighted in gray

2. Biomarkers of Exposure

Spot urine, whole blood, and serum concentrations of essential (copper, manganese, selenium, and zinc) and toxic metals (arsenic, cadmium, mercury, and lead, including arsenic and mercury species) were measured using inductively coupled plasma mass spectrometry.(122-133) Specimens were collected at the time of the laboratory exam, shipped on dry ice to the National Center for Environmental Health (NCEH) in Atlanta, GA, and stored frozen at -20°C until assayed. For metals measured in multiple biological matrices (Table XXIV), we selected the most sensitive, reliable, or stable biomarker of exposure with the exception of mercury.(134-141) Because the chemical form of mercury varies by bodily fluid, we included both blood methylmercury (an organic mercury compound) and urinary mercury, which primarily represents elemental and inorganic mercury, in our analyses.

To isolate the more toxic forms of arsenic present in urinary total arsenic concentrations, we implemented a validated residual-based approach.(142) Briefly, we regressed natural log-transformed total arsenic on natural log-transformed arsenobetaine concentrations, a non-toxic organic arsenical derived from recent seafood intake. We then added the conditional mean of total arsenic among individuals with non-detectable arsenobetaine (<1.19 μg/L) to the model residuals in order to obtain more interpretable values that can be considered approximations of inorganic arsenic exposure.

For all biomarkers, concentrations below the detection limit were substituted with the limit divided by √2, per the NHANES protocol.(143) If the limit differed between the two survey cycles, we selected the higher of the two, replacing any values below. To account for measurement error due to urine dilution, we calculated metal excretion rates for metal biomarkers measured in urine (calibrated arsenic, cadmium, and mercury) as our prior work has shown that excretion rates are a less biased method for addressing measurement error due to urine dilution in studies of obesity or obesity-related endpoints compared to creatinine corrections.(144) These rates can be interpreted as the amount of analyte excreted over the time period covered by the collected urinary voids, and were calculated using the formula below:

$$ER\ (ng/hr) = \frac{Analyte\ concntration\ (ng/mL) \times \sum Void\ volume\ (mL)}{Time\ since\ last\ void\ (hr)}$$(145)

Concentrations of the respective analyte were converted to nanograms per milliliter (ng/mL), the total amount of urine voids collected in the mobile examination center was measured in milliliters (mL), and the duration of hours between the void prior to examination as self-reported by the participant and the last collected void during the examination was recorded (hr).

3. Metabolic Syndrome and Component Conditions

Examinations were conducted by trained health technicians, health interviewers, phlebotomists, and physicians in the mobile examination center. Body weight, height, and waist circumference were

measured according to NIH guidelines.(146, 147) Three (four if a prior attempt was interrupted or incomplete) seated blood pressure measurements were obtained after a 5-minute rest using a calibrated mercury sphygmomanometer.(148, 149) The first measurement was discarded, and the average of all remaining measurements was calculated for use in analyses. Participants randomly assigned to a morning session were asked to fast for 9 hours overnight prior to the examination.(150, 151) Blood samples obtained from these participants were processed in the mobile examination center laboratory. Plasma specimens were shipped to the University of Missouri-Columbia, MO where plasma glucose was measured using a hexokinase enzymatic method (Roche Diagnostics, Indianapolis, IN).(152, 153) Serum specimens were sent to the University of Minnesota, Minneapolis, MN, for measurement of HDL cholesterol by a magnesium/dextran sulfate method and serum triglycerides by a glycerol blanking enzymatic method (Roche Diagnostics).(154-157) Trained interviewers using the Computer-Assisted Personal Interviewing (CAPI) system collected information about prescription medications taken in the past month as part of the Prescription Medication Questionnaire administered in the home.(148, 149) Participants were asked to show the interviewer medication containers, or verbally report the medication name if no container was presented. Antihypertensive, antidiabetes, and lipid-modifying medications (fibrates and niacins) were coded using the Multum Lexicon Plus® Drug Database. Dietary supplement use in the last 30 days was also assessed, thus we included supplemental use of niacins in addition to prescribed.

Metabolic syndrome was defined according to the harmonized definition as the presence of at least 3 of following components: 1) high blood pressure (systolic blood pressure ≥130 mmHg, diastolic blood pressure ≥85 mmHg, or current use of medication to treat high blood pressure); 2) high triglyceride levels (≥150 mg/dL, or current use of medication to treat elevated triglycerides); 3) low HDL cholesterol levels (<50 mg/dL for women, <40 mg/dL for men, or current use of medication to treat reduced HDL); 4) abdominal obesity (waist circumference of ≥88 centimeters for women or ≥102 centimeters for men); or 5) high fasting blood glucose levels (≥100 mg/dL or current use of medication to treat hyperglycemia).(4)

4. Covariates

Body mass index (BMI), expressed in kg/m^2, was calculated using participants' weight and height. Interviewer-administered questionnaires were used to obtain information on demographics (age, gender, race/ethnicity), educational attainment, and annual family income. Questionnaires also ascertained lifestyle factors, including cigarette use, alcohol intake, and physical activity. Cigarette use was classified as never (those who reported smoking fewer than 100 cigarettes in their lifetime), former (reported ever smoking at least 100 cigarettes in their lifetime but do not currently smoke), or current (smoked at least 100 cigarettes and currently smoke some days or every day). The average number of alcoholic drinks consumed per day in the past year was calculated based on the reported frequency and average number of drinks on a consumption day. Participants were asked to self-report frequency and duration of moderate and vigorous physical activity across work, transportation, and leisure-time domains. With these data, we derived a dichotomous variable to indicate whether or not the participant met the 2008 U.S. national physical activity guidelines of ≥150 minutes of moderate activity, ≥75 minutes of vigorous activity per week, or an equivalent combination.(158) Finally, participants underwent up to two 24-hour dietary recalls; the average total caloric intake across the 48-hour period was calculated.

5. Statistical Analyses

All statistical analyses were performed using Stata 13.1 (College Station, TX). As previously described, metal biomarkers and fasting glucose were each measured in subsamples that only partially overlapped. For this reason, we did not weight our analyses in accordance with recommendations by the National Center for Health Statistics.(159) We did, however, account for clustering and stratification when estimating variances by Taylor series linearization. At a minimum, all multivariable regression models included age, gender, race/ethnicity, and family income relative to the federal poverty line as covariates, since these variables were used to identify sampled participants.(159, 160) In addition, we

included these variables in logistic regression models to estimate the prevalence of metabolic syndrome and its component conditions in the target population via marginal standardization.(161)

We calculated distribution percentiles and geometric means for each of the following metal biomarkers: 1.) urinary arsenic excretion rates (after calibration to remove the contribution of organic arsenic from recent seafood consumption, in ng/hr); 2.) urinary cadmium excretion rates (ng/hr); 3.) serum copper concentrations (μg/dL); 4.) blood manganese concentrations (μg/L); 5.) blood methylmercury concentrations (μg/L); 6.) urinary mercury excretion rates (ng/hr); 7.) blood lead concentrations (μg/dL); 8.) blood selenium concentrations (μg/L); and 9.) serum zinc concentrations (μg/dL). Least square geometric mean biomarker excretion rates and concentrations were compared across participant characteristics using linear regression models that accounted for the complex survey design.

Separate Poisson regression models were performed to estimate the prevalence ratios for each metal, categorized into quartiles, with metabolic syndrome and its individual component conditions. P-values for linear trends were estimated by re-running these models with the biomarker quartiles parametrized ordinally. Taylor series linearization was again used to obtain design-based 95% confidence intervals. Minimally adjusted models (Model 1) included age, gender, race/ethnicity, and family income: poverty ratio, as covariates since these variables were used to select NHANES participants.(159, 160) Age and family income: poverty ratio were modeled continuously, while gender (male or female) and race/ethnicity (non-Hispanic white, non-Hispanic black, Hispanic, or other including multi-racial) were parameterized as indicator variables. Model 2 further adjusted for total caloric intake (continuous, kcal/day), educational attainment (less than high school, high school diploma or GED, or at least some college), smoking status (current, former, or never), average number of drinks per day in past year (continuous), physical activity status (met the 2008 physical activity guidelines or did not), and survey cycle (2011-2012 or 2013-2014). BMI (kg/m^2) was entered as an additional continuous covariate in Model 2, except for in models of abdominal obesity so as to avoid over-adjustment.

Pearson's correlation coefficients were calculated between all possible pairs of metal biomarkers. To derive patterns of metal exposures, we performed principal components analysis (PCA). Because all of the toxic metals and some of the essential metals (manganese and zinc) were right-skewed, we natural log-transformed these biomarkers prior to conducting PCA. The appropriateness of this transformation was confirmed by visual examination of the distributions (Figures 8-9, Appendix B). We applied an orthogonal varimax rotation to the factors, and retained those with eigenvalues greater than 1.(162) Continuous scores (linear combinations of the metal biomarkers multiplied by their respective loadings) for each of the retained components were predicted for each individual. After categorizing these scores into quartiles, all were entered simultaneously into Poisson regression models of metabolic syndrome and the individual component conditions with adjustment for age, gender, race/ethnicity, family income: poverty ratio, total caloric intake, educational attainment, smoking status, alcohol consumption, physical activity status, survey cycle, and BMI (except for in models of abdominal obesity). The distributions of the component scores were additionally compared across participant characteristics in order to describe patterns of concomitant exposures.

6. Sensitivity Analyses

We ran several sensitivity analyses to assess the robustness of our findings. First, we adjusted for serum cotinine in addition to self-reported smoking status in order to address any residual confounding. Second, we adjusted models of methylmercury for recent seafood consumption since the relationships of blood concentrations with metabolic syndrome and individual components might be confounded by certain dietary patterns. We used the frequency of seafood meals eaten in the past 30 days as a covariate, which we derived by summing types of shellfish (clams, crabs, crayfish, lobsters, mussels, oysters, scallops, shrimp, other known/unknown shellfish) and fish (breaded fish, tuna, bass, catfish, cod, flatfish, haddock, mackerel, perch, pike, pollock, porgy, salmon, sardines, sea bass, shark, swordfish, trout, walleye, and other known/unknown fish) self-reported during the first dietary recall. Third, we performed stratified analyses to better understand the influence of weight loss on associations of blood lead

concentrations with metabolic syndrome, as studies indicate this process can release lead (of which more than 90% is stored) in bone into the bloodstream.(163, 164) We defined weight loss as a measured body weight that was at least 4 kg lower than the participants' self-reported weight from one year prior to the examination date. Fourth, we additionally adjusted for bone mineral density of the femoral neck (measured by dual energy x-ray absorptiometry only within the 2013-14 cycle) in models of blood lead concentrations because of the aforementioned lead content in bones and because bone mineral density may be a risk factor for metabolic syndrome.(163, 165)

C. Results

The selected metals were detectable in the majority of participants (Table XXV). Arsenic excretion rates and blood manganese concentrations were significantly lower among older individuals, while cadmium excretion rates, blood methylmercury, and blood lead concentrations were lowest amongst younger individuals (Table XXVI). There were gender-based differences; arsenic, lead, selenium, and zinc were higher in men, but cadmium, copper, and manganese were higher in women. We further observed racial/ethnic differences for all metal biomarkers except for zinc. Cadmium excretion rates, serum copper, and blood lead concentrations were positively related to current smoking. Arsenic and lead biomarkers were higher among alcohol drinkers. Serum copper concentrations increased with body mass index, whereas blood methylmercury and blood lead concentrations declined.

A total of 514 of the 1,088 individuals analyzed satisfied the criteria for metabolic syndrome. After standardizing by age, gender, race/ethnicity, and family income: poverty ratio, the corresponding prevalence of the syndrome was 34.2% (Table XXVII). The prevalence of individual component conditions ranged from 22.2% (high blood pressure) to 47.5% (low HDL cholesterol).

In single-metal regression models, urinary arsenic excretion rates, urinary mercury excretion rates, blood selenium concentrations, and serum zinc concentrations were positively related to metabolic syndrome in a dose-dependent manner after adjustment for potential confounders and variables related to

TABLE XXV.

DISTRIBUTIONS OF METAL BIOMARKERS AND PROPORTION OF NON-DETECTS

	As (ng/hr)[a]	**Cd (ng/hr)[b]**	**Cu (µg/dL)[c]**	**Mn (µg/L)[d]**	**MeHg (µg/L)[e]**	**Hg (ng/hr)[f]**	**Pb (µg/dL)[g]**	**Se (µg/L)[h]**	**Zn (µg/L)[i]**
Essential	No	No	Yes	Yes	No	No	No	Yes	Yes
Matrix	Urine[j]	Urine[j]	Serum	Blood	Blood	Urine[j]	Blood	Blood	Serum
Geometric mean	253.5	11.1	114.9	9.4	0.59	17.8	1.08	195.5	87.3
Percentile									
10th	123.9	3.7	87.3	6.2	0.08	4.7	0.48	167.7	72.2
25th	174.3	6.2	98.5	7.5	0.24	8.9	0.70	180.6	78.6
50th	254.3	11.1	113.2	9.3	0.59	17.8	1.05	195.0	86.7
75th	372.2	20.1	132.0	11.7	1.38	36.5	1.64	210.0	96.4
90th	526.4	32.5	154.6	14.7	3.00	66.8	2.45	228.1	106.9
Percent < LOD[k]	2.8	8.3	0	0	13.1	19.3	0.9	0	0

[a] Total arsenic (after calibration to remove the contribution of organic arsenic from recent seafood consumption).
[b] Cadmium.
[c] Copper.
[d] Manganese.
[e] Methylmercury.
[f] Mercury (mainly inorganic/elemental).
[g] Lead.
[h] Selenium.
[i] Zinc.
[j] Urinary metal concentrations are expressed as excretion rates to account for urine dilution.
[k] Limit of detection.

TABLE XXVI.

GEOMETRIC MEANS OF METAL BIOMARKERS BY PARTICIPANT CHARACTERISTICS (N=1,088)

Characteristic	N	As (ng/hour)[a]	Cd (ng/hour)[b]	Cu (µg/dL)[c]	Mn (µg/L)[d]	MeHg (µg/L)[e]	Hg (ng/hour)[f]	Pb (µg/dL)[g]	Se (µg/L)[h]	Zn (µg/L)[i]
Survey cycle										
2011-2012	519	246.0 (5.9)	11.6 (0.4)	112.7 (1.1)	9.3 (0.2)	0.63 (0.05)	18.9 (0.9)	1.17 (0.04)*	192.7 (1.4)*	87.0 (0.8)
2013-2014	569	260.6 (6.1)	10.6 (0.4)	116.9 (1.9)	9.5 (0.1)	0.56 (0.05)	16.8 (1.1)	1.00 (0.03)*	198.0 (0.9)*	87.5 (1.2)
Age (years)										
20-39	390	271.6 (7.6)*	7.0 (0.3)*	112.2 (1.5)*	9.7 (0.2)*	0.51 (0.04)*	15.5 (0.9)*	0.78 (0.03)*	195.0 (1.2)	88.0 (1.0)
40-59	368	264.1 (8.3)*	13.9 (0.6)*	117.3 (1.7)*	9.4 (0.2)*	0.60 (0.05)*	21.8 (1.5)*	1.16 (0.03)*	196.5 (1.3)	87.3 (0.9)
60+	330	223.2 (7.8)*	14.8 (0.7)*	115.4 (1.3)*	9.0 (0.1)*	0.69 (0.06)*	16.7 (1.1)*	1.46 (0.05)*	194.9 (1.5)	86.4 (0.9)
Gender										
Female	515	238.3 (6.6)*	11.7 (0.4)*	128.8 (1.3)*	10.0 (0.1)*	0.59 (0.04)	17.9 (0.9)	0.90 (0.03)*	192.8 (1.2)*	85.0 (0.8)*
Male	573	268.0 (5.8)*	10.5 (0.5)*	103.6 (1.2)*	8.9 (0.1)*	0.59 (0.03)	17.7 (0.9)	1.27 (0.04)*	197.9 (1.1)*	89.3 (0.8)*
Race/ethnicity										
White	491	255.3 (6.5)*	10.8 (0.4)*	113.1 (1.6)*	9.0 (0.1)*	0.47 (0.03)*	16.6 (1.1)*	1.10 (0.03)*	195.7 (1.1)*	88.2 (0.8)
Black	220	219.7 (6.3)*	12.9 (0.9)*	124.1 (1.7)*	8.4 (0.1)*	0.68 (0.07)*	16.2 (0.9)*	1.10 (0.06)*	192.0 (1.7)*	84.7 (1.1)
Hispanic	238	266.5 (7.8)*	9.3 (0.5)*	117.3 (1.9)*	10.0 (0.2)*	0.54 (0.06)*	18.9 (2.0)*	0.96 (0.05)*	194.9 (1.6)*	88.1 (1.5)
Other	139	284.8 (16.6)*	12.5 (0.9)*	103.5 (2.0)*	11.5 (0.4)*	1.24 (0.16)*	23.4 (2.1)*	1.20 (0.06)*	201.0 (2.1)*	86.6 (1.2)
Education										
Less than high school	214	228.0 (8.3)*	12.6 (0.8)*	116.8 (2.0)	9.4 (0.3)	0.52 (0.05)*	14.0 (1.1)*	1.29 (0.09)*	193.3 (1.8)	88.0 (1.2)
High school diploma/GED	235	241.1 (9.6)*	11.3 (0.7)*	114.1 (1.5)	9.2 (0.2)	0.47 (0.03)*	14.5 (0.8)*	1.11 (0.04)*	194.8 (1.7)	87.4 (1.0)
At least some college	639	267.6 (5.4)*	10.5 (0.3)*	114.5 (1.5)	9.4 (0.1)	0.67 (0.04)*	20.8 (1.1)*	1.01 (0.02)*	196.4 (1.1)	86.9 (0.8)
Family income to poverty ratio										
Below poverty (<1)	251	253.3 (7.4)	11.1 (0.7)	120.4 (1.3)*	9.7 (0.2)	0.50 (0.05)*	15.1 (1.3)*	0.99 (0.05)*	192.5 (1.5)*	88.0 (1.5)
At or above poverty (>1)	837	254.3 (5.5)	11.1 (0.3)	113.2 (1.3)*	9.3 (0.1)	0.62 (0.03)*	18.7 (0.7)*	1.11 (0.03)*	196.4 (1.1)*	87.0 (0.6)
Smoking status										
Never smoker	610	249.5 (6.6)	8.5 (0.3)*	114.2 (1.5)*	9.7 (0.1)*	0.59 (0.03)*	18.4 (0.7)*	0.93 (0.02)*	196.0 (1.0)	86.2 (0.8)
Former smoker	267	262.1 (8.5)	14.7 (0.6)*	113.2 (1.7)*	9.1 (0.2)*	0.72 (0.07)*	20.3 (1.5)*	1.28 (0.05)*	197.2 (1.4)	88.4 (1.1)
Current smoker	211	254.3 (9.3)	16.2 (1.1)*	119.1 (1.9)*	8.9 (0.2)*	0.45 (0.04)*	13.7 (1.0)*	1.35 (0.06)*	191.9 (1.9)	88.8 (1.4)
Average drinks per day										
Non-drinker	382	234.5 (6.7)*	12.0 (0.6)	116.4 (1.9)	9.8 (0.2)*	0.47 (0.04)*	15.3 (0.9)*	1.05 (0.03)*	193.5 (1.3)*	86.7 (0.8)
1-2	655	264.3 (4.9)*	10.5 (0.4)	113.8 (1.3)	9.2 (0.2)*	0.68 (0.04)*	19.7 (0.8)*	1.07 (0.03)*	197.0 (0.9)*	87.3 (0.9)
3+	51	266.3 (19.2)*	12.3 (1.5)	117.6 (4.9)	8.4 (0.5)*	0.52 (0.08)*	14.5 (1.8)*	1.48 (0.15)*	190.7 (3.1)*	90.7 (2.6)

TABLE XXVI. (CONTINUED)

GEOMETRIC MEANS OF METAL BIOMARKERS BY PARTICIPANT CHARACTERTISTICS (N=1,088)

Characteristic	N	As (ng/hour)[a]	Cd (ng/hour)[b]	Cu (µg/dL)[c]	Mn (µg/L)[d]	MeHg (µg/L)[e]	Hg (ng/hour)[f]	Pb (µg/dL)[g]	Se (µg/L)[h]	Zn (µg/L)[i]
Body mass index (kg/m^2)										
≤25	347	257.4 (8.2)	10.7 (0.5)	109.9 (1.4)*	9.7 (0.2)	0.71 (0.06)*	18.4 (1.1)	1.2 (0.04)*	193.6 (1.4)	83.4 (1.1)
25-29.9	331	254.4 (7.3)	10.8 (0.6)	111.3 (1.6)*	9.2 (0.2)	0.61 (0.04)*	18.9 (1.3)	1.1 (0.04)*	197.1 (1.6)	87.6 (1.1)
≥30	410	249.6 (6.4)	11.6 (0.5)	122.3 (1.6)*	9.3 (0.2)	0.49 (0.03)*	16.5 (1.0)	1.0 (0.03)*	195.7 (1.3)	86.1 (0.7)
Physically active										
No	387	230.7 (6.9)*	11.8 (0.6)	119.1 (1.6)*	9.5 (0.2)	0.57 (0.04)	16.5 (1.0)	1.09 (0.04)	195.1 (1.4)	86.9 (1.0)
Yes	701	267.1 (5.1)*	10.6 (0.4)	112.6 (1.3)*	9.3 (0.2)	0.60 (0.04)	18.5 (0.8)	1.07 (0.02)	195.6 (0.9)	87.5 (0.7)
Total calories (kcal/day)										
<1550	273	229.4 (8.8)*	12.0 (0.7)	121.5 (1.8)*	9.8 (0.2)	0.68 (0.06)	17.3 (1.2)	1.13 (0.05)	195.8 (1.5)	87.5 (1.2)
1550-1972	271	246.6 (8.4)*	10.7 (0.4)	116.8 (1.7)*	9.3 (0.1)	0.57 (0.04)	16.7 (1.0)	1.05 (0.04)	193.1 (1.5)	85.9 (1.0)
1973-2554	272	267.6 (8.3)*	11.4 (0.6)	112.9 (1.7)*	9.5 (0.2)	0.59 (0.05)	18.6 (1.2)	1.05 (0.04)	197.7 (1.8)	87.0 (1.1)
2555+	272	272.9 (10.2)*	10.2 (0.6)	108.6 (1.8)*	9.0 (0.2)	0.53 (0.03)	18.6 (1.1)	1.09 (0.05)	195.0 (1.8)	88.6 (0.8)

[a] Total arsenic (after calibration to remove the contribution of organic arsenic from recent seafood consumption).
[b] Cadmium.
[c] Copper.
[d] Manganese.
[e] Methylmercury.
[f] Mercury (mainly inorganic/elemental).
[g] Lead.
[h] Selenium.
[i] Zinc.
*p-value < 0.05

TABLE XXVII.
PREVALENCE OF METABOLIC SYNDROME AND INDIVIDUAL COMPONENT CONDITIONS

Condition	Prevalence[a]
Metabolic syndrome	34.2%
High blood pressure	22.2%
High triglycerides	38.6%
Low HDL	47.5%
High glucose	35.9%
Abdominal obesity	47.2%

[a] Standardized by age, gender, race/ethnicity, and family income: poverty ratio.

the probability of selection (all $p_{trends} < 0.05$,Table XXVIII; Figure 10, Appendix B). For arsenic, the only statistically significant positive association was with high triglycerides among individuals with excretion rates in the highest quartile (PR: 1.26, 95% CI: 1.06-1.50, Table XXVIII; Figure 12, Appendix B). Urinary mercury excretion rates above the 75th percentile were associated with a 32% (95% CI: 3-69%) greater prevalence of high blood pressure and 45% (95% CI: 22-74%) greater prevalence of high triglycerides (Table XXVIII; Figures 11-12, Appendix B). Blood selenium concentrations were positively related to dyslipidemia, such that the prevalence of high triglycerides was elevated by 43% (95% CI: 13-82%) and low HDL cholesterol by 21% (95% CI: 2-43%) among those in the highest quartile compared to the lowest (Table XXVIII; Figure 12, Appendix B). Serum zinc concentrations in the highest quartile were associated with a greater likelihood of high triglycerides (PR=1.43, 95% CI: 1.23-1.66), low HDL (PR=1.14, 95% CI: 1.02-1.27) and high glucose (PR=1.17, 95% CI: 1.03-1.33) compared to the lowest quartile (Table XXVIII; Figures 12-13, Appendix B).

Blood lead concentrations were marginally associated with prevalent metabolic syndrome but the relationship was negative in direction (Table XXVIII; Figure 10, Appendix B). Significant inverse linear associations of blood lead were observed for low HDL ($PR_{Q4\ vs.\ Q1}$=0.73, 95% CI: 0.59-0.90; Figure 12, Appendix B) and abdominal obesity ($PR_{Q4\ vs.\ Q1}$=0.67, 95% CI: 0.56-0.79; Figure 14, Appendix B). We

TABLE XXVIII.

PREVALENCE RATIOS FOR METABOLIC SYNDROME AND COMPONENTS BY METAL BIOMARKER (N=1,088)

Metal biomarker	Metabolic syndrome PR (95% CI) Model 1[a]	Metabolic syndrome PR (95% CI) Model 2[b]	High blood pressure[b] PR (95% CI)	High triglycerides[b] PR (95% CI)	Low HDL[b] PR (95% CI)	High glucose[b] PR (95% CI)	Abdominal obesity[c] PR (95% CI)
Urinary arsenic (ng/hour)[d]							
Q1 (25.4-174.2)	1.00 (ref.)	1.00 (ref.)	1.00 (ref.)	1.00 (ref.)	1.00 (ref.)	1.00 (ref.)	1.00 (ref.)
Q2 (174.3-254.2)	1.07 (0.92-1.24)	1.18 (1.03-1.35)	1.15 (0.89-1.47)	1.12 (0.96-1.29)	1.12 (0.94-1.32)	1.12 (0.93-1.34)	0.99 (0.87-1.14)
Q3 (254.3-372.2)	1.12 (0.96-1.32)	1.14 (0.95-1.37)	1.00 (0.74-1.33)	1.18 (0.98-1.42)	1.11 (0.92-1.34)	1.12 (0.94-1.35)	1.08 (0.95-1.22)
Q4 (372.3-2,990.7)	1.20 (1.00-1.44)	1.27 (1.08-1.50)	1.28 (1.00-1.65)	1.26 (1.06-1.50)	1.12 (0.95-1.31)	1.03 (0.87-1.22)	1.00 (0.88-1.14)
p_{trend}	0.034	0.018	0.184	0.010	0.211	0.643	0.618
Urinary cadmium (ng/hour)							
Q1 (0.6-6.2)	1.00 (ref.)	1.00 (ref.)	1.00 (ref.)	1.00 (ref.)	1.00 (ref.)	1.00 (ref.)	1.00 (ref.)
Q2 (6.2-11.0)	1.08 (0.90-1.29)	1.01 (0.83-1.23)	0.94 (0.71-1.23)	0.94 (0.81-1.10)	0.98 (0.82-1.17)	0.82 (0.69-0.98)	1.14 (0.95-1.37)
Q3 (11.1-20.1)	1.10 (0.87-1.38)	1.07 (0.89-1.29)	0.96 (0.76-1.21)	1.07 (0.89-1.28)	1.15 (0.99-1.33)	0.91 (0.78-1.06)	1.14 (0.96-1.36)
Q4 (20.2-206.8)	1.07 (0.84-1.36)	1.03 (0.81-1.30)	1.01 (0.77-1.33)	0.89 (0.74-1.07)	0.96 (0.79-1.16)	1.06 (0.92-1.21)	1.12 (0.94-1.34)
p_{trend}	0.649	0.738	0.829	0.461	0.875	0.202	0.237
Serum copper (µg/dL)							
Q1 (24.7-98.4)	1.00 (ref.)	1.00 (ref.)	1.00 (ref.)	1.00 (ref.)	1.00 (ref.)	1.00 (ref.)	1.00 (ref.)
Q2 (98.5-113.2)	1.04 (0.91-1.18)	0.97 (0.85-1.10)	1.15 (0.88-1.51)	0.97 (0.79-1.20)	0.90 (0.77-1.05)	1.04 (0.87-1.23)	1.25 (1.02-1.52)
Q3 (113.3-132.0)	1.11 (0.91-1.35)	0.95 (0.78-1.16)	1.03 (0.82-1.29)	0.98 (0.80-1.19)	0.94 (0.79-1.12)	1.08 (0.93-1.26)	1.48 (1.17-1.87)
Q4 (132.1-295.6)	1.26 (1.09-1.46)	0.94 (0.80-1.11)	0.94 (0.67-1.31)	1.02 (0.84-1.24)	0.93 (0.79-1.10)	1.14 (0.95-1.36)	1.57 (1.26-1.97)
p_{trend}	0.007	0.528	0.528	0.866	0.580	0.118	<0.001
Blood manganese (µg/L)							
Q1 (3.4-7.5)	1.00 (ref.)	1.00 (ref.)	1.00 (ref.)	1.00 (ref.)	1.00 (ref.)	1.00 (ref.)	1.00 (ref.)
Q2 (7.6-9.3)	1.00 (0.84-1.19)	1.03 (0.91-1.17)	1.15 (0.95-1.40)	1.03 (0.92-1.17)	0.95 (0.82-1.10)	0.97 (0.83-1.12)	1.06 (0.94-1.20)
Q3 (9.4-11.7)	1.01 (0.84-1.21)	0.99 (0.84-1.18)	0.99 (0.76-1.26)	1.06 (0.88-1.28)	1.07 (0.91-1.25)	1.10 (0.96-1.26)	1.02 (0.86-1.20)
Q4 (11.8-45.5)	1.02 (0.83-1.26)	1.04 (0.87-1.23)	1.08 (0.86-1.35)	0.96 (0.81-1.14)	1.02 (0.85-1.21)	0.98 (0.82-1.17)	1.05 (0.89-1.24)
p_{trend}	0.819	0.801	0.865	0.799	0.572	0.747	0.679

TABLE XXVIII. (CONTINUED)

PREVALENCE RATIOS FOR METABOLIC SYNDROME AND COMPONENTS BY METAL BIOMARKER (N=1,088)

	Metabolic syndrome PR (95% CI)		High blood pressure[b]	High triglycerides[b]	Low HDL[b]	High glucose[b]	Abdominal obesity[c]
Metal biomarker	**Model 1[a]**	**Model 2[b]**	**PR (95% CI)**	**PR (95% CI)**	**PR (95% CI)**	**PR (95% CI)**	**PR (95% CI)**
Blood methylmercury (µg/L)							
Q1 (0.08-0.24)	1.00 (ref.)	1.00 (ref.)	1.00 (ref.)	1.00 (ref.)	1.00 (ref.)	1.00 (ref.)	1.00 (ref.)
Q2 (0.25-0.59)	1.02 (0.85-1.23)	0.97 (0.80-1.18)	0.87 (0.66-1.13)	1.07 (0.87-1.31)	0.96 (0.82-1.13)	0.92 (0.77-1.11)	0.96 (0.83-1.10)
Q3 (0.60-1.37)	0.90 (0.74,-1.10)	0.94 (0.79-1.11)	0.94 (0.72-1.22)	1.08 (0.91-1.27)	0.86 (0.71-1.04)	0.97 (0.82-1.16)	0.90 (0.79-1.02)
Q4 (1.38-25.89)	1.00 (0.82-1.22)	1.10 (0.91-1.33)	1.00 (0.78-1.28)	1.19 (0.97-1.45)	1.00 (0.83-1.20)	1.02 (0.84-1.25)	0.85 (0.71-1.01)
p_{trend}	0.657	0.418	0.808	0.102	0.679	0.735	0.039
Urinary mercury (ng/hour)							
Q1 (0.8-8.9)	1.00 (ref.)	1.00 (ref.)	1.00 (ref.)	1.00 (ref.)	1.00 (ref.)	1.00 (ref.)	1.00 (ref.)
Q2 (9.0-17.7)	1.08 (0.92-1.26)	1.14 (0.95, 1.38)	1.28 (1.03-1.60)	1.25 (1.03-1.52)	1.11 (0.95-1.28)	0.98 (0.87-1.12)	1.04 (0.90-1.20)
Q3 (17.8-36.4)	1.13 (0.94-1.37)	1.18 (0.98, 1.42)	1.22 (0.96-1.57)	1.36 (1.09-1.69)	1.08 (0.89-1.32)	1.01 (0.90-1.12)	1.05 (0.87-1.26)
Q4 (36.5-973.3)	1.14 (0.97-1.34)	1.27 (1.06, 1.50)	1.32 (1.03-1.69)	1.45 (1.22-1.74)	1.19 (1.00-1.42)	1.04 (0.90-1.21)	0.97 (0.83-1.13)
p_{trend}	0.125	0.012	0.047	<0.001	0.108	0.501	0.774
Blood lead (µg/dL)							
Q1 (0.18-0.70)	1.00 (ref.)	1.00 (ref.)	1.00 (ref.)	1.00 (ref.)	1.00 (ref.)	1.00 (ref.)	1.00 (ref.)
Q2 (0.71-1.05)	0.89 (0.73-1.09)	0.90 (0.73, 1.11)	1.10 (0.84-1.43)	0.85 (0.72-0.99)	0.90 (0.76-1.07)	1.03 (0.86-1.23)	0.94 (0.82-1.07)
Q3 (1.06-1.63)	0.79 (0.66-0.95)	0.85 (0.69, 1.05)	1.00 (0.75-1.33)	0.76 (0.64-0.91)	0.79 (0.65-0.97)	0.87 (0.69-1.09)	0.92 (0.81-1.05)
Q4 (1.64-15.98)	0.68 (0.53-0.87)	0.82 (0.54, 1.04)	1.01 (0.72-1.41)	0.82 (0.67-1.01)	0.73 (0.59-0.90)	0.95 (0.77-1.18)	0.67 (0.56-0.79)
p_{trend}	0.001	0.076	0.734	0.065	0.003	0.372	<0.001
Blood selenium (µg/L)							
Q1 (120.1-180.6)	1.00 (ref.)	1.00 (ref.)	1.00 (ref.)	1.00 (ref.)	1.00 (ref.)	1.00 (ref.)	1.00 (ref.)
Q2 (180.7-194.9)	1.21 (0.98-1.48)	1.20 (0.98-1.46)	0.86 (0.65-1.14)	1.29 (1.02-1.63)	1.08 (0.89-1.31)	1.11 (0.97-1.26)	1.10 (0.93-1.30)
Q3 (195.0-210.0)	1.16 (0.90-1.48)	1.24 (0.99-1.55)	1.01 (0.77-1.32)	1.27 (0.99-1.62)	1.12 (0.94-1.34)	1.00 (0.85-1.18)	1.02 (0.85-1.22)
Q4 (210.1-356.0)	1.32 (1.07-1.63)	1.31 (1.06-1.63)	1.13 (0.89-1.43)	1.43 (1.13-1.82)	1.21 (1.02-1.43)	1.12 (0.96-1.30)	1.20 (1.00-1.43)
p_{trend}	0.026	0.012	0.137	0.007	0.023	0.325	0.104

TABLE XXVIII. (CONTINUED)

PREVALENCE RATIOS FOR METABOLIC SYNDROME AND COMPONENTS BY METAL BIOMARKER (N=1,088)

Metal biomarker	Metabolic syndrome PR (95% CI) Model 1[a]	Metabolic syndrome PR (95% CI) Model 2[b]	High blood pressure[b] PR (95% CI)	High triglycerides[b] PR (95% CI)	Low HDL[b] PR (95% CI)	High glucose[b] PR (95% CI)	Abdominal obesity[c] PR (95% CI)
Serum zinc (µg/L)							
Q1 (49.1-78.6)	1.00 (ref.)	1.00 (ref.)	1.00 (ref.)	1.00 (ref.)	1.00 (ref.)	1.00 (ref.)	1.00 (ref.)
Q2 (78.7-86.7)	1.03 (0.87-1.22)	1.09 (0.90-1.32)	1.01 (0.80-1.26)	1.15 (0.94-1.40)	1.04 (0.87-1.23)	1.05 (0.87-1.27)	0.99 (0.84-1.17)
Q3 (86.8-96.3)	1.04 (0.86-1.26)	1.12 (0.92-1.36)	0.99 (0.79-1.24)	1.27 (1.06-1.53)	1.06 (0.90-1.25)	1.04 (0.91-1.20)	1.07 (0.93-1.22)
Q4 (96.4-232.5)	1.13 (0.96-1.34)	1.32 (1.11-1.57)	1.14 (0.90-1.44)	1.43 (1.23-1.66)	1.14 (1.02-1.27)	1.17 (1.03-1.33)	0.92 (0.78-1.09)
p_{trend}	0.187	0.004	0.362	<0.001	0.031	0.020	0.593

[a] Model 1 includes age (continuous, years), gender (male or female), race/ethnicity (non-Hispanic white, non-Hispanic black, Hispanic, or other including multi-racial), and family income: poverty ratio (continuous)

[b] Model 2 includes the variables in Model 1, with additional adjustment for total caloric intake (continuous, kcal/day), educational attainment (less than high school, high school diploma or GED, or at least some college), smoking status (current, former, or never), average number of drinks per day in past year, physical activity status (met the 2008 physical activity guidelines or did not), survey cycle (2011-2012 or 2013-2014), and body mass index (continuous, kg/m^2)

[c] Includes all variables in Model 2 except for body mass index

[d] Calibrated to remove the contribution of organic arsenic from recent seafood consumption

additionally observed an inverse relationship of blood methylmercury with abdominal obesity (p_{trend} = 0.039), and positive relationship of serum copper with abdominal obesity ($p_{trend} < 0.001$), but these did not correspond to differences in frank metabolic syndrome prevalence (Figures 10 and 14, Appendix B). Sensitivity analyses controlling for serum cotinine (Table XXXIV, Appendix B) and femoral bone mineral density (in blood lead models, Table XXXV, Appendix B) did not appreciably alter the results; stratified models of blood lead concentrations with metabolic syndrome and component conditions also did not reveal differences by recent weight loss (data not shown). The significant inverse association of blood methylmercury with abdominal obesity disappeared after adjusting for recent seafood consumption (Table XXXVI, Appendix B).

Bivariate correlations between the metal biomarkers were generally low (Table XXIX).The highest Pearson correlation coefficient observed was between blood methylmercury and mercury excretion rates (r = 0.34), indicating a common exposure source for both or that some methylmercury may be excreted via urine.(139) All other correlation coefficients were below 0.20. Five principal components explained 70.39% of the total variance (Table XXX). We characterized these into the following distinct patterns: arsenic-inorganic/elemental mercury, manganese-methylmercury, cadmium-lead, copper, and selenium-zinc. Distributions of the component scores derived from PCA are described in Table XXXI. Co-exposures to arsenic and inorganic/elemental mercury were highest amongst participants who were middle-aged (40-59 years), attended at least some college, had a family income above the federal poverty threshold, drank an average of 1-2 alcoholic beverages per day, and met the physical activity guidelines. The manganese-methylmercury pattern was positively associated with female gender, “other” race/ethnicity, at least some college education, a family income above the poverty line, never or formerly smoking, having an average of 1-2 alcoholic drinks daily, lower BMI, and lower caloric intakes during the 24-hour recalls. Cadmium-lead exposures were lower during the 2013-2014 NHANES cycle, and among younger (20-39 years), female, non-Hispanic white, less educated (less than high school or a diploma/GED), never smoker, non- and light alcohol drinker, obese (BMI ≥ 30 kg/m^2), physically active, and lower calorie consumer participants. The copper pattern was associated with middle age,

TABLE XXIX.
PEARSON CORRELATION COEFFICIENTS BETWEEN METAL BIOMARKERS

	As[a]	**Cd**[b]	**Cu**[c]	**Mn**[d]	**MeHg**[e]	**Hg**[f]	**Pb**[g]	**Se**[h]	**Zn**[i]
As[a]	1.00								
Cd[b]	0.12*	1.00							
Cu[c]	-0.08*	0.08*	1.00						
Mn[d]	-0.02	0.02	0.09*	1.00					
MeHg[e]	0.08*	0.05*	-0.08*	0.13*	1.00				
Hg[f]	0.15*	0.08*	-0.03*	0.01	0.34*	1.00			
Pb[g]	-0.05*	0.15*	-0.07	-0.05	0.06*	0.02	1.00		
Se[h]	0.01	-0.03	-0.10*	0.01	0.19*	0.01*	-0.03	1.00	
Zn[i]	0.03	0.07*	0.03	0.02	0.02	0.01	-0.02	0.10*	1.00

[a] Total arsenic (after calibration to remove the contribution of organic arsenic from recent seafood consumption, expressed as an excretion rate in ng/hr).
[b] Cadmium (ng/hr).
[c] Copper (µg/dL).
[d] Manganese (µg/L).
[e] Methylmercury (µg/L).
[f] Mercury (mainly inorganic/elemental, ng/hr).
[g] Lead (µg/dL).
[h] Selenium (µg/L).
[i] Zinc (µg/L).
*P-value < 0.05.

female gender, a family income below the federal poverty line, not drinking alcohol, obesity, physical inactivity, and lower caloric intakes. Finally, co-exposures to selenium and zinc were higher among males, non-Hispanic whites, and former/current smokers.

Regression model coefficients for the component scores, adjusted for the same covariates as Model 2 in the primary analyses, are displayed in Figure 3. The arsenic-inorganic/elemental mercury pattern was positively and linearly associated with the prevalence of metabolic syndrome ($p_{trend} < 0.001$), primarily due to an association with high triglycerides ($p_{trend} < 0.001$). Co-exposures to manganese and methylmercury appeared unrelated to metabolic syndrome and each of its component conditions. Increasing quartiles for the cadmium-lead pattern showed inverse associations with metabolic syndrome ($p_{trend} = 0.014$), high triglycerides ($p_{trend} = 0.001$), low HDL ($p_{trend} = 0.002$), and abdominal obesity ($p_{trend} < 0.001$). Associations for the copper pattern mirrored those observed in the single-metal models, with null

TABLE XXX.
STANDARDIZED ROTATED FACTOR LOADINGS FROM PCA*

Component	1	2	3	4	5
As[a]	**0.71**	-0.13	-0.12	-0.04	0.02
Cd[b]	0.30	-0.01	**0.55**	0.34	0.11
Cu[c]	-0.06	0.02	-0.05	**0.75**	0.08
Mn[d]	-0.16	**0.59**	-0.16	0.39	-0.06
MeHg[e]	0.09	**0.64**	0.17	-0.16	-0.06
Hg[f]	**0.55**	0.33	0.00	-0.07	-0.02
Pb[g]	-0.18	0.02	**0.78**	-0.14	-0.03
Se[h]	-0.15	0.31	-0.11	-0.35	**0.54**
Zn[i]	0.05	-0.11	0.03	0.12	**0.83**
Eigenvalue	1.48	1.29	1.28	1.19	1.09
Total variance (%)	16.36	14.37	14.28	13.27	12.08
Cumulative (%)	16.36	30.76	45.03	58.31	70.39

[a] Total arsenic (after calibration to remove the contribution of organic arsenic from recent seafood consumption, expressed as an excretion rate in ng/hr).
[b] Cadmium (ng/hr).
[c] Copper (µg/dL).
[d] Manganese (µg/L).
[e] Methylmercury (µg/L).
[f] Mercury (mainly inorganic/elemental, ng/hr).
[g] Lead (µg/dL).
[h] Selenium (µg/L).
[i] Zinc (µg/L).
*Loadings are bolded if **> 0.40**.

TABLE XXXI.

DISTRIBUTIONS OF COMPONENT SCORES BY PARTICIPANT CHARACTERISTICS

	As-Hg pattern[a]			Mn-MeHg pattern[b]			Cd-Pb pattern[c]			Cu pattern[d]			Se-Zn pattern[e]		
Characteristic	**Q1[f]**	**Q4[g]**	**p_{trend}**	**Q1[f]**	**Q4[g]**	**p_{trend}**	**Q1[f]**	**Q4[g]**	**p_{trend}**	**Q1[f]**	**Q4[g]**	**p_{trend}**	**Q1[f]**	**Q4[g]**	**p_{trend}**
Survey cycle			0.455			0.528			<0.001			0.210			0.130
2011-2012	46.7	47.1		46.0	48.5		38.2	53.7		50.4	43.8		53.3	42.7	
2013-2014	53.3	52.9		54.0	51.5		61.8	46.3		49.6	57.2		46.7	57.3	
Age (years)			0.011			0.108			<0.001			0.019			0.639
20-39	44.9	34.2		40.8	33.5		70.2	9.2		41.9	31.2		39.7	36.4	
40-59	25.0	44.1		31.3	37.1		23.2	40.4		31.6	42.3		30.9	36.0	
60+	30.1	21.7		27.9	29.4		6.6	30.4		26.5	26.5		29.4	27.6	
Gender			0.223			<0.001			0.001			<0.001			<0.001
Female	52.2	49.6		38.6	51.8		58.5	37.5		16.5	76.5		53.3	41.2	
Male	47.8	50.4		61.4	48.2		41.5	62.5		83.5	23.5		46.7	58.8	
Race/ethnicity			0.217			<0.001			0.936			0.115			0.039
White	48.9	41.9		57.4	36.0		46.3	45.2		50.0	41.5		41.6	48.5	
Black	19.9	16.5		19.5	15.4		14.7	25.7		14.4	26.5		25.0	17.3	
Hispanic	22.4	24.6		18.8	20.6		29.4	14.7		19.1	23.5		21.3	21.7	
Other	8.8	16.9		4.4	27.9		9.6	14.3		16.5	8.5		12.1	12.5	
Education			<0.001			0.023			0.006			0.121			0.942
Less than high school	25.4	15.4		22.1	15.1		15.8	29.8		17.6	23.5		18.8	18.4	
High school diploma/GED	27.2	16.9		26.8	15.4		21.3	23.5		21.0	22.4		22.0	22.0	
At least some college	47.4	67.7		51.1	69.5		62.9	46.7		61.4	54.1		59.2	59.6	
Family income to poverty ratio			0.159			0.047			0.017			<0.001			0.734
Below poverty (<1)	25.7	21.7		26.8	19.5		30.2	20.6		17.3	31.6		28.3	24.6	
At or above poverty (>1)	74.3	78.3		73.2	80.5		69.9	79.4		82.7	68.4		71.7	75.4	
Smoking status			0.060			0.002			<0.001			0.052			0.024
Never smoker	61.4	54.8		51.5	66.2		76.1	32.7		62.9	53.0		59.2	50.4	
Former smoker	16.2	28.3		22.1	25.0		14.0	37.1		24.6	20.2		19.5	26.5	
Current smoker	22.4	16.9		26.5	8.8		9.9	30.2		12.5	26.8		21.3	23.1	

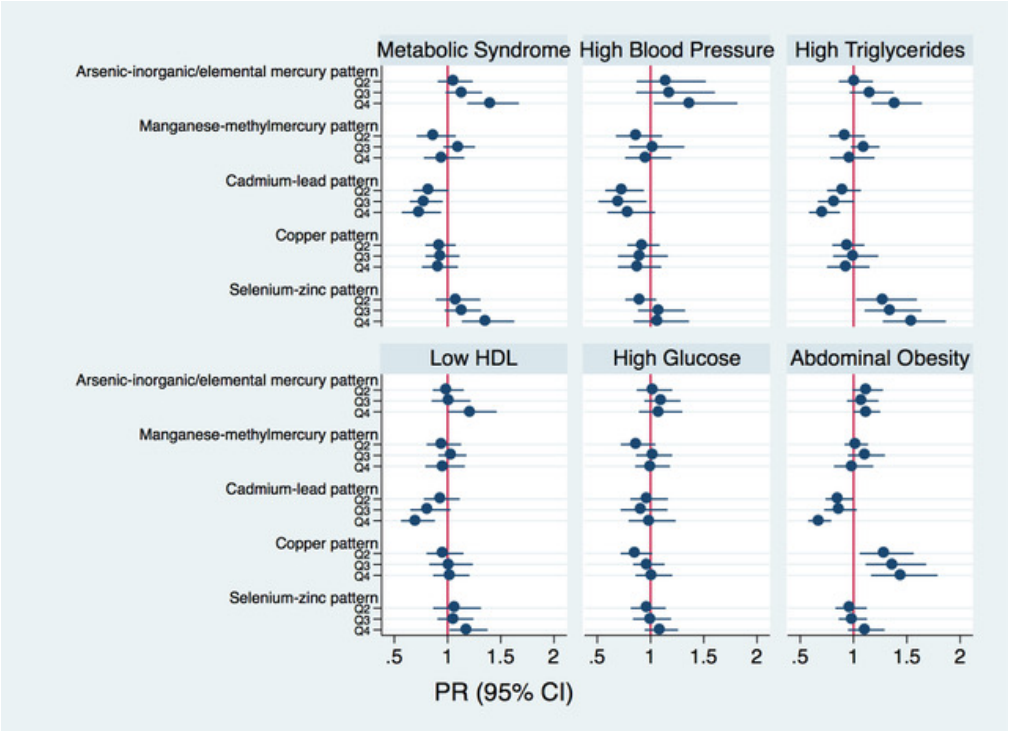
TABLE XXXI. (CONTINUED)

DISTRIBUTIONS OF COMPONENT SCORES BY PARTICIPANT CHARACTERISTICS

	As-Hg pattern[a]			Mn-MeHg pattern[b]			Cd-Pb pattern[c]			Cu pattern[d]			Se-Zn pattern[e]		
Characteristic	**Q1[f]**	**Q4[g]**	**p_{trend}**	**Q1[f]**	**Q4[g]**	**p_{trend}**	**Q1[f]**	**Q4[g]**	**p_{trend}**	**Q1[f]**	**Q4[g]**	**p_{trend}**	**Q1[f]**	**Q4[g]**	**p_{trend}**
Average drinks per day			0.001			0.021			0.882			0.002			0.125
Non-drinker	43.4	27.6		38.2	32.0		35.3	34.2		28.3	46.0		38.6	33.1	
1-2	52.2	67.6		55.2	65.4		62.5	58.4		66.5	48.5		57.0	62.5	
3+	4.4	4.8		6.6	2.6		2.2	7.4		5.2	5.5		4.4	4.4	
Body mass index (kg/m^2)			0.770			0.040			0.013			0.024			0.978
≤25	35.3	35.3		32.4	41.2		28.7	37.8		40.8	27.9		29.8	30.9	
25-29.9	26.1	29.4		27.9	29.8		28.3	30.2		34.2	23.2		30.2	32.0	
≥30	38.6	35.3		39.7	29.0		43.0	32.0		25.0	48.9		40.0	37.1	
Physically active			0.090			0.802			0.020			0.016			0.675
No	39.3	32.0		30.5	32.4		31.6	41.9		28.7	42.7		36.8	37.9	
Yes	60.7	68.0		69.5	67.7		68.4	58.1		71.3	57.4		63.2	62.1	
Total calories (kcal/day)			0.061			0.009			0.016			<0.001			0.489
<1550	27.9	23.5		21.7	30.5		21.7	28.3		18.0	29.8		21.3	26.5	
1550-1972	27.9	22.1		27.9	26.1		26.5	23.5		19.9	27.2		26.1	19.5	
1973-2554	21.4	26.1		23.2	21.7		25.3	25.0		27.6	24.2		25.7	26.1	
2555+	22.8	28.3		27.2	21.7		26.5	23.2		34.5	18.8		26.9	27.9	

[a] Arsenic-inorganic/elemental mercury pattern.
[b] Manganese-methylmercury pattern.
[c] Cadmium-lead pattern.
[d] Copper pattern.
[e] Selenium-zinc pattern.
[f] Quartile 1.
[g] Quartile 4.

Figure 3. Adjusted prevalence ratios (95% CI) for metabolic syndrome and individual component conditions by principal component score quartiles in NHANES[a]



[a] Adjusted for age (continuous, years), gender (male or female), race/ethnicity (non-Hispanic white, non-Hispanic black, Hispanic, or other including multi-racial), family income: poverty ratio (continuous), total caloric intake (continuous, kcal/day), educational attainment (less than high school, high school diploma or GED, or at least some college), smoking status (current, former, or never), average number of drinks per day in past year, and physical activity status (met the 2008 physical activity guidelines or did not), survey cycle (2011-2012 or 2013-2014), with additional adjustment for body mass index (continuous, kg/m^2) in all models except for those of abdominal obesity

relationships observed for metabolic syndrome and all component conditions except for abdominal obesity ($p_{trend} = 0.002$). Lastly, selenium-zinc scores were monotonically related to the prevalence of metabolic syndrome ($p_{trend} < 0.001$), but only the positive association with high triglycerides was statistically significant ($p_{trend} < 0.001$).

D. Discussion

This is the first study to assess exposures to multiple metals with metabolic syndrome in the United States. We found higher levels of arsenic, inorganic/elemental mercury, selenium, and zinc biomarkers to be cross-sectionally associated with an increased prevalence of metabolic syndrome. These relationships persisted after adjusting for various sociodemographic and lifestyle characteristics. When assessing patterns of co-exposures through principal component analysis, we again observed increasing exposures to arsenic, inorganic/elemental mercury, selenium, and zinc were associated with a greater burden of metabolic syndrome. Conversely, blood lead concentrations, especially when coupled with urinary cadmium excretion rates, were consistently inversely related to metabolic syndrome, low HDL cholesterol levels, and abdominal obesity.

Prior research studies assessing metal exposures in isolation have found arsenic and inorganic/elemental mercury exposures to be associated with high blood pressure, whereas selenium has been associated with high triglycerides.(52, 104, 166) The identified combinations of arsenic with inorganic/elemental mercury and selenium with zinc, however, are a novel contribution of the present analysis. Arsenic and inorganic/elemental mercury co-exposures may be due to a shared exposure pathway. For example, diets heavy in contaminated foods like rice are one possible source.(167, 168) The selenium-zinc pattern could similarly be the result of dietary patterns (e.g., meat is rich in both minerals) or multi-mineral supplement use.(169) Toxic metals arsenic and mercury could influence the development of cardiometabolic abnormalities by an assortment of mechanisms, including epigenetic changes, oxidative stress, and/or inflammation.(52, 111) The essential metals selenium and zinc, on the other hand, are considered to defend against oxidative stress. There are, however, mechanistic data to

suggest excessive selenium does not necessarily correlate with enhanced antioxidant activity and that excessive zinc can actually increase the expression of pro-inflammatory cytokines.(170-173) Hence, our findings may reflect possible harmful effects of selenium and zinc at exposure levels above certain thresholds.

The consistent inverse associations of blood lead with metabolic syndrome was unexpected, as was the lack of an association with high blood pressure given the hypertensive effect of lead exposure is widely documented.(112) That being said, lead concentrations in blood may only be weakly related to blood pressure – a meta-analysis found that a doubling of blood concentrations corresponds to a 0.6-1.0 mmHg increase in diastolic and systolic pressures, respectively.(174) Nearly all analyzed individuals had low-level blood lead concentrations (<5 μg/dL), and the estimated prevalence of high blood pressure was only 22.2%. Thus, we may have been underpowered to detect an association. In addition, other NHANES analyses have shown lead and cadmium are both negatively associated with measures of obesity, congruent with our finding for abdominal obesity.(175, 176) An inverse link between blood lead concentrations and HDL cholesterol has also been reported by a small recent Taiwanese study (n=677), which the authors posited might be related to metallothionein activity based on their observation of effect modification by genetic variants of MT2A (which encodes the metallothionein-2 protein).(177) The results of our analyses of the cadmium-lead pattern derived from principal component analysis, in which we observed negative associations with metabolic syndrome, high triglycerides, low HDL cholesterol, and abdominal obesity, and a U-shaped association with high blood pressure, were also surprising. However, inverse associations of urinary cadmium with blood pressure have been reported in the literature, and thus divergent relationships of lead and cadmium may explain the apparent U-shaped dose-response curve.(53) We cannot rule out the possibility that the negative relationships of blood lead/cadmium with dyslipidemia and abdominal obesity could be due to reverse causation. For example, lifestyle changes in response to these conditions may lead to weight loss, which has been found to mobilize lead stored in bones and increase the amount circulating in the bloodstream.(164, 178) While we

attempted to address the impact of recent weight loss in sensitivity analyses, we were limited by the self-reported nature of the weight history data available.

While novel and comprehensive, the present study has several limitations worth nothing. The cross-sectional study design precludes the establishment of temporality. Higher levels of arsenic, inorganic/elemental mercury, copper, selenium, and zinc and lower levels of cadmium and lead could be found in their respective biologic matrices as a consequence of metabolic syndrome or any number of the individual component conditions. Additionally, the biomarkers assessed in this study were measured only at one point in time and thus provide only a snapshot of exposures. Future studies should consider repeatedly measuring metal biomarkers to better elucidate longitudinal associations with metabolic syndrome. Likewise, other biologic matrices such as toenails or hair might serve as better long-term markers of metal exposures.(179)

Despite these drawbacks, the current analysis has several strengths. It is the first to evaluate essential and toxic metal exposures – individually and jointly – with metabolic syndrome among U.S. adults. NHANES collected a range of biospecimens, thereby allowing for improvements over other studies that are often relegated to measuring metals in only one matrix. Furthermore, although we did not use NHANES sampling weights and thus our results may not be entirely generalizable, our analytic sample included racially and ethnically diverse men and women, aged 20-74 years, from across the country. In summary, this work provides insights on manifold metal exposures and their interrelationships with adverse cardiometabolic health. Future prospective studies are needed to confirm whether are findings represent causal associations.

IV. CHANGES IN BLOOD PRESSURE ASSOCIATED WITH LEAD, MANGANESE, AND SELENIUM IN A BANGLADESHI COHORT

A. Background

Lead contamination is a major public health problem in Bangladesh where unsafe levels have been recorded in drinking water supplies and in ambient air.(180, 181) Leaded gasoline was banned in 1999, but background contamination persists.(182) In addition, leaded paint and industrial emissions are widespread.(183)Toxic metal exposures are increasingly being recognized as potentially important risk factors for the development of cardiovascular disease.(3) For lead in particular, increases in blood pressure and hypertension are among the most widely studied clinical manifestations.(184) The evidence suggests that even low-level exposures to lead (i.e., blood lead concentrations below 10 μg/dL) have a hypertensive effect.(47)

In contrast to toxic metals, other metals are needed at trace amounts for normal physiologic functioning. Manganese is one such essential micronutrient that can be a toxicant when exposures are excessive. Recent studies have observed associations of manganese body burden (as measured in blood and toenails) and recent exposure (as measured from dietary recalls) with blood pressure.(185-187) However, these studies have been limited by cross-sectional designs and have found inconsistent patterns. Like lead, elevated concentrations of manganese have been documented in Bangladesh's groundwater.(180) In general though, the most common sources of manganese are dietary, with grains and vegetables primary contributors to dietary intakes.(188) Selenium is another antioxidant trace element that may play a role in the development of cardiovascular disease. A cross-sectional analysis of serum selenium concentrations observed a positive relationship with hypertension, but the opposite was found in a prospective study evaluating selenium in whole blood.(189, 190)

Given both the essentiality and potential toxicity of manganese and selenium, previous studies may have observed conflicting results by assuming linear relationships with blood pressure, or because different study populations may be at differing points of dose-response curves. In addition, there is a lack

of prospective data for the manganese-blood pressure relationship. To date, it is unclear how these micronutrients affect blood pressure over time, or if joint exposures with lead could modify any effects. To that end, we evaluated the longitudinal associations of lead, manganese, and selenium with blood pressure in a prospective cohort of Bangladeshi adults.

B. Methods

1. Study Population

The Bangladesh Vitamin E and Selenium Trial (BEST) participants are residents of rural communities in Bangladesh enrolled in a 2x2 factorial, randomized controlled trial of 7,000 adults with arsenic-induced skin lesions. The trial was designed to evaluate the efficacy of vitamin E and selenium supplementation as chemopreventive agents for skin cancer. Enrollment procedures are described elsewhere.(191) Briefly, individuals were permanent residents in the arsenic-endemic study area (Araihazar districts of Narayanganj, Comilla, Noakhali, and the Matlab district of Chandpur), and provided signed informed consent. Individuals who were pregnant, unwilling to discontinue current vitamin use, had a prior history of cancer, were too ill to participate, or were unwilling to provide blood and urine samples were excluded. Participants were randomized between April 2006 and August 2009, and underwent biennial follow-up clinical examinations for a 6-year period. For the purposes of this study, we restricted analyses to participants randomized to the placebo arm (n=1,753).

2. Biomarkers of Exposure

Of placebo-assigned participants enrolled in the study, 255 were randomly selected for blood measurements of lead, manganese, and selenium at the baseline examination. Venous blood samples were collected in 10 mL vacutainer tubes, stored in portable 4°C coolers immediately after collection, and processed within 2 to 8 hours of collection in the field laboratory. Upon receipt, samples were stored at -80°C until analysis. Samples were thawed, thoroughly mixed, and diluted 50 times. Concentrations of lead, manganese, and selenium were measured in whole blood using ICP Mass Spectrophotometer, model

ELAN DRC II, manufactured by Perkin Elmer. Detection limits were 0.02 µg/dL for lead, 0.4 µg/L for manganese, and 0.4 µg/L for selenium. Since BEST was designed as a clinical trial for the prevention of non-melanoma skin cancer among individuals with visible arsenic toxicity, urinary arsenic concentrations were additionally measured. After enrollment to the trial, participants whose primary drinking water source contained unsafe levels of arsenic were provided with filters to reduce their exposure.(191) We therefore considered arsenic exposure to be a potential confounder, rather than a main exposure of interest.

3. Blood Pressure, Other Clinical Parameters, and Sociodemographic Factors

General clinical examinations of participants were conducted at baseline, 2-, 4-, and 6-years follow-up by trained study physicians. Blood pressure and anthropomorphic measurements were collected following standard protocols.(191) At each examination, after a 5 minute period of rest, the study physician obtained two seated blood pressure measurements using an automated sphygmomanometer. The average systolic and diastolic value at each examination was calculated for use in subsequent analyses; pulse pressure was calculated as the difference between the average systolic and diastolic blood pressure measurements at each visit. Body weight was measured to the nearest kilogram at baseline and every biennial clinical evaluation. Height was recorded to the nearest centimeter, and was measured only at baseline.

A health and lifestyle questionnaire that included demographic characteristics (age, sex, educational duration, among others), medication use, and health history, was administered by the study physician in Bengali. Participants were specifically asked about all prescription and over-the-counter medications regularly taken, and were asked to bring their respective containers to each examination for review by the study physician. Medications were then standardized to generic names, and sorted into categories including antihypertensives and antidiabetes.(192) At the last follow-up visit, blood specimens from all participants were measured for glycated hemoglobin (HbA1c) in the field laboratory on a Lambda UV/ViS spectrophotometer (Perkin-Elmer, Waltham, MA). Using these sources of information,

we defined diabetes as either self-report of a physician diagnosis, antidiabetes medication use, or HbA1c at or above 6.1%. The 6.1% cutpoint, which is lower than the 6.5% value recommended by the American Diabetes Association, was selected based on prior studies of HbA1c within South Asian populations.(193, 194)

4. <u>Statistical Analyses</u>

Analyses were confined to the random subset of placebo-assigned participants with measured concentrations of lead, manganese, and selenium at baseline 255 of 1,753 individuals). Mixed-effects regression models of systolic, diastolic, and pulse pressure were performed separately. The general model forms were as follows:

$$BP_{ij} = \alpha + X_{i0}\beta + Time_{ij}\,\beta + X_{i0}(Time_{ij})\beta + Z_{ij}\beta + \mu_{i0} + e_{ij}$$

where BP_{ij} is the blood (or pulse) pressure of the *i*th individual at time *j*. X_{i0} is a row vector of the baseline biomarker(s), which were modeled using dummy variables for quartiles of exposure with the lowest serving as the referent category; we additionally calculated p-values for linear trends by modeling quartiles as ordinal variables. Time is the duration between the baseline examination and the blood pressure measurement (i.e., 0, 2, 4, or 6 years). The β coefficients for $X_{i0}(Time_{ij})$ are the estimated annual changes in blood or pulse pressure corresponding to the respective biomarker quartile. Z_{ij} is a matrix of potential confounders, representing either covariates measured at baseline or at each examination (i.e., time-varying). μ_{i0} is a random intercept for each individual used to account for the within-subject correlation of the repeated blood pressure measures, and e_{ij} is the error term.

Potential confounders were selected based on *a priori* knowledge of factors associated with exposures to metals and blood pressure. All models were sex- and age-adjusted. Single biomarker models were performed to evaluate the individual effect of each biomarker on changes in blood pressure. Mutually adjusted models included baseline blood concentrations of lead, manganese, and selenium to

control for confounding by co-exposures. Fully adjusted models further included study site (Araihazar or Matlab), smoking status (current, former, or never), educational duration (years), creatinine-corrected urinary arsenic concentration (μg/g), body mass index (kg/m2), diabetes status (yes or no), and antihypertensive medication use (yes or no). Body mass index, diabetes status, and antihypertensive medication use were allowed to vary over time. Indicator variables were used to model categorical variables, while continuous variables (i.e., age, educational duration, urinary arsenic concentrations, and body mass index) were modeled as restricted cubic splines with knots at the 10th, 50th, and 90th percentiles to allow for non-linearity. Tests for linear trends in the longitudinal associations between the biomarkers and blood pressure were conducted by modeling concentration quartiles as ordinal variables. Interactions between lead, manganese, and selenium (i.e., all possible combinations, including an interaction between all three biomarkers over time) were evaluated using likelihood ratio tests. All analyses were conducted in Stata version 14.2 (College Station, TX).

5. Sensitivity Analyses

Because antihypertensive medications can lower blood pressure, we conducted sensitivity analyses to evaluate the robustness of our results. First, we repeated analyses excluding individuals who reported antihypertensive medication use at baseline or at any follow-up visit. Second, we added a constant value (10 mmHg for systolic, 5 mmHg for diastolic) to blood pressure among those using antihypertensive medications, and reanalyzed the corrected blood and pulse pressure measurements.(195)

In addition to analyzing blood pressure as a continuous variable, we also evaluated hypertensive status in order to gain insights into the clinical relevance of lead, manganese, and selenium exposures. We defined hypertension as a systolic blood pressure ≥140 mmHg, diastolic blood pressure ≥90 mmHg, antihypertensive medication use, or self-reported physician diagnosed hypertension. Participants classified as hypertensive at the baseline examination were excluded from these analyses (n=77). Among the remaining 178 participants, exposure biomarker quartiles were redefined. Discrete-time hazard models were used to estimate hazard ratios with 95% confidence intervals for hypertension

incidence.(196) The models were based on the probability of becoming hypertensive during each two-year follow-up period, conditional on being normotensive during the prior follow-up interval. Participants who were lost to follow-up were censored; participants who remained normotensive were additionally censored at the end of study participation. The proportional-hazards assumption was checked by testing interaction terms with time. The confounders included in the discrete-time hazard models were identical to those from our main analyses.

C. Results

Of the study participants, 234 (91.8%) had all four blood pressure measurements (baseline and 3 follow-ups), 16 had three measurements (baseline and 2 follow-ups), and 5 individuals had two measurements (baseline and 1 follow-up). On average, systolic and diastolic blood pressures were relatively stable over the study period, while antihypertensive medication use increased (Table XXXII).

TABLE XXXII.
BLOOD PRESSURE, PULSE PRESSURE, AND ANTIHYPERTENSIVE MEDICATION USE OVER TIME

Visit	N	Systolic blood pressure (mmHg) *Mean ± SD*	Diastolic blood pressure (mmHg) *Mean ± SD*	Pulse pressure (mmHg) *Mean ± SD*	Antihypertensive medication use *n (%)*
Baseline	255	119 ± 16	78 ± 10	41 ± 11	19 (7.5)
Visit 1	255	115 ± 17	76 ± 10	40 ± 11	22 (8.6)
Visit 2	248	113 ± 17	74 ± 11	39 ± 11	27 (10.9)
Visit 3	236	118 ± 18	77 ± 10	40 ± 12	36 (15.3)

Overall, median blood concentrations at baseline were 8.5 µg/dL for lead, 10.0 µg/L for manganese, and 122.0 µg/L for selenium. Lead concentrations were weakly positively correlated with manganese (Pearson correlation = 0.13, p-value = 0.04) and selenium concentrations (Pearson correlation = 0.21, p-value < 0.001). No association was observed between blood concentrations of selenium and manganese (Pearson correlation = 0.03, p-value = 0.67). Table XXXIII describes how concentrations differed by sociodemographic and clinical characteristics. Levels of blood lead were greater among individuals from Araihazar, males, and current and former smokers. Participants with greater concentrations of manganese in their blood at baseline tended to be women, ages 25-37 years, and former or never smokers. Selenium concentrations at baseline were higher among males, and were positively associated with body mass index.

Likelihood ratio tests indicated that concentrations of lead, manganese, and selenium did not modify the effects of one another on blood pressure trajectories (p-values > 0.10). Single biomarker sex- and age-adjusted point estimates differed substantially (<10%) from the models with mutual adjustment for all three biomarkers, suggesting lead, manganese, and selenium confound one another (Tables XXXVII-XXXIX, Appendix C). For example, lead concentrations in the highest quartile were more strongly associated with longitudinal increases in systolic blood pressure after accounting for manganese and selenium. Estimates from mutually adjusted models, however, were similar when comparing only sex- and age-adjustment to full adjustment for all additional covariates (smoking status, educational duration, creatinine-corrected urinary arsenic concentration, diabetes, body mass index, and antihypertensive use).

In fully adjusted models blood lead concentrations in the highest quartile were associated with longitudinal increases of 1.16 mmHg per year (95% CI: 0.21, 2.11) in systolic blood pressure, 0.53 mmHg per year (95% CI: -0.10, 1.16) in diastolic blood pressure, and 0.63 mmHg per year (-0.08, 1.34) in pulse pressure (Figure 4). The association between lead exposure and systolic blood pressure appeared to be monotonic ($P_{trend} = 0.037$). Mid-range manganese concentrations were associated with declines in blood pressure; systolic blood pressure decreased -1.64 (95% CI: -2.56, -0. 72) mmHg annually among

TABLE XXXIII.

BLOOD CONCENTRATIONS BY PARTICIPANT CHARACTERISTICS AT BASELINE

		Lead (µg/dL)		Manganese (µg/L)		Selenium (µg/L)	
Characteristic	**N**	**Median (IQR)[a]**	**p-value**	**Median (IQR)[a]**	**p-value**	**Median (IQR)[a]**	**p-value**
Overall	255	8.5 (4.2-13.8)		10.0 (8.2-12.4)		122.0 (111.0-136.0)	
Study site			<0.01		0.19		0.45
Araihazar	155	11.9 (8.9-15.4)		9.7 (8.2-12.3)		123.0 (111.0-139.0)	
Matlab	100	3.5 (2.6-5.1)		10.3 (8.4-13.1)		122.0 (110.0-136.0)	
Gender			<0.01		<0.01		0.02
Female	140	5.8 (3.3-10.5)		10.8 (8.6-13.8)		121.0 (107.0-134.5)	
Male	115	11.6 (5.9-15.1)		9.1 (7.9-11.0)		125.0 (114.0-140.0)	
Age (years)			0.50		0.01		0.33
25-37	88	8.0 (3.6-13.5)		10.7 (8.7-13.8)		121.5 (111.5-132.5)	
38-46	82	8.1 (4.3-14.4)		9.4 (8.2-11.7)		127.5 (109.0-140.0)	
47-64	85	9.2 (5.0-12.8)		9.4 (7.8-12.0)		120.0 (110.0-130.0)	
Smoking status			<0.01		0.01		0.59
Current	60	11.9 (6.1-15.1)		9.2 (7.8-10.6)		125.0 (112.5-140.0)	
Former	24	10.9 (5.4-16.7)		10.2 (8.3-12.5)		120.0 (112.5-140.5)	
Never	171	7.1 (3.5-11.8)		10.3 (8.4-13.1)		122.0 (110.0-136.0)	
Education duration (years)			0.21		0.86		0.85
0	101	9.9 (5.0-14.4)		9.8 (8.2-12.4)		122.0 (110.0-139.0)	
1-5	83	7.8 (3.9-12.8)		10.0 (8.6-12.3)		122.0 (110.0-138.0)	
6-15	71	7.4 (3.7-13.4)		10.1 (8.1-12.3)		124.0 (116.0-134.0)	
Urinary arsenic (µg/g creatinine)			<0.01		0.02		<0.01
22-99	66	10.4 (7.1-15.3)		8.7 (7.7-11.5)		128.0 (121.0-140.0)	
100-399	90	10.0 (5.0-14.8)		10.0 (8.4-12.9)		125.5 (113.0-141.0)	
400-2240	99	5.4 (2.8-10.3)		10.4 (8.6-12.5)		117.0 (104.0-128.0)	
Body mass index (kg/m^2)			0.07		0.32		<0.01
17.0-18.6	101	7.6 (3.5-11.9)		10.1 (8.7-12.3)		117.0 (107.0-128.0)	
18.7-21.5	83	7.4 (4.2-14.4)		10.1 (8.1-12.9)		125.0 (118.0-143.0)	
21.6-32.9	71	9.7 (5.6-14.9)		9.4 (8.0-11.8)		129.0 (118.0-143.0)	

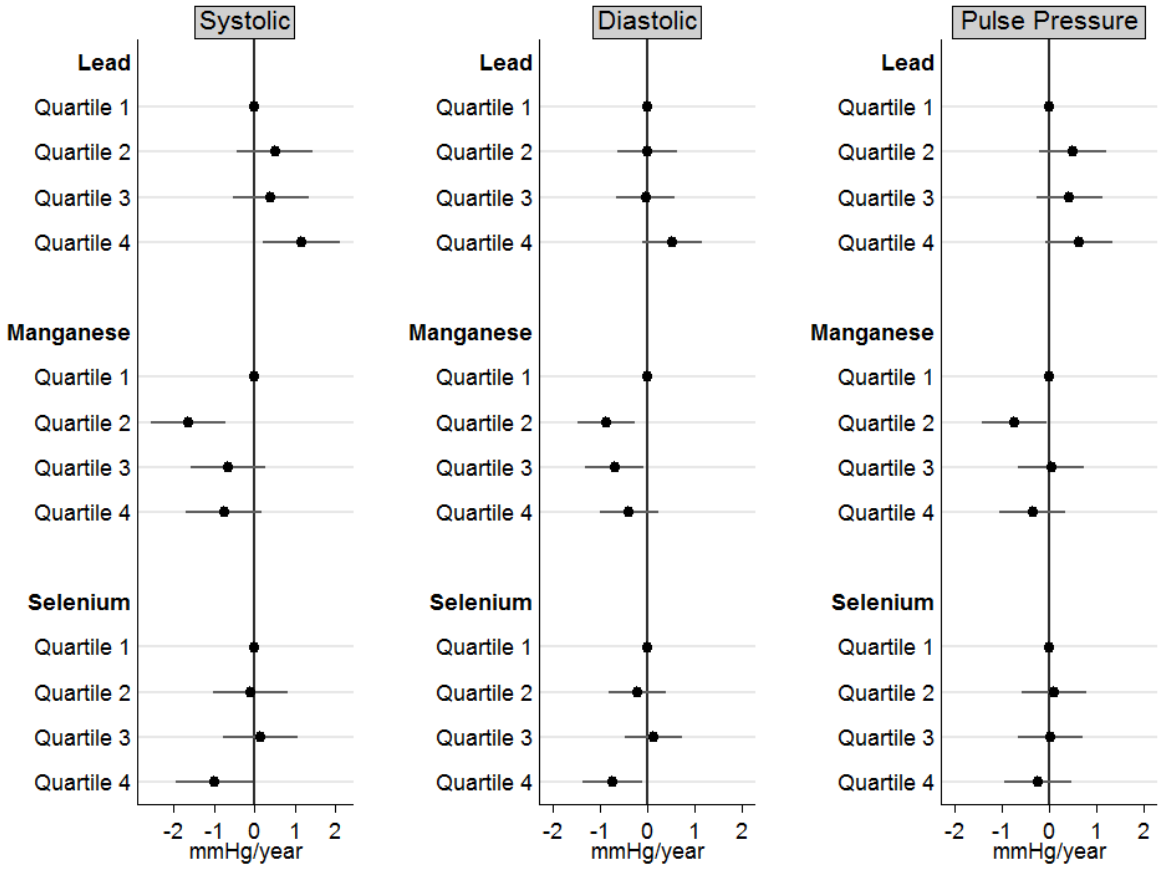
TABLE XXXIII. (CONTINUED)

BLOOD CONCENTRATIONS BY PARTICIPANT CHARACTERISTICS AT BASELINE

		Lead (µg/dL)		Manganese (µg/L)		Selenium (µg/L)	
Characteristic	**N**	**Median (IQR)[a]**	**p-value**	**Median (IQR)[a]**	**p-value**	**Median (IQR)[a]**	**p-value**
Diabetes			0.69		0.05		0.13
No	251	8.5 (4.2-13.8)		10.1 (8.3-12.4)		122.0 (110.0-136.0)	
Yes	4	9.9 (7.2-12.3)		8.2 (7.9-8.2)		135.0 (127.5-141.5)	
Antihypertensive medication use			0.31		0.27		0.87
No	236	8.7 (4.3-13.9)		10.1 (8.3-12.4)		122.0 (111.0-136.0)	
Yes	19	6.4 (4.2-10.0)		9.0 (8.1-11.4)		122.0 (110.0-141.0)	

[a] IQR = interquartile range (25^{th}-75^{th} percentiles).

Figure 4. Adjusted longitudinal changes in systolic, diastolic, and pulse pressure by quartiles of whole blood lead, manganese, and selenium concentrations in BEST[a]



[a] Mutually adjusted for baseline blood lead, manganese, and selenium, in addition to age, sex, site, smoking status, educational duration, creatinine-corrected urinary arsenic concentration, diabetes, body mass index, and antihypertensive use.

individuals in the second quartile, diastolic blood pressures decreased -0.88 (95% CI: -1.48, -0.27) and -0.70 (95% CI: -1.31, -0.08) mmHg annually among individuals in the second and third quartiles, respectively, and pulse pressure decreased -0.74 (95% CI: -1.42, -0.06) mmHg annually among individuals in the second quartile. Yearly decreases of 0.99 (95% CI: -1.95, -0.04) mmHg in systolic blood pressure and 0.73 (95% CI: -1.36, -0.09) mmHg in diastolic blood pressure were observed among those in the highest quartile of selenium exposure, but no associations were observed with pulse pressure.

Sensitivity analyses to address biases from antihypertensive medication use yielded similar results to our main analyses (Figures 15-16, Appendix C). Between 2006 and 2015, 46 incident hypertension cases were identified among the 178 participants analyzed – 19 cases at the first follow-up, 9 at the second, and 18 at the third (Figure 17, Appendix C). Median (25^{th}, 75^{th} percentile) values of the biomarkers were as follows: 7.8 (4.0-13.8) µg/dL for lead, 10.1 (8.3-13.0) µg/L for manganese, and 121.0 (110.0-135.0 µg/L for selenium. Only manganese was significantly associated with hypertension risk; baseline manganese concentrations in the second quartile were associated with a 79% decrease in the incidence of hypertension in the fully adjusted model relative to the lowest quartile (Table XXXX, model 3, HR: 0.21, 95% CI: 0.06-0.81, Appendix C). The hazard ratio estimates for manganese concentrations in the third and fourth quartiles, as well as for lead and selenium concentrations were largely null.

D. Discussion

Within this population of rural Bangladeshi adults, the median blood lead concentration was 8.5 µg/dL blood lead. For adults, concentrations above 5.0 µg/dL are considered elevated, thus this population has exposure levels of clinical and public health concern.(197) Without a formal exposure risk assessment, the sources of lead exposure are unclear but may be due to industrial (e.g., lead acid battery manufacturing, leaded paint, ceramics) and agricultural (e.g., pesticides) applications of lead, which are not currently regulated in Bangladesh.(198, 199) Concentrations of manganese were generally normal (4-15 µg/L) in the study sample.(200, 201) Whole blood selenium does not have established reference levels, but the observed concentrations were similar to those reported in previous studies.(202, 203) The

results of our analyses suggest that both manganese and selenium have the potential to lower blood pressure when exposures are within certain ranges. For manganese, blood concentrations within the second and third quartiles (8.3-12.4 μg/L) were consistently associated with significant annual declines in blood pressure and pulse pressure, suggesting a U- or J-shaped dose-response. For selenium, blood concentrations in the highest quartile (137-214 μg/L) were the only range of exposure to have a significant blood pressure-lowering effect. Without evidence of a linear trend, these results indicate the possibility of a threshold effect for selenium exposure. In contrast, lead exposures raised blood pressures annually in a dose-dependent manner.

This study is the first to our knowledge to prospectively evaluate the collective role of essential micronutrients and toxic metals with blood pressure. We found no statistical evidence of effect modification, but did observe manganese and selenium exerted lowering effects on blood pressure in contrast to the increases associated with greater lead exposure. These results are consistent with prior longitudinal studies of lead, and somewhat consistent with a longitudinal study of selenium that observed a significant inverse association with hypertension, but only among men.(190, 204, 205) Our finding of non-linear associations for manganese with blood and pulse pressure is novel, and may have been missed by other studies if linearity was assumed or if the exposure distribution of the study population was different. The precise mechanisms underlying these relationships have not yet been entirely elucidated. It has been hypothesized that lead exposure triggers acute responses from the autonomic nervous system, or stimulates more chronic oxidative stress and inflammation pathways.(206-208) The essential micronutrients manganese and selenium both function through enzymes (manganese metalloenzymes and selenoproteins, respectively), of which several possess antioxidant properties that may offer protection from oxidative stress.(188, 209)

There are several limitations to this study. For one, the biomarkers used to evaluate exposure status may be suboptimal. Blood concentrations of lead are routinely used in environmental epidemiologic studies as an indicator of recent exposure, but the half-life of lead in blood is only 36 days. Bone concentrations of lead are preferable as they are indicative of cumulative exposures, however,

testing requires specialized imaging equipment.(210) Blood may be a particularly poor biological matrix for the measurement of manganese, with a half-life of only 2 hours reported in rats, whereas the half-life for selenium has been estimated to be 100 days.(201, 211) Nevertheless, we found significant associations between blood concentrations of these elements and changes in blood pressure over time, for which there is biologic plausibility. Furthermore, since manganese and selenium are essential micronutrients that are consumed daily rather than episodically, we do not expect large variations in the amounts consumed from dietary sources. Blood concentrations were only measured at baseline, thus we could not evaluate changes in exposure levels over time, or how increases or reductions in exposure levels could affect blood pressure trajectories. However, we expect changes in exposure levels to be minimal for two reasons: (1) metals are persistent environmental toxicants, and (2) food consumption patterns, while broadly shifting in Bangladesh in recent years to incorporate more non-starch foods, were likely relatively stable at the individual-level during the 6-year follow-up period.(212) We did not evaluate other micronutrients, such as iron, zinc, or copper that may protect against the toxicity of lead by reducing absorption or retention.(210) Lastly, this study was conducted within a sample of adults highly exposed to arsenic, so the results may not be generalizable to other populations.

In a relatively short study period, we observed opposing changes in blood pressure associated with lead, manganese, and selenium exposures. These changes were relatively small (<2 mmHg/year), but would be clinically meaningful over the course of many years. With Bangladesh currently in the midst of transitioning from a public health burden of communicable to non-communicable disease, an estimated 20% of the adult population is already suffering from hypertension.(213). This number is only expected to grow, thus, if confirmed, our results could be of great public health importance. The toxic effects of lead are well-documented, but our findings suggest that many Bangladeshis have elevated levels of exposure.(197, 210) In addition to hypertension, kidney damage and neurocognitive effects are of concern at levels these high, particularly for vulnerable populations like pregnant women and children. Ongoing efforts to reduce arsenic exposure in the area may also want to consider remediation of lead from water sources. More research is necessary to determine the major sources of current lead exposure. If exposures

are predominantly from workplace exposures or cigarette smoking, regulatory measures targeted at reducing lead in occupational settings or in tobacco products may prove more effective. Our findings additionally indicated that optimizing intakes of nutritionally essential micronutrients could promote the lowering of blood pressures. Future studies should assess the efficacy of dietary or supplemental interventions on micronutrient intakes for hypertension prevention.

V. DISCUSSION

This body of work evaluated the relationships of metal exposures from various sources with cardiometabolic phenotypes in three distinct populations. While we observed significant positive and negative associations for certain metals with specific conditions in some populations, these relationships were null in others. Nevertheless, our findings improve upon the current understanding of metals as non-traditional risk factors for chronic disease and can be used to generate hypotheses.

A. Summary and Discussion of Aims

The major findings from Aim 1, which assessed selected essential metals from dietary and supplemental sources with metabolic syndrome among diverse U.S. Hispanic/Latino adults, were as follows: 1.) greater total intakes of copper and zinc were each associated with a reduced risk of incident high blood pressure; 2.) greater total intakes of manganese and zinc were each associated with a reduced risk of incident high fasting glucose; and 3.) greater total intakes of selenium were associated with an increased risk of incident high triglycerides and marginally increased risk of incident low HDL cholesterol. We were unable to distinguish which source (food/beverages or supplements) if either was more important as a protective or risk factor in these preliminary analyses. However, we plan on re-evaluating once complete data on the 6-year follow-up visit are made available.

The second Aim of this dissertation was to look at how exposures to multiple metals, including toxic ones, may be related to metabolic syndrome among U.S. adults. Through traditional regression modeling and the application of principal component analysis, we found increasing exposures to inorganic arsenic-inorganic and elemental mercury were associated with a higher burden of metabolic syndrome, and with high blood pressure, high triglycerides, and low HDL cholesterol in particular. We also observed positive associations for selenium and zinc exposures with prevalent high triglycerides and low HDL cholesterol. In contrast, individuals with higher exposures to lead, including when

concomitantly exposed to more cadmium, were significantly less likely to have experienced metabolic syndrome, dyslipidemia, and abdominal obesity.

In our final Aim, we analyzed how whole blood concentrations of manganese, lead, and selenium related to longitudinal changes in blood and pulse pressure in a cohort of Bangladeshi adults. Over a 6-year period, both manganese and selenium were associated with reductions in blood pressure, but the associations appeared to be non-linear (for manganese, a U- or J-shaped dose-response curve; for selenium, a possible threshold). In contrast, a linear trend was seen for lead exposure, with an average increase of over 1 mmHg in systolic blood pressure annually for the most highly exposed (blood lead concentrations >13.8 μg/dL). However, exposures to lead (and arsenic) were much higher in this population than those seen among U.S. adults. While we found no statistical evidence of lead exposures modifying the blood pressure-lowering effects of the essential metals manganese and selenium, it is possible that these associations are confined to individuals highly exposed to toxic metals. As such, the generalizability of the findings from Aim 3 is unclear.

In both Aims 1 and 2, selenium was consistently positively associated with high triglycerides. Within the HCHS/SOL population, daily total intakes of selenium above 149.1 μg were linked with the highest likelihood of developing high triglycerides, whereas in the NHANES, blood concentrations above 210.0 μg/L were associated with an elevated prevalence of the condition. Both cut-points correspond to the experiences of the upper 25% of individuals. We were unable to evaluate triglycerides within the BEST population, but our findings suggest selenium might exert a modest blood pressure-lowering effect not seen in the two U.S. populations. In general, blood selenium concentrations in Bangladesh (median: 122 μg/L) tended to be lower than those seen in the U.S. (median: 195 μg/L). Although the differences in selenium concentrations between these two populations may reflect inter-laboratory variation, our findings could instead suggest the role of selenium as a cardiometabolic protective or harmful risk factor is dependent upon selenium status. In fact, there is a growing body of literature suggesting selenium increases the risk of cardiometabolic conditions only among selenium-replete populations.(171)

Like with selenium, each of our 3 analyses was able to evaluate manganese within the context of cardiometabolic health. Results from the first Aim indicated increasing dietary and supplemental intakes of manganese could confer protection against the development of high fasting glucose, but not frank metabolic syndrome or other related conditions, among diverse U.S. Hispanic/Latino adults. In Aim 2, neither manganese itself nor in combination with methylmercury was associated with blood pressure, dyslipidemia, hyperglycemia, or abdominal obesity within the general U.S. population. Finally, in our third Aim, we observed mid-range manganese exposures to be associated with a reduced risk of hypertension and a narrowing of pulse pressure (a marker of arterial stiffness and useful predictor for cardiovascular disease). The seemingly protective concentrations of blood manganese indicated in these Bangladeshi adults were between 8.2 and 12.4 μg/L. While the general U.S. population had a similar range of blood manganese concentrations, we found no association with pre-existing hypertension analyses using data from NHANES. Furthermore, we saw null associations for dietary and supplemental intakes with incident hypertension within the HCHS/SOL, despite a similar duration of follow-up. Discrepancies between these three analyses could be due to differences in study design, including exposure assessment, measurement error, and statistical power, or inherent heterogeneity between the US-based and Bangladeshi study populations. Furthermore, we did not adjust for multiple comparisons across the three studies (or for the various endpoints addressed within each study) as our hypotheses were specified *a priori*. Thus, it should be noted that some of our results may be false positives.

Zinc exposures were also analyzed in Aims 1 and 2. Although not directly comparable, as Aim 1 relied on estimates of dietary and supplemental intakes whereas Aim 2 objectively measured concentrations in serum samples, the results were incongruent. For example, greater zinc intakes were related to a lower risk of developing high blood pressure and high glucose over a 6-year period among U.S. Hispanic/Latino adults. Cross-sectional analyses using data from NHANES, however, indicated higher zinc exposures were associated with a greater burden of prevalent dyslipidemia and hyperglycemia. It is worth noting that intakes of essential metals from dietary sources reflect only one possible route of exposure. In contrast, biomarkers integrate exposures from multiple pathways, thus

serum concentrations could represent zinc inhaled from polluted ambient air or from occupational settings. It is therefore possible that only excessive zinc exposures from environmental sources are responsible for the relationships seen within the NHANES population. Prospective studies with biomarkers are needed to disentangle the influence of zinc on glucose levels and lipid profiles at the population-level.

Our findings regarding lead exposures were also mixed. The hypertensive effects of lead are widely recognized, but we found no cross-sectional association between blood lead concentrations and high blood pressure in U.S. adults.(210) Conversely, we observed a linear trend with longitudinal increases systolic blood pressure among Bangladeshi adults, although blood lead concentrations were several orders of magnitude higher in this population. For most Americans, lead exposures have decreased substantially in recent years.(197) The levels of lead exposure experienced by the general U.S. population may be so low that no measureable effect on blood pressure can be detected. Our results suggesting lead is inversely associated with low HDL cholesterol and abdominal obesity are more difficult to interpret, especially because so few epidemiologic studies have evaluated lead exposures with these endpoints. Longitudinal data on lead biomarkers, including lead content in bones, bone mineral density, and anthropometric measurements are needed to confirm our findings and clarify possible mechanisms.

Despite somewhat inconsistent findings, this work is an important contribution to the study of environmental chemicals as risk factors for adverse cardiometabolic health. On top of evaluating the associations of 8 metals and metalloids – both essential and toxic – with a multitude of cardiometabolic conditions, we were able to estimate usual intakes of selected minerals among diverse Hispanics/Latinos (one of the fastest growing minority populations in the US) and identify exposures to metal mixtures within the general U.S. population. Furthermore, our work in Bangladesh highlighted the significant burden of toxic lead exposures there, an issue that may be overshadowed by a focus on arsenic contamination in this region. By leveraging data from three distinct study populations, we were able to assess the impact of environmental and dietary metal exposures on a variety of chronic conditions in a

way that a single data source would have been unlikely to capture. As such, this dissertation represents a comprehensive body of research addressing key questions regarding metals and cardiometabolic health from different vantage points.

B. Future Directions

Future studies should consider moving beyond analyses of individual metals to focus on multiple exposures, keeping in mind that individuals are exposed to many metals simultaneously on a daily basis. Likewise, investigators should assess the impacts of essential metals given their continual intake through foods and potential intake from dietary supplements, in addition to metals recognized as toxic. Prospective chronic disease cohorts with extant specimens and/or the resources to collect and test for biomarkers would benefit from technologies like inductively coupled plasma mass spectrometry that have the capability to measure panels of metals quickly and at a low cost.

Measuring metal biomarkers and cardiometabolic parameters at different time points would also help to clarify the directionality of observed associations and identify critical windows of exposure. An ideal study of metals as causal factors for metabolic syndrome would collect temporal data on exposures, outcomes, and time-varying confounders, in addition to conducting formal risk assessments to identify sources and routes of exposure. There are ongoing epidemiologic efforts, notably the Strong Heart Study, presently conducting such investigations.(214) Animal studies on metals, especially those with a focus on oxidative stress and systemic inflammation, would also aid in elucidating their underlying mechanisms of action on the cardiovascular system and metabolic processes.

A foremost challenge in examining multiple environmental and dietary exposures is how to best quantify their collective impact. We used several approaches in this dissertation, including creating "scores" reflecting combinations of dietary and supplemental intakes, principal component analysis, and assessing statistical interaction. More advanced methodologies, including those that allow for non-linearity, are currently under development with some are already being applied to similar research questions.(215) Implementing a Bayesian statistical framework, novel frequentist models like weighted

quantile sum regression, or relying on machine learning might allow for more accurate identification of metal-induced cardiometabolic phenotypes and the development of public health interventions in response.

C. Conclusions

The studies included in this dissertation have broadened the current knowledge regarding the impacts of exposures to metals and suggest that both toxic and essential metals from dietary and environmental sources may be determinants of cardiometabolic risk. Future research is needed to validate our findings and to shed light on instances where our results were equivocal. The information garnered from this work and related studies could ultimately prove useful in guiding future public health interventions. At the population-level, such research could have far-reaching impacts by identifying specific metals (or metal mixtures) that require environmental remediation or minerals that should be optimized through changes in diets or through supplementation. Ultimately, this research could be a valuable first step for the development of policy efforts addressing environmental contamination and/or fortification of the food supply.

CITED LITERATURE

1. Aguilar, M., T. Bhuket, S. Torres, B. Liu, and R.J. Wong, *Prevalence of the metabolic syndrome in the United States, 2003-2012.* Jama, 2015. **313**(19): p. 1973-4.

2. Grundy, S.M., *Metabolic syndrome update.* Trends Cardiovasc Med, 2016. **26**(4): p. 364-73.

3. Lamas, G.A., A. Navas-Acien, D.B. Mark, and K.L. Lee, *Heavy Metals, Cardiovascular Disease, and the Unexpected Benefits of Chelation Therapy.* J Am Coll Cardiol, 2016. **67**(20): p. 2411-8.

4. Alberti, K.G., R.H. Eckel, S.M. Grundy, P.Z. Zimmet, J.I. Cleeman, K.A. Donato, J.C. Fruchart, W.P. James, C.M. Loria, S.C. Smith, Jr., E. International Diabetes Federation Task Force on, Prevention, L. Hational Heart, I. Blood, A. American Heart, F. World Heart, S. International Atherosclerosis, and O. International Association for the Study of, *Harmonizing the metabolic syndrome: a joint interim statement of the International Diabetes Federation Task Force on Epidemiology and Prevention; National Heart, Lung, and Blood Institute; American Heart Association; World Heart Federation; International Atherosclerosis Society; and International Association for the Study of Obesity.* Circulation, 2009. **120**(16): p. 1640-5.

5. Qu, X., K. Huang, L. Deng, and H. Xu, *Selenium deficiency-induced alterations in the vascular system of the rat.* Biol Trace Elem Res, 2000. **75**(1-3): p. 119-28.

6. Davis, C.D., D.M. Ney, and J.L. Greger, *Manganese, iron and lipid interactions in rats.* J Nutr, 1990. **120**(5): p. 507-13.

7. Klevay, L.M., *Hypertension in Rats Due to Copper Deficiency.* Nutrition Reports International, 1987. **35**(5): p. 999-1005.

8. Medeiros, D.M., *Hypertension in the Wistar-Kyoto Rat as a Result of Post-Weaning Copper Restriction.* Nutrition Research, 1987. **7**(2): p. 231-235.

9. Baly, D.L., D.L. Curry, C.L. Keen, and L.S. Hurley, *Dynamics of Insulin and Glucagon-Release in Rats - Influence of Dietary Manganese.* Endocrinology, 1985. **116**(5): p. 1734-1740.

10. Cohen, A.M., A. Teitelbaum, E. Miller, V. Bentor, R. Hirt, and M. Fields, *Effect of Copper on Carbohydrate-Metabolism in Rats.* Israel Journal of Medical Sciences, 1982. **18**(8): p. 840-844.

11. Hurley, L.S., C.L. Keen, and D.L. Baly, *Manganese Deficiency and Toxicity - Effects on Carbohydrate-Metabolism in the Rat.* Neurotoxicology, 1984. **5**(1): p. 97-104.

12. Klevay, L.M., W.K. Canfield, S.K. Gallagher, L.K. Henriksen, H.C. Lukaski, W. Bolonchuk, L.K. Johnson, D.B. Milne, and H.H. Sandstead, *Decreased Glucose-Tolerance in 2 Men during Experimental Copper Depletion.* Nutrition Reports International, 1986. **33**(2): p. 371-382.

13. Baer, M.T., J.C. King, T. Tamura, S. Margen, R.B. Bradfield, W.L. Weston, and N.A. Daugherty, *Nitrogen-Utilization, Enzyme-Activity, Glucose-Intolerance and Leukocyte Chemotaxis in Human Experimental Zinc Depletion.* American Journal of Clinical Nutrition, 1985. **41**(6): p. 1220-1235.

14. Katyakatya, M., A. Ensminger, L. Mejean, and G. Debry, *The Effect of Zinc Supplementation on Plasma-Cholesterol Levels.* Nutrition Research, 1984. **4**(4): p. 633-638.

15. Klevay, L.M., *Hypercholesterolemia in rats produced by an increase in the ratio of zinc to copper ingested.* Am J Clin Nutr, 1973. **26**(10): p. 1060-8.

16. Liu, C.C.F. and D.M. Medeiros, *Excess Diet Copper Increases Systolic Blood-Pressure in Rats.* Biological Trace Element Research, 1986. **9**(1): p. 15-24.

17. Friedman, B.J., J.H. Freelandgraves, C.W. Bales, F. Behmardi, R.L. Shoreykutschke, R.A. Willis, J.B. Crosby, P.C. Trickett, and S.D. Houston, *Manganese Balance and Clinical Observations in Young Men Fed a Manganese-Deficient Diet.* Journal of Nutrition, 1987. **117**(1): p. 133-143.

18. Lukaski, H.C., L.M. Klevay, and D.B. Milne, *Effects of Dietary Copper on Human Autonomic Cardiovascular Function.* European Journal of Applied Physiology and Occupational Physiology, 1988. **58**(1-2): p. 74-80.

19. Park, J.H.Y., C.J. Grandjean, M.H. Hart, S.H. Erdman, P. Pour, and J.A. Vanderhoof, *Effect of Pure Zinc-Deficiency on Glucose-Tolerance and Insulin and Glucagon-Levels.* American Journal of Physiology, 1986. **251**(3): p. E273-E278.

20. Stranges, S., J.R. Marshall, R. Natarajan, R.P. Donahue, M. Trevisan, G.F. Combs, F.P. Cappuccio, A. Ceriello, and M.E. Reid, *Effects of long-term selenium supplementation on the incidence of type 2 diabetes - A randomized trial.* Annals of Internal Medicine, 2007. **147**(4): p. 217-223.

21. Coudray, C., A.M. Roussel, F. Mainard, J. Arnaud, A. Favier, and E.S. Grp, *Lipid peroxidation level and antioxidant micronutrient status in a pre-aging population; Correlation with chronic disease prevalence in a French epidemiological study (Nantes, France).* Journal of the American College of Nutrition, 1997. **16**(6): p. 584-591.

22. Stranges, S., S. Sieri, M. Vinceti, S. Grioni, E. Guallar, M. Laclaustra, P. Muti, F. Berrino, and V. Krogh, *A prospective study of dietary selenium intake and risk of type 2 diabetes.* Bmc Public Health, 2010. **10**.

23. Sun, Q., R. Van Dam, W.C. Willett, and F.B. Hu, *A Prospective Study of Zinc Intake and Risk of Type 2 Diabetes: The Nurses' Health Study.* Circulation, 2009. **119**(10): p. E291-E291.

24. Seo, J.A., S.W. Song, K. Han, K.J. Lee, and H.N. Kim, *The Associations between Serum Zinc Levels and Metabolic Syndrome in the Korean Population: Findings from the 2010 Korean National Health and Nutrition Examination Survey.* Plos One, 2014. **9**(8).

25. Eshak, E.S., H. Iso, K. Maruyama, I. Muraki, and A. Tamakoshi, *Associations between dietary intakes of iron, copper and zinc with risk of type 2 diabetes mellitus: A large population-based prospective cohort study.* Clinical Nutrition, 2018. **37**(2): p. 667-674.

26. Zhou, B.A., X.F. Su, D.T. Su, F.F. Zeng, M.H. Wang, L.C. Huang, E.S. Huang, Y.B. Zhu, D. Zhao, D.H. He, X.H. Zhu, E. Yeoh, R.H. Zhang, and G.Q. Ding, *Dietary intake of manganese and the risk of the metabolic syndrome in a Chinese population.* British Journal of Nutrition, 2016. **116**(5): p. 853-863.

27. Shan, Z., S. Chen, T. Sun, C. Luo, Y. Guo, X. Yu, W. Yang, F.B. Hu, and L. Liu, *U-Shaped Association between Plasma Manganese Levels and Type 2 Diabetes.* Environ Health Perspect, 2016.

28. Obeid, O., M. Elfakhani, S. Hlais, M. Iskandar, M. Batal, Y. Mouneimne, N. Adra, and N. Hwalla, *Plasma copper, zinc, and selenium levels and correlates with metabolic syndrome components of lebanese adults.* Biological Trace Element Research, 2008. **123**(1-3): p. 58-65.

29. Feng, W., X. Cui, B. Liu, C. Liu, Y. Xiao, W. Lu, H. Guo, M. He, X. Zhang, J. Yuan, W. Chen, and T. Wu, *Association of urinary metal profiles with altered glucose levels and diabetes risk: a population-based study in China.* PLoS One, 2015. **10**(4): p. e0123742.

30. Lee, M., B. Jung, S. Chung, J. Chung, and O. Bae, *Arsenic-induced dysfunction in relaxation of blood vessels.* Toxicological Sciences, 2003. **72**: p. 17-17.

31. Fu, J.Q., C.G. Woods, E. Yehuda-Shnaidman, Q.A. Zhang, V. Wong, S. Collins, G.F. Sun, M.E. Andersen, and J.B. Pi, *Low-Level Arsenic Impairs Glucose-Stimulated Insulin Secretion in Pancreatic Beta Cells: Involvement of Cellular Adaptive Response to Oxidative Stress.* Environmental Health Perspectives, 2010. **118**(6): p. 864-870.

32. Afolabi, O.K., A.D. Wusu, O.O. Ogunrinola, E.O. Abam, D.O. Babayemi, O.A. Dosumu, O.B. Onunkwor, E.A. Balogun, O.O. Odukoya, and O. Ademuyiwa, *Arsenic-induced dyslipidemia in male albino rats: comparison between trivalent and pentavalent inorganic arsenic in drinking water.* Bmc Pharmacology & Toxicology, 2015. **16**.

33. El Muayed, M., M.R. Raja, X.M. Zhang, K.W. MacRenaris, S. Bhatt, X.J. Chen, M. Urbanek, T.V. O'Halloran, and W.L. Lowe, *Accumulation of cadmium in insulin-producing beta cells.* Islets, 2012. **4**(6): p. 405-416.

34. Messner, B., M. Knoflach, A. Seubert, A. Ritsch, K. Pfaller, B. Henderson, Y.H. Shen, I. Zeller, J. Willeit, G. Laufer, G. Wick, S. Kiechl, and D. Bernhard, *Cadmium Is a Novel and Independent Risk Factor for Early Atherosclerosis Mechanisms and In Vivo Relevance.* Arteriosclerosis Thrombosis and Vascular Biology, 2009. **29**(9): p. 1392-1398.

35. Larregle, E.V., S.M. Varas, L.B. Oliveros, L.D. Martinez, R. Anton, E. Marchevsky, and M.S. Gimenez, *Lipid metabolism in liver of rat exposed to cadmium.* Food and Chemical Toxicology, 2008. **46**(5): p. 1786-1792.

36. Gonick, H.C., Y.X. Ding, S.C. Bondy, Z.M. Ni, and N.D. Vaziri, *Lead-induced hypertension - Interplay of nitric oxide and reactive oxygen species.* Hypertension, 1997. **30**(6): p. 1487-1492.

37. Chen, K.L., S.H. Liu, C.C. Su, C.C. Yen, C.Y. Yang, K.I. Lee, F.C. Tang, Y.W. Chen, T.H. Lu, Y.C. Su, and C.F. Huang, *Mercuric Compounds Induce Pancreatic Islets Dysfunction and Apoptosis in Vivo.* International Journal of Molecular Sciences, 2012. **13**(10): p. 12349-12366.

38. Wildemann, T.M., N. Mirhosseini, S.D. Siciliano, and L.P. Weber, *Cardiovascular responses to lead are biphasic, while methylmercury, but not inorganic mercury, monotonically increases blood pressure in rats.* Toxicology, 2015. **328**: p. 1-11.

39. Moreira, E.L., J. de Oliveira, M.F. Dutra, D.B. Santos, C.A. Goncalves, E.M. Goldfeder, A.F. de Bem, R.D. Prediger, M. Aschner, and M. Farina, *Does Methylmercury-Induced Hypercholesterolemia Play a Causal Role in Its Neurotoxicity and Cardiovascular Disease?* Toxicological Sciences, 2012. **130**(2): p. 373-382.

40. Heo, Y., P.J. Parsons, and D.A. Lawrence, *Lead differentially modifies cytokine production in vitro and in vivo.* Toxicol Appl Pharmacol, 1996. **138**(1): p. 149-57.

41. Gump, B.B., J.A. MacKenzie, A.K. Dumas, C.D. Palmer, P.J. Parsons, Z.M. Segu, Y.S. Mechref, and K.G. Bendinskas, *Fish consumption, low-level mercury, lipids, and inflammatory markers in children.* Environ Res, 2012. **112**: p. 204-11.

42. Dutta, K., P. Prasad, and D. Sinha, *Chronic low level arsenic exposure evokes inflammatory responses and DNA damage.* Int J Hyg Environ Health, 2015. **218**(6): p. 564-74.

43. Eckel, R.H., K.G. Alberti, S.M. Grundy, and P.Z. Zimmet, *The metabolic syndrome.* Lancet, 2010. **375**(9710): p. 181-3.

44. Navas-Acien, A., E.K. Silbergeld, R. Pastor-Barriuso, and E. Guallar, *Arsenic exposure and prevalence of type 2 diabetes in US adults.* JAMA, 2008. **300**(7): p. 814-22.

45. Moon, K.A., E. Guallar, J.G. Umans, R.B. Devereux, L.G. Best, K.A. Francesconi, W. Goessler, J. Pollak, E.K. Silbergeld, B.V. Howard, and A. Navas-Acien, *Association between exposure to low to moderate arsenic levels and incident cardiovascular disease. A prospective cohort study.* Ann Intern Med, 2013. **159**(10): p. 649-59.

46. Tellez-Plaza, M., A. Navas-Acien, A. Menke, C.M. Crainiceanu, R. Pastor-Barriuso, and E. Guallar, *Cadmium exposure and all-cause and cardiovascular mortality in the U.S. general population.* Environ Health Perspect, 2012. **120**(7): p. 1017-22.

47. Gambelunghe, A., G. Sallsten, Y. Borne, N. Forsgard, B. Hedblad, P. Nilsson, B. Fagerberg, G. Engstrom, and L. Barregard, *Low-level exposure to lead, blood pressure, and hypertension in a population-based cohort.* Environ Res, 2016. **149**: p. 157-63.

48. Vupputuri, S., M.P. Longnecker, J.L. Daniels, X. Guo, and D.P. Sandler, *Blood mercury level and blood pressure among US women: results from the National Health and Nutrition Examination Survey 1999-2000.* Environ Res, 2005. **97**(2): p. 195-200.

49. Moon, S.S., *Additive effect of heavy metals on metabolic syndrome in the Korean population: the Korea National Health and Nutrition Examination Survey (KNHANES) 2009-2010.* Endocrine, 2014. **46**(2): p. 263-71.

50. Lee, B.K. and Y. Kim, *Blood cadmium, mercury, and lead and metabolic syndrome in South Korea: 2005-2010 Korean National Health and Nutrition Examination Survey.* Am J Ind Med, 2013. **56**(6): p. 682-92.

51. Maull, E.A., H. Ahsan, J. Edwards, M.P. Longnecker, A. Navas-Acien, J. Pi, E.K. Silbergeld, M. Styblo, C.H. Tseng, K.A. Thayer, and D. Loomis, *Evaluation of the association between arsenic and diabetes: a National Toxicology Program workshop review.* Environ Health Perspect, 2012. **120**(12): p. 1658-70.

52. Abhyankar, L.N., M.R. Jones, E. Guallar, and A. Navas-Acien, *Arsenic Exposure and Hypertension: A Systematic Review.* Environ Health Perspect, 2012. **120**(4): p. 494-500.

53. Gallagher, C.M. and J.R. Meliker, *Blood and urine cadmium, blood pressure, and hypertension: a systematic review and meta-analysis.* Environ Health Perspect, 2010. **118**(12): p. 1676-84.

54. Cheng, Y., J. Schwartz, D. Sparrow, A. Aro, S.T. Weiss, and H. Hu, *Bone lead and blood lead levels in relation to baseline blood pressure and the prospective development of hypertension: the Normative Aging Study.* Am J Epidemiol, 2001. **153**(2): p. 164-71.

55. He, K., P. Xun, K. Liu, S. Morris, J. Reis, and E. Guallar, *Mercury exposure in young adulthood and incidence of diabetes later in life: the CARDIA Trace Element Study.* Diabetes Care, 2013. **36**(6): p. 1584-9.

56. Ballatori, N., *Transport of toxic metals by molecular mimicry.* Environ Health Perspect, 2002. **110 Suppl 5**: p. 689-94.

57. Sigel, A., H. Sigel, R.K.O. Sigel, and Royal Society of Chemistry (Great Britain), *Metallothioneins and related chelators*. Metal ions in life sciences,. 2009, Cambridge: Royal Society of Chemistry. xxix, 514 p.

58. Archer, S.L., J. Stamler, A. Moag-Stahlberg, L. Van Horn, D. Garside, Q. Chan, J.J. Buffington, and A.R. Dyer, *Association of dietary supplement use with specific micronutrient intakes among middle-aged American men and women: the INTERMAP Study.* J Am Diet Assoc, 2005. **105**(7): p. 1106-14.

59. Abdulla, M. and J. Chmielnicka, *New aspects on the distribution and metabolism of essential trace elements after dietary exposure to toxic metals.* Biol Trace Elem Res, 1989. **23**: p. 25-53.

60. Kordas, K., B. Lonnerdal, and R.J. Stoltzfus, *Interactions between nutrition and environmental exposures: effects on health outcomes in women and children.* J Nutr, 2007. **137**(12): p. 2794-7.

61. Trumbo, P., A.A. Yates, S. Schlicker, and M. Poos, *Dietary reference intakes: vitamin A, vitamin K, arsenic, boron, chromium, copper, iodine, iron, manganese, molybdenum, nickel, silicon, vanadium, and zinc.* Journal of the American Dietetic Association, 2001. **101**(3): p. 294-301.

62. Monsen, E.R., *Dietary reference intakes for the antioxidant nutrients: vitamin C, vitamin E, selenium, and carotenoids.* Journal of the American Dietetic Association, 2000. **100**(6): p. 637-40.

63. Capdor, J., M. Foster, P. Petocz, and S. Samman, *Zinc and glycemic control: a meta-analysis of randomised placebo controlled supplementation trials in humans.* J Trace Elem Med Biol, 2013. **27**(2): p. 137-42.

64. Klein, E.A., I. Thompson, C.M. Tangen, M.S. Lucia, P. Goodman, L.M. Minasian, L.G. Ford, H.L. Parnes, J.M. Gaziano, D.D. Karp, M.M. Lieber, P.J. Walther, J.K. Parsons, J. Chin, A.K. Darke, S.M. Lippman, G.E. Goodman, F.L. Meyskens, and L.H. Baker, *Vitamin E and the risk of prostate cancer: Updated results of the Selenium and Vitamin E Cancer Prevention Trial (SELECT).* Journal of clinical oncology : official journal of the American Society of Clinical Oncology, 2012. **30**(5_suppl): p. 7.

65. Eshak, E.S., H. Iso, K. Maruyama, I. Muraki, and A. Tamakoshi, *Associations between dietary intakes of iron, copper and zinc with risk of type 2 diabetes mellitus: A large population-based prospective cohort study.* Clin Nutr, 2017.

66. Stranges, S., S. Sieri, M. Vinceti, S. Grioni, E. Guallar, M. Laclaustra, P. Muti, F. Berrino, and V. Krogh, *A prospective study of dietary selenium intake and risk of type 2 diabetes.* BMC Public Health, 2010. **10**: p. 564.

67. Schwab, S., A. Zierer, M. Heier, B. Fischer, C. Huth, J. Baumert, C. Meisinger, A. Peters, and B. Thorand, *Intake of Vitamin and Mineral Supplements and Longitudinal Association with HbA1c Levels in the General Non-Diabetic Population--Results from the MONICA/KORA S3/F3 Study.* PloS one, 2015. **10**(10): p. e0139244.

68. Lavange, L.M., W.D. Kalsbeek, P.D. Sorlie, L.M. Aviles-Santa, R.C. Kaplan, J. Barnhart, K. Liu, A. Giachello, D.J. Lee, J. Ryan, M.H. Criqui, and J.P. Elder, *Sample design and cohort selection in the Hispanic Community Health Study/Study of Latinos.* Ann Epidemiol, 2010. **20**(8): p. 642-9.

69. Sorlie, P.D., L.M. Aviles-Santa, S. Wassertheil-Smoller, R.C. Kaplan, M.L. Daviglus, A.L. Giachello, N. Schneiderman, L. Raij, G. Talavera, M. Allison, L. Lavange, L.E. Chambless, and G. Heiss, *Design and implementation of the Hispanic Community Health Study/Study of Latinos.* Ann Epidemiol, 2010. **20**(8): p. 629-41.

70. Feskanich, D., B.H. Sielaff, K. Chong, and I.M. Buzzard, *Computerized collection and analysis of dietary intake information.* Comput Methods Programs Biomed, 1989. **30**(1): p. 47-57.

71. Harnack, L., M. Stevens, N. Van Heel, S. Schakel, J.T. Dwyer, and J. Himes, *A computer-based approach for assessing dietary supplement use in conjunction with dietary recalls.* Journal of food composition and analysis : an official publication of the United Nations University, International Network of Food Data Systems, 2008. **21**(Suppliment 1): p. S78-S82.

72. Johnson, R.K., P. Driscoll, and M.I. Goran, *Comparison of multiple-pass 24-hour recall estimates of energy intake with total energy expenditure determined by the doubly labeled water method in young children.* J Am Diet Assoc, 1996. **96**(11): p. 1140-4.

73. Sievert, Y.A., S.F. Schakel, and I.M. Buzzard, *Maintenance of a nutrient database for clinical trials.* Control Clin Trials, 1989. **10**(4): p. 416-25.

74. Schakel, S.F., Y.A. Sievert, and I.M. Buzzard, *Sources of data for developing and maintaining a nutrient database.* J Am Diet Assoc, 1988. **88**(10): p. 1268-71.

75. World Health Organization. *Global Physical Activity Surveillance*. Available from: http://www.who.int/chp/steps/GPAQ/en/.

76. World Health Organization. *Global Physical Activity Surveillance*. 2018 [cited 2018 January 2]; Available from: http://www.who.int/ncds/surveillance/steps/GPAQ/en/.

77. Carriquiry, A.L., *Estimation of usual intake distributions of nutrients and foods.* The Journal of nutrition, 2003. **133**(2): p. 601S-8S.

78. Tooze, J.A., V. Kipnis, D.W. Buckman, R.J. Carroll, L.S. Freedman, P.M. Guenther, S.M. Krebs-Smith, A.F. Subar, and K.W. Dodd, *A mixed-effects model approach for estimating the distribution of usual intake of nutrients: the NCI method.* Statistics in medicine, 2010. **29**(27): p. 2857-68.

79. National Cancer Institute. *Usual Dietary Intakes: SAS Macros for Analysis of a Single Dietary Component*. 2015 [cited 2017 September 1]; Available from: https://epi.grants.cancer.gov/diet/usualintakes/macros_single.html.

80. Siega-Riz, A.M., D. Sotres-Alvarez, G.X. Ayala, M. Ginsberg, J.H. Himes, K. Liu, C.M. Loria, Y. Mossavar-Rahmani, C.L. Rock, B. Rodriguez, M.D. Gellman, and L. Van Horn, *Food-group and nutrient-density intakes by Hispanic and Latino backgrounds in the Hispanic Community Health Study/Study of Latinos.* Am J Clin Nutr, 2014. **99**(6): p. 1487-98.

81. Bailey, R.L., V.L. Fulgoni, 3rd, D.R. Keast, and J.T. Dwyer, *Dietary supplement use is associated with higher intakes of minerals from food sources.* The American journal of clinical nutrition, 2011. **94**(5): p. 1376-81.

82. Garriguet, D., *Combining nutrient intake from food/beverages and vitamin/mineral supplements.* Health Rep, 2010. **21**(4): p. 71-84.

83. U.S. Department of Health and Human Services. *Usual Dietary Intakes: The NCI Method*. 2015; Available from: https://epi.grants.cancer.gov/diet/usualintakes/method.html.

84. Rao, J.N.K. and C.F.J. Wu, *Resampling Inference with Complex Survey Data.* Journal of the American Statistical Association, 1988. **83**(401): p. 231-241.

85. Park, Y.-W., S. Zhu, L. Palaniappan, S. Heshka, M.R. Carnethon, and S.B. Heymsfield, *The metabolic syndrome: prevalence and associated risk factor findings in the US population from the Third National Health and Nutrition Examination Survey, 1988-1994.* Archives of internal medicine, 2003. **163**(4): p. 427-36.

86. Sun, K., J. Liu, and G. Ning, *Active smoking and risk of metabolic syndrome: a meta-analysis of prospective studies.* PLoS One, 2012. **7**(10): p. e47791.

87. Ayala, G.X., B. Baquero, and S. Klinger, *A systematic review of the relationship between acculturation and diet among Latinos in the United States: implications for future research.* J Am Diet Assoc, 2008. **108**(8): p. 1330-44.

88. Archer, S.L., J. Stamler, A. Moag-Stahlberg, L. Van Horn, D. Garside, Q. Chan, J.J. Buffington, and A.R. Dyer, *Association of dietary supplement use with specific micronutrient intakes among middle-aged American men and women: the INTERMAP Study.* Journal of the American Dietetic Association, 2005. **105**(7): p. 1106-14.

89. Vashum, K.P., M. McEvoy, Z. Shi, A.H. Milton, M.R. Islam, D. Sibbritt, A. Patterson, J. Byles, D. Loxton, and J. Attia, *Is dietary zinc protective for type 2 diabetes? Results from the Australian longitudinal study on women's health.* BMC Endocr Disord, 2013. **13**: p. 40.

90. Wu, W., S. Jiang, Q. Zhao, K. Zhang, X. Wei, T. Zhou, D. Liu, H. Zhou, Q. Zeng, L. Cheng, X. Miao, and Q. Lu, *Environmental exposure to metals and the risk of hypertension: A cross-sectional study in China.* Environ Pollut, 2018. **233**: p. 670-678.

91. Bergomi, M., S. Rovesti, M. Vinceti, R. Vivoli, E. Caselgrandi, and G. Vivoli, *Zinc and copper status and blood pressure.* J Trace Elem Med Biol, 1997. **11**(3): p. 166-9.

92. Canatan, H., I. Bakan, M. Akbulut, I. Halifeoglu, G. Cikim, G. Baydas, and N. Kilic, *Relationship among levels of leptin and zinc, copper, and zinc/copper ratio in plasma of patients with essential hypertension and healthy normotensive subjects.* Biological Trace Element Research, 2004. **100**(2): p. 117-123.

93. Olatunbosun, D.A., J.O. Bolodeoku, T.O. Cole, and B.K. Adadevoh, *Relationship of Serum Copper and Zinc to Human Hypertension in Nigerians.* Bulletin of the World Health Organization, 1976. **53**(1): p. 134-135.

94. Shan, Z.L., S.J. Chen, T.P. Sun, C. Luo, Y.J. Guo, X.F. Yu, W. Yang, F.B. Hu, and L.G. Liu, *U-Shaped Association between Plasma Manganese Levels and Type-2 Diabetes.* Environmental Health Perspectives, 2016. **124**(12): p. 1876-1881.

95. Koh, E.S., S.J. Kim, H.E. Yoon, J.H. Chung, S. Chung, C.W. Park, Y.S. Chang, and S.J. Shin, *Association of blood manganese level with diabetes and renal dysfunction: a cross-sectional study of the Korean general population.* Bmc Endocrine Disorders, 2014. **14**.

96. Anetor, J.I., O.A. Asiribo, K.S. Adedapo, T.S. Akingbola, O.S. Olorunnisola, and F.A.A. Adeniyi, *Increased plasma manganese, partially reduced ascorbate,1 and absence of mitochondrial oxidative stress in type 2 diabetes mellitus: Implications for the superoxide uncoupling protein 2 (Ucp-2) pathway.* Biological Trace Element Research, 2007. **120**(1-3): p. 19-27.

97. Zelko, I.N., T.J. Mariani, and R.J. Folz, *Superoxide dismutase multigene family: a comparison of the CuZn-SOD (SOD1), Mn-SOD (SOD2), and EC-SOD (SOD3) gene structures, evolution, and expression.* Free Radic Biol Med, 2002. **33**(3): p. 337-49.

98. Davis, C.D. and J.L. Greger, *Longitudinal Changes of Manganese-Dependent Superoxide-Dismutase and Other Indexes of Manganese and Iron Status in Women.* American Journal of Clinical Nutrition, 1992. **55**(3): p. 747-752.

99. Uauy, R., C. Castilloduran, M. Fisberg, N. Fernandez, and A. Valenzuela, *Red-Cell Superoxide-Dismutase Activity as an Index of Human Copper Nutrition.* Journal of Nutrition, 1985. **115**(12): p. 1650-1655.

100. Davis, C.D., D.B. Milne, and F.H. Nielsen, *Changes in dietary zinc and copper affect zinc-status indicators of postmenopausal women, notably, extracellular superoxide dismutase and amyloid precursor proteins.* American Journal of Clinical Nutrition, 2000. **71**(3): p. 781-788.

101. Houstis, N., E.D. Rosen, and E.S. Lander, *Reactive oxygen species have a causal role in multiple forms of insulin resistance.* Nature, 2006. **440**(7086): p. 944-948.

102. Touyz, R.M., *Reactive oxygen species, vascular oxidative stress, and redox signaling in hypertension - What is the clinical significance?* Hypertension, 2004. **44**(3): p. 248-252.

103. Rayman, M.P., *Selenium in cancer prevention: a review of the evidence and mechanism of action.* Proc Nutr Soc, 2005. **64**(4): p. 527-42.

104. Bleys, J., A. Navas-Acien, S. Stranges, A. Menke, E.R. Miller, 3rd, and E. Guallar, *Serum selenium and serum lipids in US adults.* Am J Clin Nutr, 2008. **88**(2): p. 416-23.

105. Laclaustra, M., S. Stranges, A. Navas-Acien, J.M. Ordovas, and E. Guallar, *Serum selenium and serum lipids in US adults: National Health and Nutrition Examination Survey (NHANES) 2003-2004.* Atherosclerosis, 2010. **210**(2): p. 643-8.

106. Christensen, K., M. Werner, and K. Malecki, *Serum selenium and lipid levels: Associations observed in the National Health and Nutrition Examination Survey (NHANES) 2011-2012.* Environ Res, 2015. **140**: p. 76-84.

107. Brown, K.M. and J.R. Arthur, *Selenium, selenoproteins and human health: a review.* Public Health Nutr, 2001. **4**(2B): p. 593-9.

108. Mueller, A.S., S.D. Klomann, N.M. Wolf, S. Schneider, R. Schmidt, J. Spielmann, G. Stangl, K. Eder, and J. Pallauf, *Redox regulation of protein tyrosine phosphatase 1B by manipulation of dietary selenium affects the triglyceride concentration in rat liver.* J Nutr, 2008. **138**(12): p. 2328-36.

109. Delibegovic, M., D. Zimmer, C. Kauffman, K. Rak, E.-G. Hong, Y.-R. Cho, J.K. Kim, B.B. Kahn, B.G. Neel, and K.K. Bence, *Liver-specific deletion of protein-tyrosine phosphatase 1B (PTP1B) improves*

metabolic syndrome and attenuates diet-induced endoplasmic reticulum stress. Diabetes, 2009. **58**(3): p. 590-9.

110. Johnson, C.C., F.M. Fordyce, and M.P. Rayman, *Symposium on 'Geographical and geological influences on nutrition': Factors controlling the distribution of selenium in the environment and their impact on health and nutrition.* The Proceedings of the Nutrition Society, 2010. **69**(1): p. 119-32.

111. Planchart, A., A. Green, C. Hoyo, and C.J. Mattingly, *Heavy Metal Exposure and Metabolic Syndrome: Evidence from Human and Model System Studies.* Curr Environ Health Rep, 2018.

112. Navas-Acien, A., B.S. Schwartz, S.J. Rothenberg, H. Hu, E.K. Silbergeld, and E. Guallar, *Bone lead levels and blood pressure endpoints: a meta-analysis.* Epidemiology, 2008. **19**(3): p. 496-504.

113. Franceschini, N., R.C. Fry, P. Balakrishnan, A. Navas-Acien, C. Oliver-Williams, A.G. Howard, S.A. Cole, K. Haack, E.M. Lange, B.V. Howard, L.G. Best, K.A. Francesconi, W. Goessler, J.G. Umans, and M. Tellez-Plaza, *Cadmium body burden and increased blood pressure in middle-aged American Indians: the Strong Heart Study.* J Hum Hypertens, 2017. **31**(3): p. 225-230.

114. Sun, Q., R.M. van Dam, W.C. Willett, and F.B. Hu, *Prospective study of zinc intake and risk of type 2 diabetes in women.* Diabetes Care, 2009. **32**(4): p. 629-34.

115. Lee, B.K. and Y. Kim, *Relationship between blood manganese and blood pressure in the Korean general population according to KNHANES 2008.* Environmental Research, 2011. **111**(6): p. 797-803.

116. Bleys, J., A. Navas-Acien, and E. Guallar, *Serum selenium and diabetes in U.S. adults.* Diabetes Care, 2007. **30**(4): p. 829-34.

117. Monsen, E.R., *Dietary reference intakes for the antioxidant nutrients: vitamin C, vitamin E, selenium, and carotenoids.* J Am Diet Assoc, 2000. **100**(6): p. 637-40.

118. Bridges, C.C. and R.K. Zalups, *Molecular and ionic mimicry and the transport of toxic metals.* Toxicol Appl Pharmacol, 2005. **204**(3): p. 274-308.

119. Johnson, C.L., S.M. Dohrmann, V.L. Burt, and L.K. Mohadjer, *National health and nutrition examination survey: sample design, 2011-2014.* Vital Health Stat 2, 2014(162): p. 1-33.

120. Centers for Disease Control and Prevention, N.C.f.H.S. *NHANES 2013–2014 Questionnaires, Datasets, and Related Documentation.* . 2014 [cited 2018 March 5]; Available from: https://wwwn.cdc.gov/nchs/nhanes/ContinuousNhanes/Default.aspx?BeginYear=2013.

121. Centers for Disease Control and Prevention, N.C.f.H.S. *NHANES 2011–2012 Questionnaires, Datasets, and Related Documentation.* . 2012 [cited 2018 March 5]; Available from: https://wwwn.cdc.gov/nchs/nhanes/continuousnhanes/default.aspx?BeginYear=2011.

122. Centers for Disease Control and Prevention, N.C.f.H.S. *National Health and Nutrition Examination Survey: Laboratory Procedure Manual - Urinary Metals and Total Arsenic.* . 2014 [cited 2018 March 5]; Available from: https://www.cdc.gov/Nchs/Data/Nhanes/Nhanes_13_14/UM_UMS_UTAS_UTASS_H_MET.pdf.

123. Centers for Disease Control and Prevention, N.C.f.H.S. *National Health and Nutrition Examination Survey: Laboratory Procedure Manual - Serum Copper, Selenium, and Zinc*. 2014 [cited 2018 March 5]; Available from: https://wwwn.cdc.gov/nchs/data/nhanes/2013-2014/labmethods/CUSEZN_H_met.pdf.

124. Centers for Disease Control and Prevention, N.C.f.H.S. *National Health and Nutrition Examination Survey: Laboratory Procedure Manual - Blood Cadmium, Lead, Manganese, Mercury, and Selenium.* 2014 [cited 2018 March 5]; Available from: https://wwwn.cdc.gov/nchs/data/nhanes/2013-2014/labmethods/PbCd_H_MET.pdf.

125. Centers for Disease Control and Prevention, N.C.f.H.S. *National Health and Nutrition Examination Survey: Laboratory Procedure Manual - Urinary arsenobetaine, arsenocholine, trimethylarsine oxide, monomethylarsonic acid, dimethylarsinic acid, arsenous (III) acid, arsenic (V) acid*. 2014 [cited 2018 March 5]; Available from: https://wwwn.cdc.gov/nchs/data/nhanes/2013-2014/labmethods/UAS_UASS_H_MET.pdf.

126. Centers for Disease Control and Prevention, N.C.f.H.S. *National Health and Nutrition Examination Survey: Laboratory Procedure Manual - Urinary Arsenobetaine, Arsenocholine, Trimethylarsine oxide, Monomethylarsonic acid, Dimethylarsinic acid, Arsenous (III) acid, Arsenic (V) acid*. 2011 [cited 2018 March 5]; Available from: https://wwwn.cdc.gov/nchs/data/nhanes/2011-2012/labmethods/uas_g_met_arsenics.pdf.

127. Centers for Disease Control and Prevention, N.C.f.H.S. *National Health and Nutrition Examination Survey: Laboratory Procedure Manual - Urinary Metals and Total Arsenic.* . 2012 [cited 2018 March 5]; Available from: https://wwwn.cdc.gov/nchs/data/nhanes/2011-2012/labmethods/uhm_g_met_heavy_metals.pdf.

128. Centers for Disease Control and Prevention, N.C.f.H.S. *National Health and Nutrition Examination Survey: Laboratory Procedure Manual - Serum Copper, Selenium, and Zinc*. 2013 [cited 2018 March 5]; Available from: https://wwwn.cdc.gov/nchs/data/nhanes/2011-2012/labmethods/CUSEZN_G_met_serum_elements.pdf.

129. Centers for Disease Control and Prevention, N.C.f.H.S. *National Health and Nutrition Examination Survey: Laboratory Procedure Manual - Blood Cadmium, Lead, Manganese, Mercury, and Selenium.* 2013 [cited 2018 March 5]; Available from: https://wwwn.cdc.gov/nchs/data/nhanes/2011-2012/labmethods/pbcd_g_met_blood-metals.pdf.

130. Centers for Disease Control and Prevention, N.C.f.H.S. *National Health and Nutrition Examination Survey: Laboratory Procedure Manual - Blood Inorganic mercury, Methyl mercury, Ethyl mercury*. 2013 [cited 2018 March 5]; Available from: https://wwwn.cdc.gov/nchs/data/nhanes/2011-2012/labmethods/ihgem_met_g_mercuryspecies.pdf.

131. Centers for Disease Control and Prevention, N.C.f.H.S. *National Health and Nutrition Examination Survey: Laboratory Procedure Manual - Blood Inorganic mercury, Methyl mercury, Ethyl mercury*. 2014 [cited 2018 March 5]; Available from: https://wwwn.cdc.gov/nchs/data/nhanes/2013-2014/labmethods/IHgEM_H_MET.pdf.

132. Centers for Disease Control and Prevention, N.C.f.H.S. *National Health and Nutrition Examination Survey: Laboratory Procedure Manual - Urinary Iodine and Mercury*. 2014 [cited 2018 March 5]; Available from: https://wwwn.cdc.gov/nchs/data/nhanes/2013-2014/labmethods/UHG_UIO_URIO_H_MET.pdf.

133. Centers for Disease Control and Prevention, N.C.f.H.S. *National Health and Nutrition Examination Survey: Laboratory Procedure Manual - Urinary Iodine and Mercury*. 2013 [cited 2018 March 5]; Available from: https://wwwn.cdc.gov/nchs/data/nhanes/2011-2012/labmethods/uiouhg_g_met_iodine_mercury.pdf.

134. Centers for Disease Control and Prevention, A.f.T.S.a.D.R.A. *Toxicological profile for arsenic*. 2007 [cited 2018 March 5]; Available from: https://www.atsdr.cdc.gov/toxprofiles/tp2.pdf.

135. Centers for Disease Control and Prevention, A.f.T.S.a.D.R.A. *Toxicological profile for cadmium*. 2012 [cited 2018 March 5]; Available from: https://www.atsdr.cdc.gov/toxprofiles/tp5.pdf.

136. Danzeisen, R., M. Araya, B. Harrison, C. Keen, M. Solioz, D. Thiele, and H.J. McArdle, *How reliable and robust are current biomarkers for copper status?* The British journal of nutrition, 2007. **98**(4): p. 676-83.

137. Ashton, K., L. Hooper, L.J. Harvey, R. Hurst, A. Casgrain, and S.J. Fairweather-Tait, *Methods of assessment of selenium status in humans: a systematic review.* The American journal of clinical nutrition, 2009. **89**(6): p. 2025S-2039S.

138. Centers for Disease Control and Prevention, A.f.T.S.a.D.R.A. *Toxicological profile for manganese*. 2012 [cited 2018 March 5]; Available from: https://www.atsdr.cdc.gov/toxprofiles/tp151.pdf.

139. Centers for Disease Control and Prevention, A.f.T.S.a.D.R.A. *Toxicological profile for mercury*. 1999 [cited 2018 March 5]; Available from: https://www.atsdr.cdc.gov/toxprofiles/tp46.pdf.

140. Centers for Disease Control and Prevention, A.f.T.S.a.D.R.A. *Toxicological profile for lead*. 2007 [cited 2018 March 5]; Available from: https://www.atsdr.cdc.gov/toxprofiles/tp13.pdf.

141. Centers for Disease Control and Prevention, A.f.T.S.a.D.R.A. *Toxicological profile for zinc*. 2005 [cited 2018 March 5]; Available from: https://www.atsdr.cdc.gov/toxprofiles/tp60.pdf.

142. Jones, M.R., M. Tellez-Plaza, D. Vaidya, M. Grau, K.A. Francesconi, W. Goessler, E. Guallar, W.S. Post, J.D. Kaufman, and A. Navas-Acien, *Estimation of Inorganic Arsenic Exposure in Populations With Frequent Seafood Intake: Evidence From MESA and NHANES.* American journal of epidemiology, 2016. **184**(8): p. 590-602.

143. CDC (Centers for Disease Control and Prevention). *Fourth National Report on Human Exposure to Environmental Chemicals. Updated Tables, September, 2017.*; Available from: https://www.cdc.gov/exposurereport/index.html

144. Bulka, C.M., S.L. Mabila, J.P. Lash, M.E. Turyk, and M. Argos, *Arsenic and Obesity: A Comparison of Urine Dilution Adjustment Methods.* Environ Health Perspect, 2017. **125**(8): p. 087020.

145. Hays, S.M., L.L. Aylward, and B.C. Blount, *Variation in urinary flow rates according to demographic characteristics and body mass index in NHANES: potential confounding of associations between health outcomes and urinary biomarker concentrations.* Environ Health Perspect, 2015. **123**(4): p. 293-300.

146. Centers for Disease Control and Prevention, N.C.f.H.S. *National Health and Nutrition Examination Survey (NHANES): Anthropometry Procedures Manual*. 2011 [cited 2018 March 5]; Available from: https://wwwn.cdc.gov/nchs/data/nhanes/2011-2012/manuals/Anthropometry_Procedures_Manual.pdf.

147. Centers for Disease Control and Prevention, N.C.f.H.S. *National Health and Nutrition Examination Survey (NHANES): Anthropometry Procedures Manual*. 2013 [cited 2018 March 5]; Available from: https://wwwn.cdc.gov/nchs/data/nhanes/2013-2014/manuals/2013_Anthropometry.pdf.

148. Centers for Disease Control and Prevention, N.C.f.H.S. *National Health and Nutrition Examination Survey (NHANES): Physical Examination Procedures Manual*. 2013 [cited 2018 March 5]; Available from: https://wwwn.cdc.gov/nchs/data/nhanes/2013-2014/manuals/Phys_Exam_Manual_2013.pdf.

149. Centers for Disease Control and Prevention, N.C.f.H.S. *National Health and Nutrition Examination Survey (NHANES): Physical Examination Procedures Manual*. 2011 [cited 2018 March 5]; Available from: https://wwwn.cdc.gov/nchs/data/nhanes/2011-2012/manuals/Physician_Exam_Manual.pdf.

150. Centers for Disease Control and Prevention, N.C.f.H.S. *National Health and Nutrition Examination Survey (NHANES): Laboratory Procedures Manual*. 2011 [cited 2018 March 5]; Available from: https://wwwn.cdc.gov/nchs/data/nhanes/2011-2012/manuals/2011-12_Laboratory_Procedures_Manual.pdf.

151. Centers for Disease Control and Prevention, N.C.f.H.S. *National Health and Nutrition Examination Survey (NHANES): Laboratory Procedures Manual*. 2013 [cited 2018 March 5]; Available from: https://wwwn.cdc.gov/nchs/data/nhanes/2013-2014/manuals/2013_MEC_Laboratory_Procedures_Manual.pdf.

152. Centers for Disease Control and Prevention, N.C.f.H.S. *National Health and Nutrition Examination Survey: Laboratory Procedure Manual - Plasma Fasting Glucose*. 2013 [cited 2018 March 5]; Available from: https://wwwn.cdc.gov/nchs/data/nhanes/2011-2012/labmethods/glu_g_met_fasting_glucose.pdf.

153. Centers for Disease Control and Prevention, N.C.f.H.S. *National Health and Nutrition Examination Survey: Laboratory Procedure Manual - Plasma Fasting Glucose*. 2015 [cited 2018 March 5]; Available from: https://wwwn.cdc.gov/nchs/data/nhanes/2013-2014/labmethods/GLU_H_MET_Fasting_Glucose.pdf.

154. Centers for Disease Control and Prevention, N.C.f.H.S. *National Health and Nutrition Examination Survey: Laboratory Procedure Manual - Serum HDL Cholesterol*. 2013 [cited 2018 March 5]; Available from: https://wwwn.cdc.gov/nchs/data/nhanes/2011-2012/labmethods/hdl_g_met_hdl.pdf.

155. Centers for Disease Control and Prevention, N.C.f.H.S. *National Health and Nutrition Examination Survey: Laboratory Procedure Manual - Serum Triglycerides*. 2013 [cited 2018 March 5]; Available from: https://wwwn.cdc.gov/nchs/data/nhanes/2011-2012/labmethods/trigly_g_met.pdf.

156. Centers for Disease Control and Prevention, N.C.f.H.S. *National Health and Nutrition Examination Survey: Laboratory Procedure Manual - Serum HDL Cholesterol*. 2015 [cited 2018 March 5]; Available from: https://wwwn.cdc.gov/nchs/data/nhanes/2013-2014/labmethods/HDL_H_MET_COBAS.pdf.

157. Centers for Disease Control and Prevention, N.C.f.H.S. *National Health and Nutrition Examination Survey: Laboratory Procedure Manual - Serum Triglycerides*. 2015 [cited 2018 March 5]; Available from: https://wwwn.cdc.gov/nchs/data/nhanes/2013-2014/labmethods/BIOPRO_H_MET_TRIGLYCERIDES.pdf.

158. *Physical activity guidelines for Americans.* Okla Nurse, 2008. **53**(4): p. 25.

159. Mirel, L.B., L.K. Mohadjer, S.M. Dohrmann, J. Clark, V.L. Burt, C.L. Johnson, and L.R. Curtin, *National Health and Nutrition Examination Survey: estimation procedures, 2007-2010.* Vital Health Stat 2, 2013(159): p. 1-17.

160. Korn, E.L. and B.I. Graubard, *Epidemiologic studies utilizing surveys: accounting for the sampling design.* American journal of public health, 1991. **81**(9): p. 1166-73.

161. Muller, C.J. and R.F. MacLehose, *Estimating predicted probabilities from logistic regression: different methods correspond to different target populations.* Int J Epidemiol, 2014. **43**(3): p. 962-70.

162. Kaiser, H.F., *Citation Classic - Varimax Criterion for Analytic Rotation in Factor-Analysis.* Current Contents/Social & Behavioral Sciences, 1979(24): p. 20-20.

163. Hu, H., R. Shih, S. Rothenberg, and B.S. Schwartz, *The epidemiology of lead toxicity in adults: Measuring dose and consideration of other methodologic issues.* Environmental Health Perspectives, 2007. **115**(3): p. 455-462.

164. Riedt, C.S., B.T. Buckley, R.E. Brolin, H. Ambia-Sobhan, G.G. Rhoads, and S.A. Shapses, *Blood lead levels and bone turnover with weight reduction in women.* J Expo Sci Environ Epidemiol, 2009. **19**(1): p. 90-6.

165. Muka, T., K. Trajanoska, J.C. Kiefte-de Jong, L. Oei, A.G. Uitterlinden, A. Hofman, A. Dehghan, M.C. Zillikens, O.H. Franco, and F. Rivadeneira, *The Association between Metabolic Syndrome, Bone Mineral Density, Hip Bone Geometry and Fracture Risk: The Rotterdam Study.* PLoS One, 2015. **10**(6): p. e0129116.

166. Kobal, A.B., M. Horvat, M. Prezelj, A.S. Briski, M. Krsnik, T. Dizdarevic, D. Mazej, I. Falnoga, V. Stibilj, N. Arneric, D. Kobal, and J. Osredkar, *The impact of long-term past exposure to elemental mercury on antioxidative capacity and lipid peroxidation in mercury miners.* J Trace Elem Med Biol, 2004. **17**(4): p. 261-74.

167. Davis, M.A., A.J. Signes-Pastor, M. Argos, F. Slaughter, C. Pendergrast, T. Punshon, A. Gossai, H. Ahsan, and M.R. Karagas, *Assessment of human dietary exposure to arsenic through rice.* Sci Total Environ, 2017. **586**: p. 1237-1244.

168. Zhang, H., X. Feng, T. Larssen, L. Shang, and P. Li, *Bioaccumulation of methylmercury versus inorganic mercury in rice (Oryza sativa L.) grain.* Environ Sci Technol, 2010. **44**(12): p. 4499-504.

169. Pennington, J.A.T. and B. Young, *Iron, zinc, copper, manganese, selenium, and iodine in foods from the United States total diet study.* Journal of Food Composition and Analysis, 1990. **3**(2): p. 166-184.

170. Powell, S.R., *The antioxidant properties of zinc.* The Journal of nutrition, 2000. **130**(5S Suppl): p. 1447S-54S.

171. Stranges, S., A. Navas-Acien, M.P. Rayman, and E. Guallar, *Selenium status and cardiometabolic health: state of the evidence.* Nutrition, metabolism, and cardiovascular diseases : NMCD, 2010. **20**(10): p. 754-60.

172. Navas-Acien, A., J. Bleys, and E. Guallar, *Selenium intake and cardiovascular risk: what is new?* Current opinion in lipidology, 2008. **19**(1): p. 43-9.

173. Suzuki, T., S. Katsumata, H. Matsuzaki, and K. Suzuki, *Dietary zinc supplementation increased TNFalpha and IL1beta-induced RANKL expression, resulting in a decrease in bone mineral density in rats.* J Clin Biochem Nutr, 2016. **58**(1): p. 48-55.

174. Nawrot, T.S., L. Thijs, E.M. Den Hond, H.A. Roels, and J.A. Staessen, *An epidemiological re-appraisal of the association between blood pressure and blood lead: a meta-analysis.* J Hum Hypertens, 2002. **16**(2): p. 123-31.

175. Padilla, M.A., M. Elobeid, D.M. Ruden, and D.B. Allison, *An examination of the association of selected toxic metals with total and central obesity indices: NHANES 99-02.* Int J Environ Res Public Health, 2010. **7**(9): p. 3332-47.

176. Scinicariello, F., M.C. Buser, M. Mevissen, and C.J. Portier, *Blood lead level association with lower body weight in NHANES 1999-2006.* Toxicology and Applied Pharmacology, 2013. **273**(3): p. 516-523.

177. Yang, C.-C., C.-S. Chuang, C.-I. Lin, C.-L. Wang, Y.-C. Huang, and H.-Y. Chuang, *The association of the blood lead level and serum lipid concentrations may be modified by the genetic combination of the metallothionein 2A polymorphisms rs10636GC and rs28366003 AA.* Journal of clinical lipidology, 2017. **11**(1): p. 234-241.

178. Han, S., W. Li, U. Jamil, K. Dargan, M. Orefice, F.W. Kemp, and J.D. Bogden, *Effects of weight loss and exercise on the distribution of lead and essential trace elements in rats with prior lead exposure.* Environ Health Perspect, 1999. **107**(8): p. 657-62.

179. Gil, F. and A.F. Hernandez, *Toxicological importance of human biomonitoring of metallic and metalloid elements in different biological samples.* Food Chem Toxicol, 2015. **80**: p. 287-97.

180. Frisbie, S.H., R. Ortega, D.M. Maynard, and B. Sarkar, *The concentrations of arsenic and other toxic elements in Bangladesh's drinking water.* Environ Health Perspect, 2002. **110**(11): p. 1147-53.

181. Salam, A., T. Hossain, M.N.A. Siddique, and A.M.S. Alam, *Characteristics of atmospheric trace gases, particulate matter, and heavy metal pollution in Dhaka, Bangladesh.* Air Quality Atmosphere and Health, 2008. **1**(2): p. 101-109.

182. Begum, B.A., P.K. Hopke, and A. Markwitz, *Air pollution by fine particulate matter in Bangladesh.* Atmospheric Pollution Research, 2013. **4**(1): p. 75-86.

183. Mitra, A.K., A. Haque, M. Islam, and S.A. Bashar, *Lead poisoning: an alarming public health problem in Bangladesh.* Int J Environ Res Public Health, 2009. **6**(1): p. 84-95.

184. Navas-Acien, A., E. Guallar, E.K. Silbergeld, and S.J. Rothenberg, *Lead exposure and cardiovascular disease--a systematic review.* Environ Health Perspect, 2007. **115**(3): p. 472-82.

185. Choi, M.K. and Y.J. Bae, *Relationship between dietary magnesium, manganese, and copper and metabolic syndrome risk in Korean adults: the Korea National Health and Nutrition Examination Survey (2007-2008).* Biol Trace Elem Res, 2013. **156**(1-3): p. 56-66.

186. Mordukhovich, I., R.O. Wright, H. Hu, C. Amarasiriwardena, A. Baccarelli, A. Litonjua, D. Sparrow, P. Vokonas, and J. Schwartz, *Associations of toenail arsenic, cadmium, mercury, manganese, and lead with blood pressure in the normative aging study.* Environ Health Perspect, 2012. **120**(1): p. 98-104.

187. Lee, B.K. and Y. Kim, *Relationship between blood manganese and blood pressure in the Korean general population according to KNHANES 2008.* Environ Res, 2011. **111**(6): p. 797-803.

188. in *Dietary Reference Intakes for Vitamin A, Vitamin K, Arsenic, Boron, Chromium, Copper, Iodine, Iron, Manganese, Molybdenum, Nickel, Silicon, Vanadium, and Zinc*. 2001: Washington (DC).

189. Laclaustra, M., A. Navas-Acien, S. Stranges, J.M. Ordovas, and E. Guallar, *Serum selenium concentrations and hypertension in the US Population.* Circ Cardiovasc Qual Outcomes, 2009. **2**(4): p. 369-76.

190. Nawrot, T.S., J.A. Staessen, H.A. Roels, E. Den Hond, L. Thijs, R.H. Fagard, A.F. Dominiczak, and H.A. Struijker-Boudier, *Blood pressure and blood selenium: a cross-sectional and longitudinal population study.* Eur Heart J, 2007. **28**(5): p. 628-33.

191. Argos, M., M. Rahman, F. Parvez, J. Dignam, T. Islam, I. Quasem, K.H. S, T.H. A, Z. Hossain, I.P. T, M. Rakibuz-Zaman, G. Sarwar, P. La Porte, J. Harjes, K. Anton, M.G. Kibriya, F. Jasmine, R. Khan, M. Kamal, C.R. Shea, M. Yunus, J.A. Baron, and H. Ahsan, *Baseline comorbidities in a skin cancer prevention trial in Bangladesh.* Eur J Clin Invest, 2013. **43**(6): p. 579-88.

192. Jiang, J., M. Liu, F. Parvez, B. Wang, F. Wu, M. Eunus, S. Bangalore, J.D. Newman, A. Ahmed, T. Islam, M. Rakibuz-Zaman, R. Hasan, G. Sarwar, D. Levy, V. Slavkovich, M. Argos, M. Scannell Bryan, S.F. Farzan, R.B. Hayes, J.H. Graziano, H. Ahsan, and Y. Chen, *Association between Arsenic Exposure from Drinking Water and Longitudinal Change in Blood Pressure among HEALS Cohort Participants.* Environ Health Perspect, 2015. **123**(8): p. 806-12.

193. Mohan, V., V. Vijayachandrika, K. Gokulakrishnan, R.M. Anjana, A. Ganesan, M.B. Weber, and K.M. Narayan, *A1C cut points to define various glucose intolerance groups in Asian Indians.* Diabetes Care, 2010. **33**(3): p. 515-9.

194. Bhowmik, B., L.M. Diep, S.B. Munir, M. Rahman, E. Wright, and S. Mahmood, *HbA1c as a diagnostic tool for diabetes and pre-diabetes: the Bangladesh experience (vol 30, pg e70, 2013).* Diabetic Medicine, 2016. **33**(2): p. 271-271.

195. Tobin, M.D., N.A. Sheehan, K.J. Scurrah, and P.R. Burton, *Adjusting for treatment effects in studies of quantitative traits: antihypertensive therapy and systolic blood pressure.* Stat Med, 2005. **24**(19): p. 2911-35.

196. Chizmar, J.F., *A discrete-time hazard analysis of the role of gender in persistence in the economics major.* Journal of Economic Education, 2000. **31**(2): p. 107-118.

197. Alarcon, W.A., E. State Adult Blood Lead, I. Surveillance Program, S. Davidson, B. Dufour, M. Roach, K. Tsang, S.F. Payne, A.M. DeLoreto, T. St Louis, S. Rajagopalan, S. Watkins, J. Chalmers, T. Shen, J.M. Turner, K. Leinenkugel, M. Asamoah, J. Lewis, E. Keyvan, K. Roseman, J. Kica, S. Yendell, C.R. Braun, D. Stover, M. Borjan, M.E. Lumia, D. Singh, E.O. Irobi, H. Krapfl, A.M. Fletcher, S. Higgins, S.J. Quigley, C.T. Benner, D. Dreher, O.H. Authority, T. Richardson, M. Sullivan, C. Tomasallo, and S. Melia, *Elevated Blood Lead Levels Among Employed Adults - United States, 1994-2013.* MMWR Morbidity and mortality weekly report, 2016. **63**(55): p. 59-65.

198. Biswas, S.K., S.A. Tarafdar, A. Islam, M. Khaliquzzaman, H. Tervahattu, and K. Kupiainen, *Impact of unleaded gasoline introduction on the concentration of lead in the air of Dhaka, Bangladesh.* Journal of the Air & Waste Management Association, 2003. **53**(11): p. 1355-1362.

199. Ahmad, S.A., M.H. Khan, S. Khandker, A.F.M. Sarwar, N. Yasmin, M.H. Faruquee, and R. Yasmin, *Blood Lead Levels and Health Problems of Lead Acid Battery Workers in Bangladesh.* Scientific World Journal, 2014.

200. Association of Occupational and Environmental Clinics. *Medical management guidelines for lead-exposed adults*. 2013; Available from: http://www.aoec.org/documents/positions/mmg_revision_with_cste_2013.pdf.

201. Agency for Toxic Substances and Disease Registry (ATSDR), *Toxicological profile for manganese*. 2012.

202. Agency for Toxic Substances and Disease Registry (ATSDR), *Toxicological profile for selenium*. 2003.

203. Chen, Y., M. Hall, J.H. Graziano, V. Slavkovich, A. van Geen, F. Parvez, and H. Ahsan, *A prospective study of blood selenium levels and the risk of arsenic-related premalignant skin lesions.* Cancer Epidemiol Biomarkers Prev, 2007. **16**(2): p. 207-13.

204. Glenn, B.S., K. Bandeen-Roche, B.K. Lee, V.M. Weaver, A.C. Todd, and B.S. Schwartz, *Changes in systolic blood pressure associated with lead in blood and bone.* Epidemiology, 2006. **17**(5): p. 538-44.

205. Glenn, B.S., W.F. Stewart, J.M. Links, A.C. Todd, and B.S. Schwartz, *The longitudinal association of lead with blood pressure.* Epidemiology, 2003. **14**(1): p. 30-6.

206. Kim, H.C., T.W. Jang, H.J. Chae, W.J. Choi, M.N. Ha, B.J. Ye, B.G. Kim, M.J. Jeon, S.Y. Kim, and Y.S. Hong, *Evaluation and management of lead exposure.* Ann Occup Environ Med, 2015. **27**: p. 30.

207. Cosselman, K.E., A. Navas-Acien, and J.D. Kaufman, *Environmental factors in cardiovascular disease.* Nat Rev Cardiol, 2015. **12**(11): p. 627-42.

208. Chang, H.R., D.A. Tsao, H.S. Yu, and C.K. Ho, *The change of beta-adrenergic system after cessation of lead exposure.* Toxicology, 2005. **207**(1): p. 73-80.

209. in *Dietary Reference Intakes for Vitamin C, Vitamin E, Selenium, and Carotenoids*. 2000: Washington (DC).

210. Agency for Toxic Substances and Disease Registry (ATSDR), *Toxicological profile for lead*. 2007.

211. Reilly, C., *Selenium in food and health.* 1996, London ; New York: Blackie Academic & Professional. xvi, 338 p.

212. Waid, J.L., M. Ali, S.H. Thilstedc, and S. Gabryscha, *Dietary change in Bangladesh from 1985 to 2010.* Global Food Security, 2017.

213. Islam, A.K. and A.A. Majumder, *Hypertension in Bangladesh: a review.* Indian Heart J, 2012. **64**(3): p. 319-23.

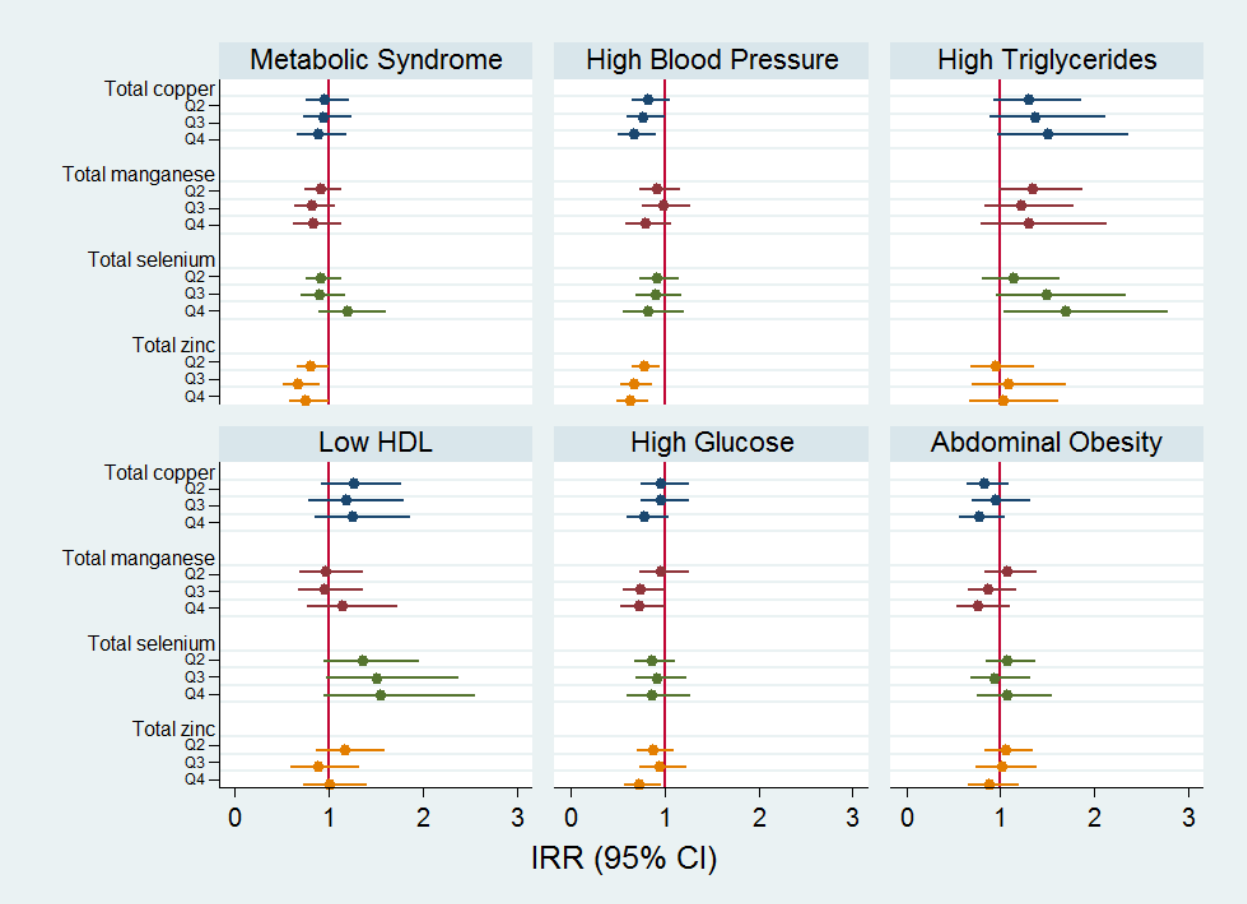
214. Spratlen, M.J. and A. Navas-Acien. *Recently Approved Ancillary Studies/Collaborations by Strong Heart Study*. 2016 [cited 2018 March 30]; Available from: http://strongheart.ouhsc.edu/Research/AncilliaryandSub-Studies/OngoingAncillaryandSub-studies.aspx.

215. Taylor, K.W., B.R. Joubert, J.M. Braun, C. Dilworth, C. Gennings, R. Hauser, J.J. Heindel, C.V. Rider, T.F. Webster, and D.J. Carlin, *Statistical Approaches for Assessing Health Effects of Environmental Chemical Mixtures in Epidemiology: Lessons from an Innovative Workshop.* Environ Health Perspect, 2016. **124**(12): p. A227-A229.

APPENDICES

APPENDIX A

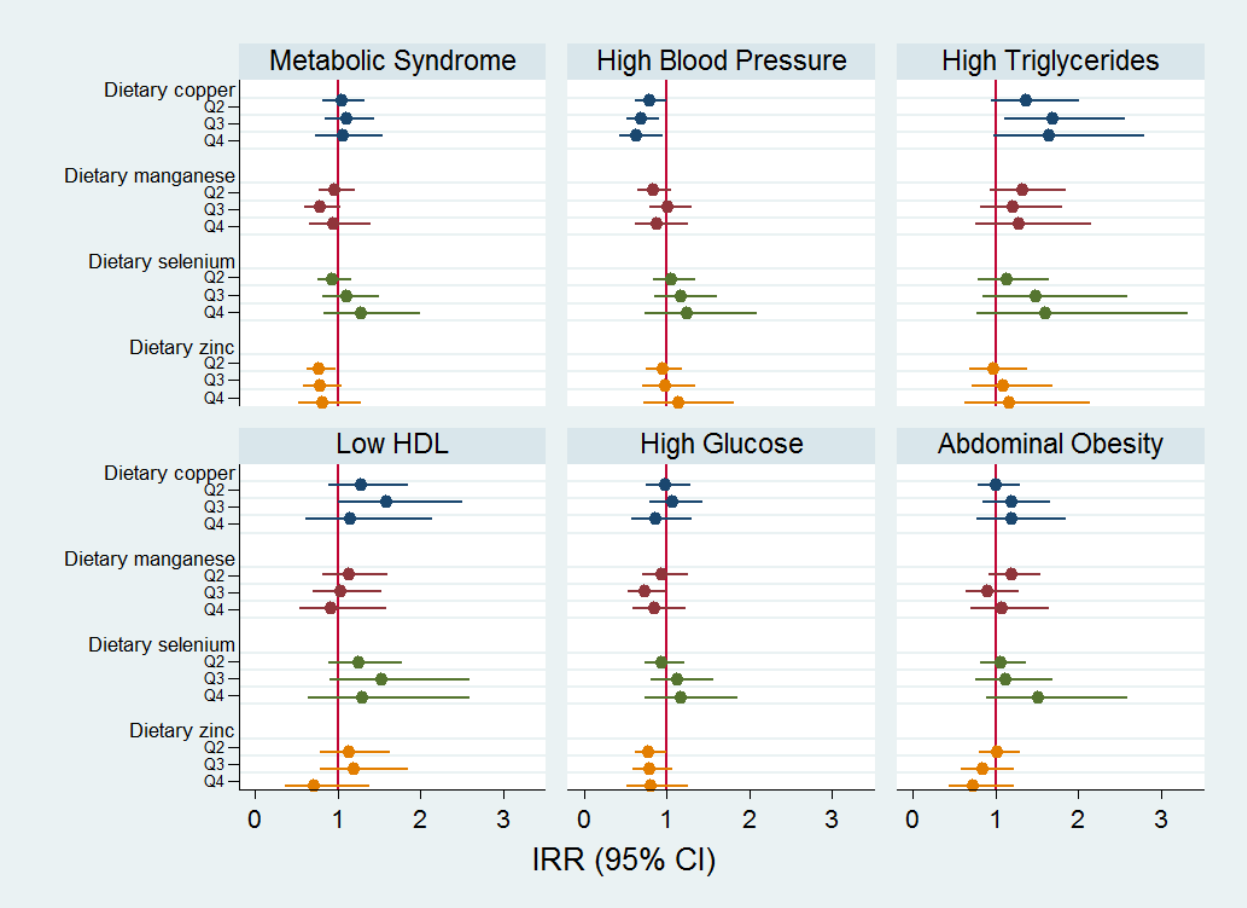
Figure 5. Incidence rate ratios (95% CI) for metabolic syndrome and components by usual total intakes of the selected minerals at baseline in HCHS/SOL[a]



[a] Adjusted for total energy intake (usual kilocalories per day from food/beverages and supplements, continuous), age (continuous), gender (male or female), Hispanic/Latino background (Central American, Cuban, Dominican , Mexican, Puerto Rican, South American, or more than one/other), study center (Bronx, Chicago, Miami, or San Diego), education (less than high school, high school or equivalent, or greater than high school), years lived in the mainland U.S. (born in mainland, <5, 5-9, 10-14, 15-19, or ≥20), alcohol use level (no current use, low/moderate, or high), cigarette smoking (never, former, or current), and physical activity level (inactive, low, medium, or high).

APPENDIX A (CONTINUED)

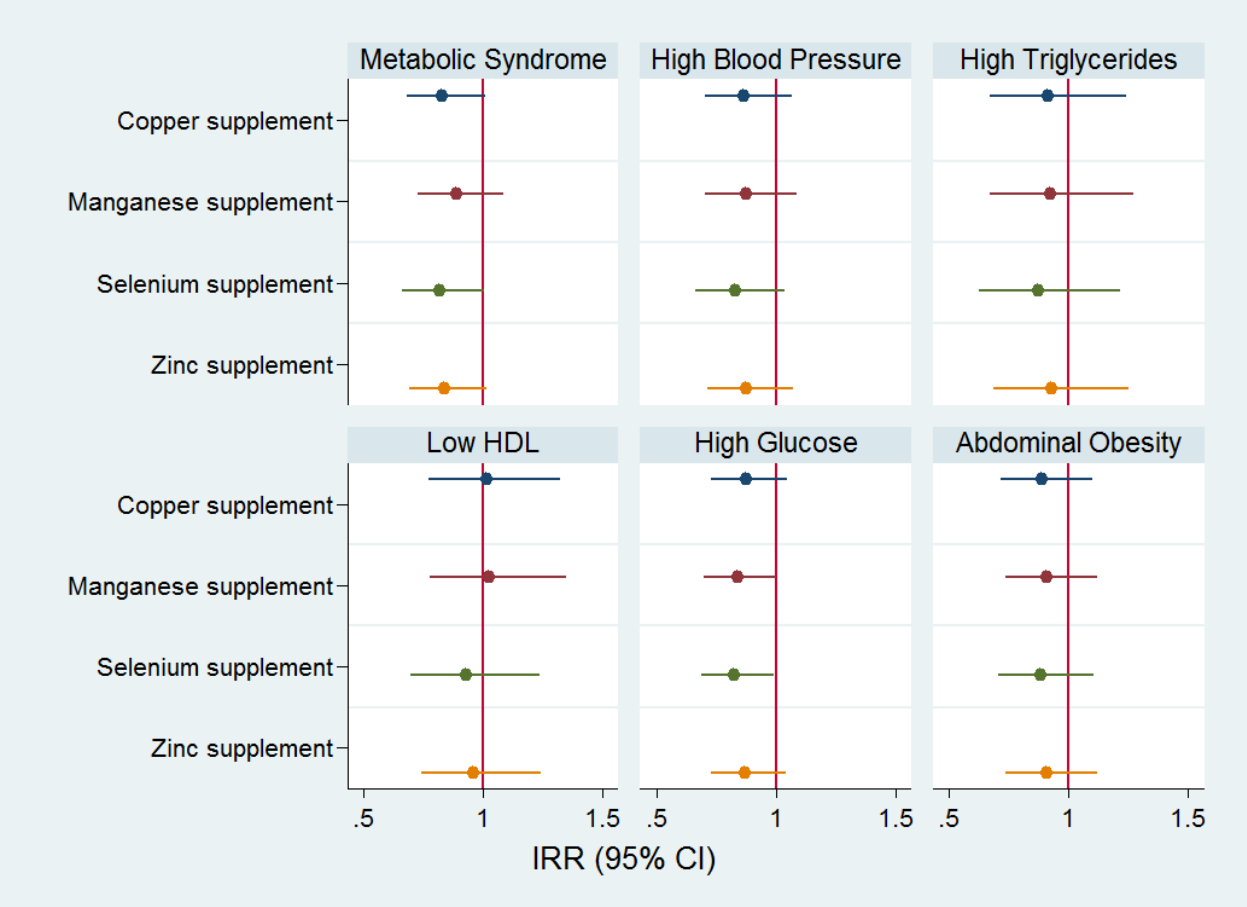
Figure 6. Incidence rate ratios (95% CI) for metabolic syndrome and components by usual dietary intakes of the selected minerals at baseline in HCHS/SOL[a]



[a] Adjusted for supplement use (i.e., use of any supplement containing copper, manganese, selenium or zinc), total energy intake (usual kilocalories per day from food/beverages and supplements, continuous), age (continuous), gender (male or female), Hispanic/Latino background (Central American, Cuban, Dominican , Mexican, Puerto Rican, South American, or more than one/other), study center (Bronx, Chicago, Miami, or San Diego), education (less than high school, high school or equivalent, or greater than high school), years lived in the mainland U.S. (born in mainland, <5, 5-9, 10-14, 15-19, or ≥20), alcohol use level (no current use, low/moderate, or high), cigarette smoking (never, former, or current), and physical activity level (inactive, low, medium, or high).

APPENDIX A (CONTINUED)

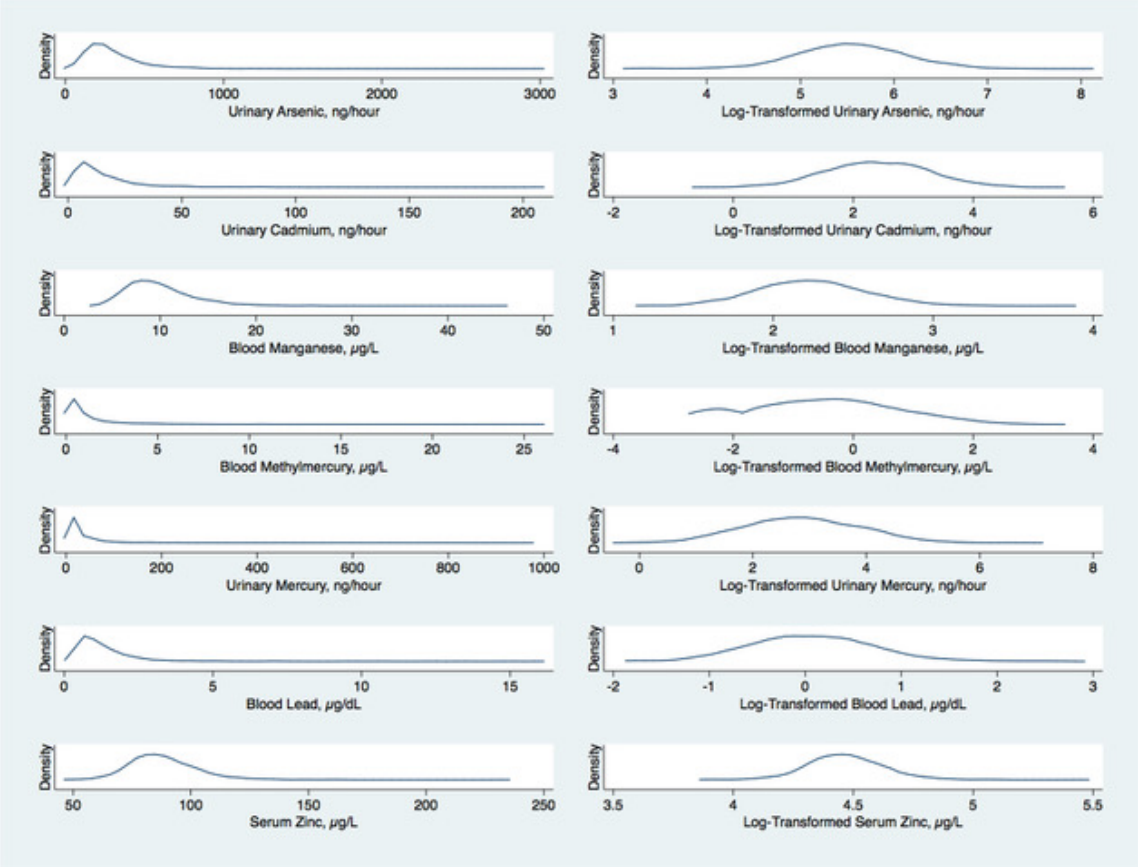
Figure 7. Incidence rate ratios (95% CI) for metabolic syndrome and components by supplemental use of the selected minerals at baseline in HCHS/SOL[a]



[a] Adjusted for usual dietary intakes of the respective mineral counterpart (copper [mg/day], manganese [mg/day], selenium [µg/day], or zinc [mg/day], continuous), total energy intake (usual kilocalories per day from food/beverages and supplements, continuous), age (continuous), gender (male or female), and Hispanic/Latino background (Central American, Cuban, Dominican, Mexican, Puerto Rican, South American, or more than one/other), study center (Bronx, Chicago, Miami, or San Diego), education (less than high school, high school or equivalent, or greater than high school), years lived in the mainland U.S. (born in mainland, <5, 5-9, 10-14, 15-19, or ≥20), alcohol use level (no current use, low/moderate, or high), cigarette smoking (never, former, or current), and physical activity level (inactive, low, medium, or high).

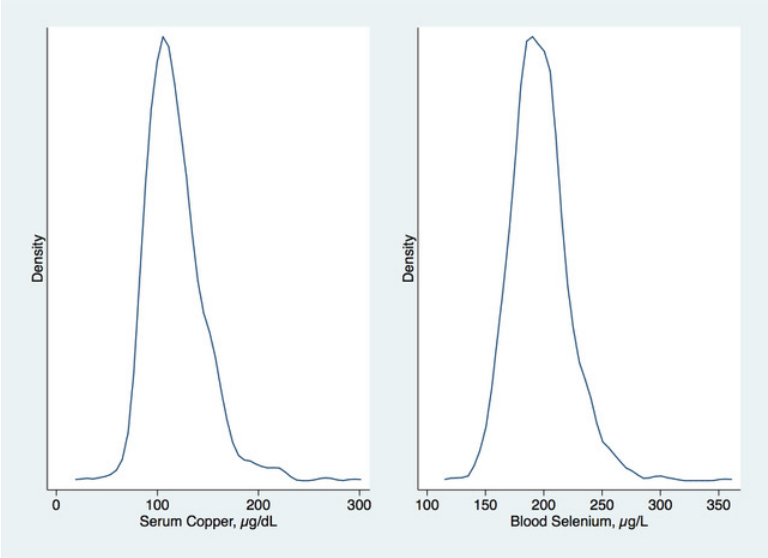
APPENDIX B

Figure 8. Distributions of skewed metal biomarkers before and after natural log-transformation in NHANES



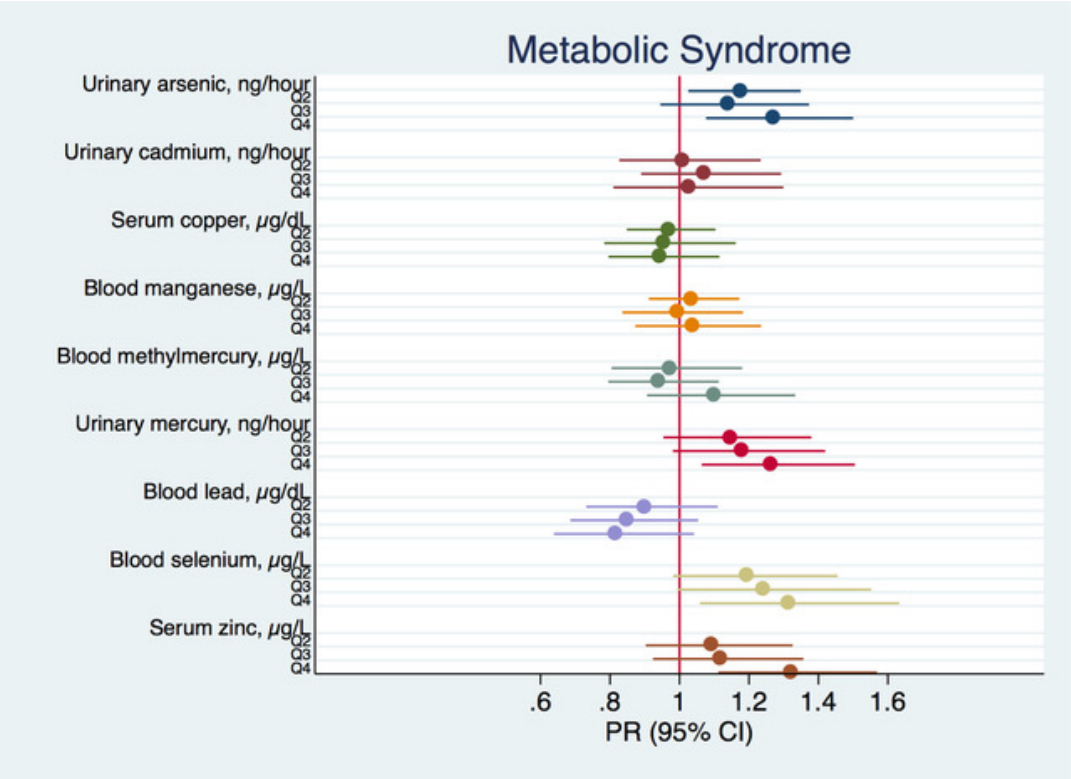
APPENDIX B (CONTINUED)

Figure 9. Distributions of normally-distributed metal biomarkers in NHANES



APPENDIX B (CONTINUED)

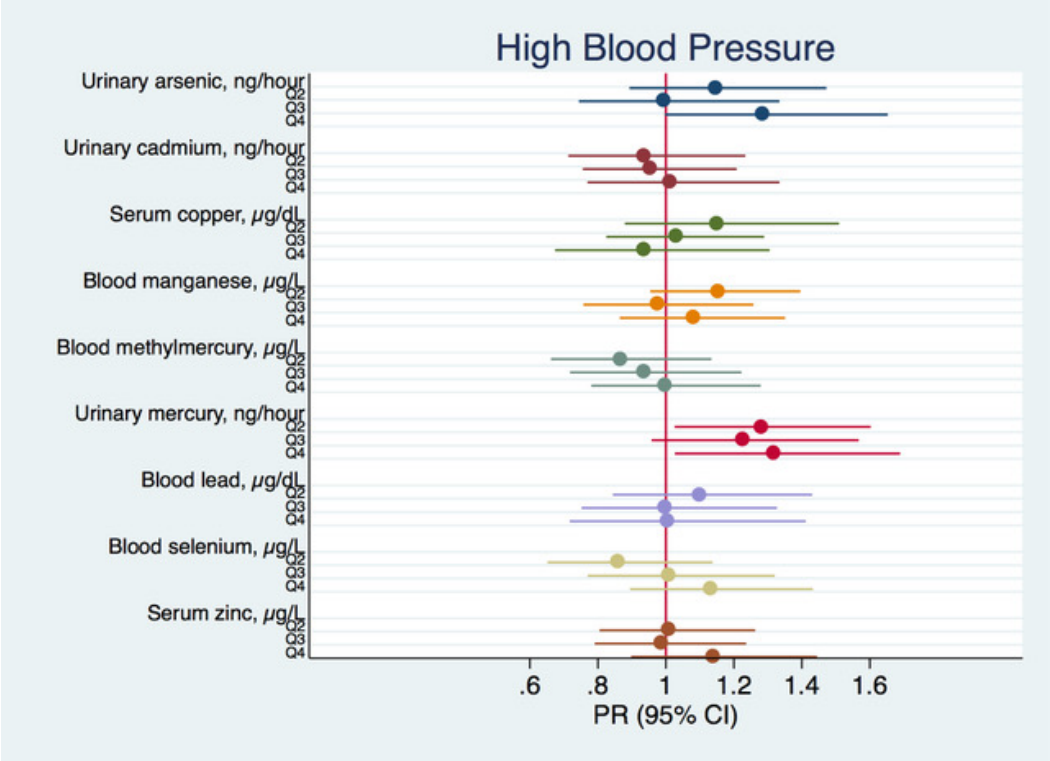
Figure 10. Adjusted prevalence ratios (95% CI) for metabolic syndrome in NHANES[a]



[a] Adjusted for age (continuous, years), gender (male or female), race/ethnicity (non-Hispanic white, non-Hispanic black, Hispanic, or other including multi-racial), family income: poverty ratio (continuous), total caloric intake (continuous, kcal/day), educational attainment (less than high school, high school diploma or GED, or at least some college), smoking status (current, former, or never), average number of drinks per day in past year, and physical activity status (met the 2008 physical activity guidelines or did not), survey cycle (2011-2012 or 2013-2014), and body mass index (continuous, kg/m^2)

APPENDIX B (CONTINUED)

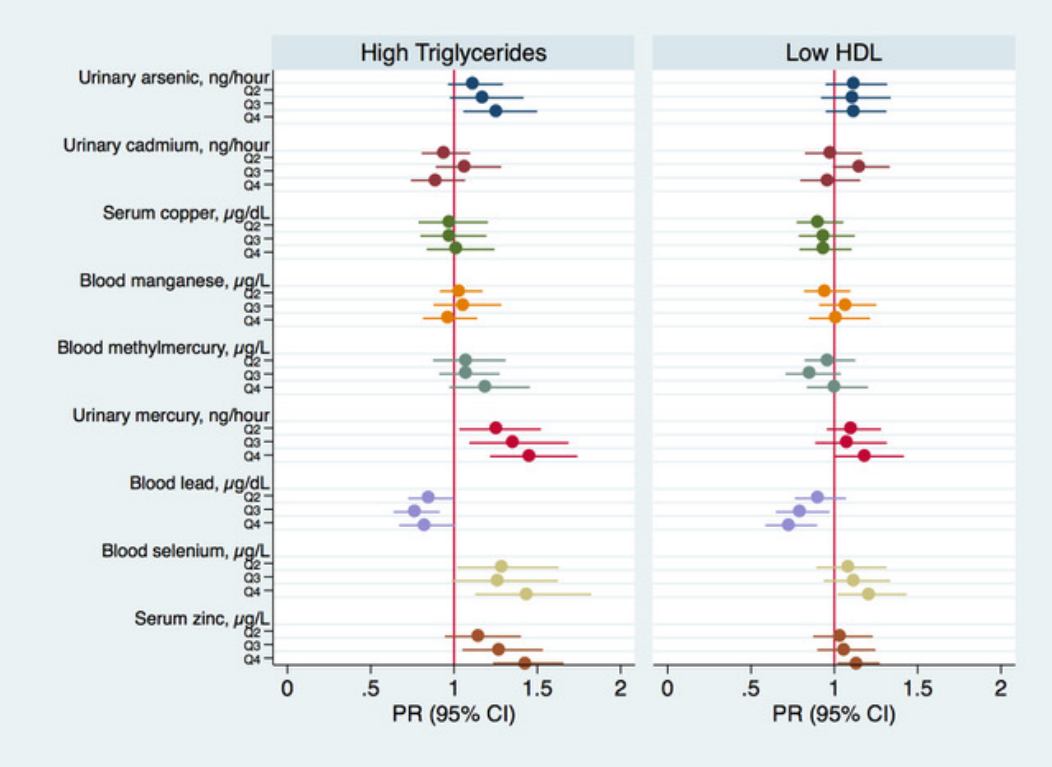
Figure 11. Adjusted prevalence ratios (95% CI) for high blood pressure in NHANES[a]



[a] Adjusted for age (continuous, years), gender (male or female), race/ethnicity (non-Hispanic white, non-Hispanic black, Hispanic, or other including multi-racial), family income: poverty ratio (continuous), total caloric intake (continuous, kcal/day), educational attainment (less than high school, high school diploma or GED, or at least some college), smoking status (current, former, or never), average number of drinks per day in past year, and physical activity status (met the 2008 physical activity guidelines or did not), survey cycle (2011-2012 or 2013-2014), and body mass index (continuous, kg/m^2)

APPENDIX B (CONTINUED)

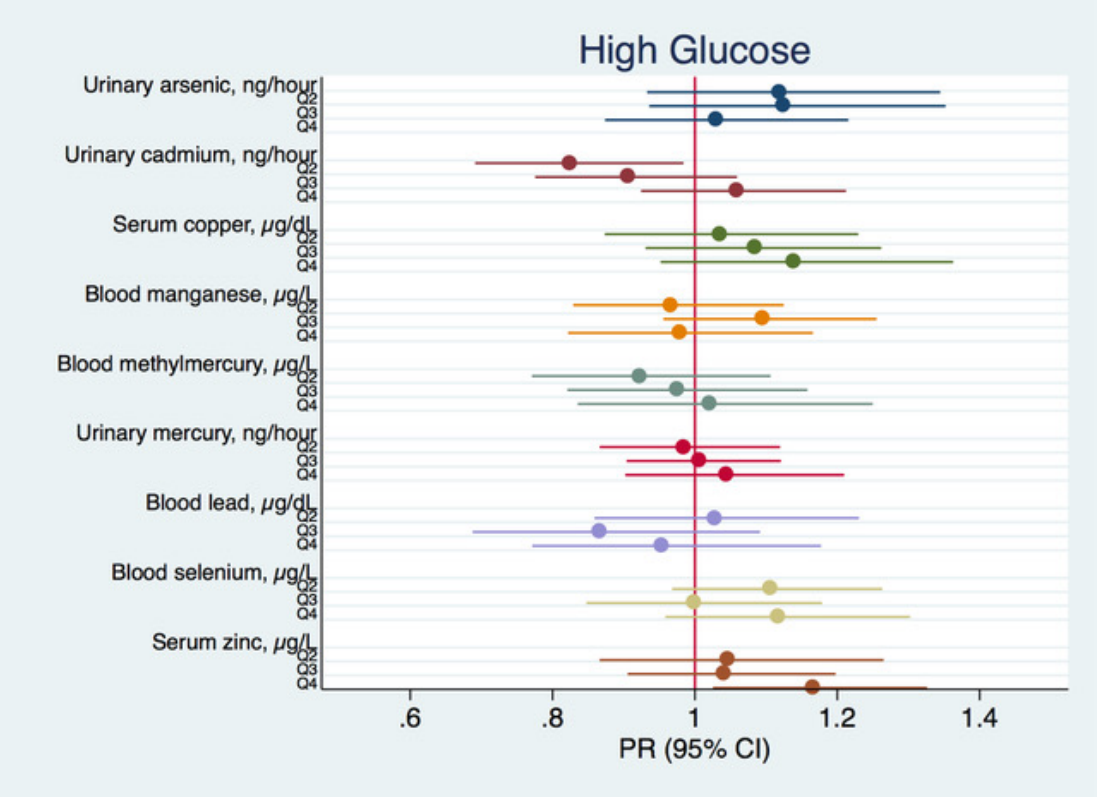
Figure 12. Adjusted prevalence ratios (95% CI) for high triglycerides and low HDL in NHANES[a]



[a] Adjusted for age (continuous, years), gender (male or female), race/ethnicity (non-Hispanic white, non-Hispanic black, Hispanic, or other including multi-racial), family income: poverty ratio (continuous), total caloric intake (continuous, kcal/day), educational attainment (less than high school, high school diploma or GED, or at least some college), smoking status (current, former, or never), average number of drinks per day in past year, and physical activity status (met the 2008 physical activity guidelines or did not), survey cycle (2011-2012 or 2013-2014), and body mass index (continuous, kg/m^2)

APPENDIX B (CONTINUED)

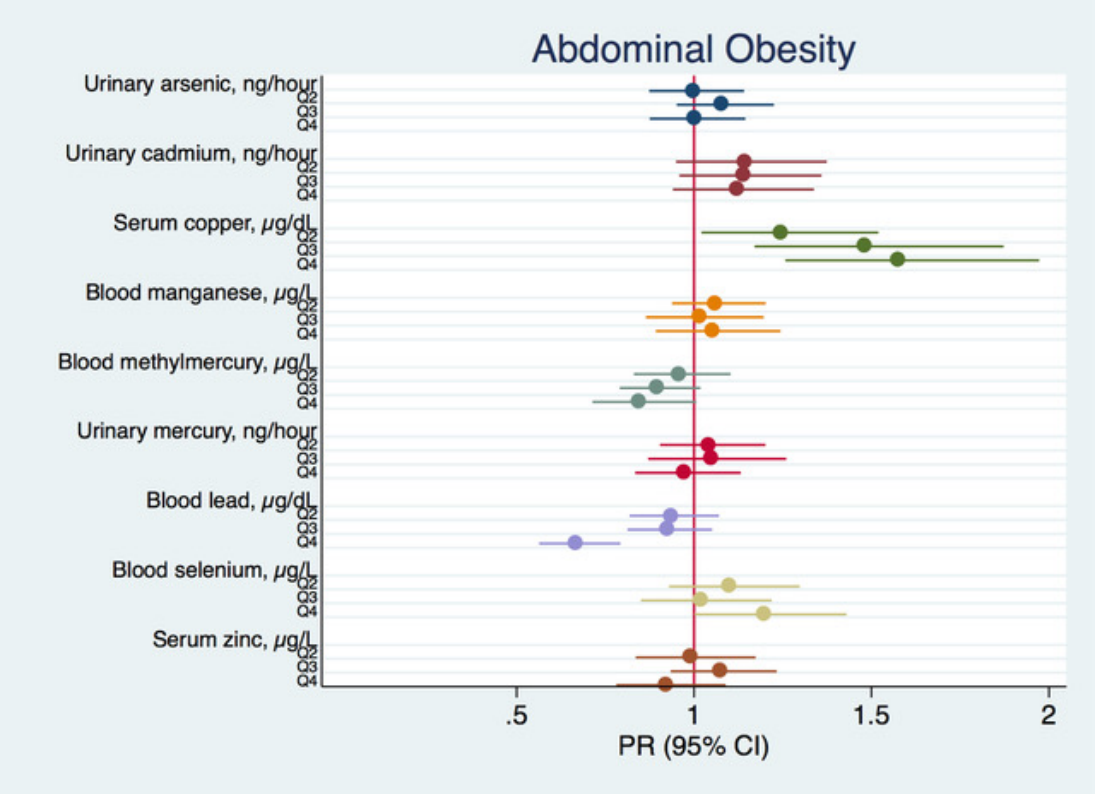
Figure 13. Adjusted prevalence ratios (95% CI) for high glucose in NHANES[a]



[a] Adjusted for age (continuous, years), gender (male or female), race/ethnicity (non-Hispanic white, non-Hispanic black, Hispanic, or other including multi-racial), family income: poverty ratio (continuous), total caloric intake (continuous, kcal/day), educational attainment (less than high school, high school diploma or GED, or at least some college), smoking status (current, former, or never), average number of drinks per day in past year, and physical activity status (met the 2008 physical activity guidelines or did not), survey cycle (2011-2012 or 2013-2014), and body mass index (continuous, kg/m^2)

APPENDIX B (CONTINUED)

Figure 14. Adjusted prevalence ratios (95% CI) for abdominal obesity in NHANES[a]



[a] Adjusted for age (continuous, years), gender (male or female), race/ethnicity (non-Hispanic white, non-Hispanic black, Hispanic, or other including multi-racial), family income: poverty ratio (continuous), total caloric intake (continuous, kcal/day), educational attainment (less than high school, high school diploma or GED, or at least some college), smoking status (current, former, or never), average number of drinks per day in past year, and physical activity status (met the 2008 physical activity guidelines or did not), and survey cycle (2011-2012 or 2013-2014)

APPENDIX B (CONTINUED)

TABLE XXXIV.

SENSITIVITY ANALYSIS: PREVALENCE RATIOS FOR METABOLIC SYNDROME AND COMPONENTS BY METAL BIOMARKERS WITH ADDITIONAL ADJUSTMENT FOR SERUM COTININE (N=1,088)

Metal biomarker	Metabolic syndrome[a] PR (95% CI)	High blood pressure[a] PR (95% CI)	High triglycerides[a] PR (95% CI)	Low HDL[a] PR (95% CI)	High glucose[a] PR (95% CI)	Abdominal obesity[b] PR (95% CI)
Urinary arsenic (ng/hour)[c]						
Q1 (25.4-174.2)	1.00 (ref.)	1.00 (ref.)	1.00 (ref.)	1.00 (ref.)	1.00 (ref.)	1.00 (ref.)
Q2 (174.3-254.2)	1.18 (1.03-1.36)	1.15 (0.90-1.48)	1.11 (0.96-1.29)	1.12 (0.95-1.31)	1.12 (0.93-1.36)	1.00 (0.88-1.14)
Q3 (254.3-372.2)	1.14 (0.95-1.38)	1.01 (0.75-1.34)	1.17 (0.97-1.42)	1.11 (0.92-1.34)	1.13 (0.94-1.36)	1.08 (0.96-1.23)
Q4 (372.3-2,990.7)	1.28 (1.08-1.51)	1.30 (1.01-1.68)	1.26 (1.05-1.50)	1.11 (0.95-1.31)	1.04 (0.88-1.22)	1.01 (0.88-1.15)
p_{trend}	0.017	0.162	0.011	0.218	0.599	0.568
Urinary cadmium (ng/hour)						
Q1 (0.6-6.2)	1.00 (ref.)	1.00 (ref.)	1.00 (ref.)	1.00 (ref.)	1.00 (ref.)	1.00 (ref.)
Q2 (6.2-11.0)	1.01 (0.83-1.23)	0.94 (0.72-1.23)	0.94 (0.81-1.10)	0.98 (0.82-1.17)	0.82 (0.69-0.98)	1.14 (0.95-1.36)
Q3 (11.1-20.1)	1.07 (0.89-1.29)	0.96 (0.76-1.22)	1.07 (0.89-1.28)	1.15 (0.99-1.33)	0.91 (0.78-1.06)	1.14 (0.96-1.35)
Q4 (20.2-206.8)	1.03 (0.81-1.30)	1.02 (0.77-1.34)	0.89 (0.74-1.07)	0.95 (0.80-1.15)	1.06 (0.93-1.20)	1.12 (0.94-1.33)
p_{trend}	0.735	0.796	0.463	0.858	0.186	0.234
Serum copper (µg/dL)						
Q1 (24.7-98.4)	1.00 (ref.)	1.00 (ref.)	1.00 (ref.)	1.00 (ref.)	1.00 (ref.)	1.00 (ref.)
Q2 (98.5-113.2)	0.96 (0.85-1.10)	1.15 (0.88-1.50)	0.97 (0.79-1.21)	0.90 (0.78-1.05)	1.03 (0.87-1.22)	1.24 (1.02-1.52)
Q3 (113.3-132.0)	0.95 (0.78-1.16)	1.02 (0.82-1.27)	0.98 (0.80-1.19)	0.94 (0.89-1.12)	1.08 (0.93-1.26)	1.47 (1.16-1.87)
Q4 (132.1-295.6)	0.94 (0.79-1.12)	0.94 (0.67-1.30)	1.02 (0.84-1.24)	0.94 (0.79-1.10)	1.14 (0.95-1.36)	1.57 (1.25-1.97)
p_{trend}	0.518	0.507	0.855	0.590	0.121	<0.001
Blood manganese (µg/L)						
Q1 (3.4-7.5)	1.00 (ref.)	1.00 (ref.)	1.00 (ref.)	1.00 (ref.)	1.00 (ref.)	1.00 (ref.)
Q2 (7.6-9.3)	1.04 (0.92-1.18)	1.17 (0.96-1.43)	1.03 (0.91-1.17)	0.94 (0.81-1.10)	0.97 (0.83-1.14)	1.07 (0.95-1.21)
Q3 (9.4-11.7)	1.00 (0.84-1.18)	0.99 (0.76-1.28)	1.06 (0.87-1.28)	1.06 (0.90-1.25)	1.10 (0.96-1.27)	1.02 (0.87-1.20)
Q4 (11.8-45.5)	1.04 (0.88-1.24)	1.09 (0.88-1.36)	0.96 (0.81-1.14)	1.01 (0.85-1.21)	0.99 (0.83-1.18)	1.06 (0.90-1.25)
p_{trend}	0.763	0.801	0.779	0.585	0.688	0.640

APPENDIX B (CONTINUED)

TABLE XXXIV. (CONTINUED)

SENSITIVITY ANALYSIS: PREVALENCE RATIOS FOR METABOLIC SYNDROME AND COMPONENTS BY METAL BIOMARKERS WITH ADDITIONAL ADJUSTMENT FOR SERUM COTININE (N=1,088)

Metal biomarker	Metabolic syndrome[a] PR (95% CI)	High blood pressure[a] PR (95% CI)	High triglycerides[a] PR (95% CI)	Low HDL[a] PR (95% CI)	High glucose[a] PR (95% CI)	Abdominal obesity[b] PR (95% CI)
Blood methylmercury (µg/L)						
Q1 (0.08-0.24)	1.00 (ref.)	1.00 (ref.)	1.00 (ref.)	1.00 (ref.)	1.00 (ref.)	1.00 (ref.)
Q2 (0.25-0.59)	0.97 (0.80-1.18)	0.87 (0.66-1.14)	1.07 (0.87-1.31)	0.96 (0.82-1.12)	0.92 (0.77-1.10)	0.96 (0.83-1.11)
Q3 (0.60-1.37)	0.94 (0.79-1.11)	0.93 (0.72-1.21)	1.08 (0.91-1.27)	0.86 (0.71-1.04)	0.97 (0.82-1.15)	0.90 (0.79-1.02)
Q4 (1.38-25.89)	1.10 (0.91-1.34)	1.00 (0.78-1.28)	1.19 (0.97-1.45)	1.00 (0.83-1.20)	1.02 (0.84-1.25)	0.85 (0.72-1.01)
p_{trend}	0.414	0.809	0.104	0.671	0.728	0.038
Urinary mercury (ng/hour)						
Q1 (0.8-8.9)	1.00 (ref.)	1.00 (ref.)	1.00 (ref.)	1.00 (ref.)	1.00 (ref.)	1.00 (ref.)
Q2 (9.0-17.7)	1.14 (0.95-1.37)	1.27 (1.01-1.59)	1.25 (1.03-1.53)	1.11 (0.95-1.28)	0.98 (0.86-1.12)	1.04 (0.90-1.20)
Q3 (17.8-36.4)	1.18 (0.98-1.41)	1.21 (0.95-1.55)	1.36 (1.09-1.69)	1.08 (0.89-1.32)	1.00 (0.90-1.11)	1.04 (0.87-1.26)
Q4 (36.5-973.3)	1.28 (1.07-1.51)	1.32 (1.03-1.69)	1.45 (1.22-1.74)	1.19 (1.00-1.41)	1.05 (0.90-1.21)	0.97 (0.84-1.13)
p_{trend}	0.012	0.046	<0.001	0.108	0.500	0.794
Blood lead (µg/dL)						
Q1 (0.18-0.70)	1.00 (ref.)	1.00 (ref.)	1.00 (ref.)	1.00 (ref.)	1.00 (ref.)	1.00 (ref.)
Q2 (0.71-1.05)	0.90 (0.73-1.11)	1.10 (0.84-1.43)	0.85 (0.72-0.99)	0.90 (0.76-1.07)	1.03 (0.86-1.23)	0.93 (0.82-1.07)
Q3 (1.06-1.63)	0.84 (0.69-1.05)	0.99 (0.75-1.31)	0.76 (0.64-0.92)	0.79 (0.65-0.97)	0.86 (0.68-1.08)	0.91 (0.80-1.04)
Q4 (1.64-15.98)	0.81 (0.64-1.03)	1.00 (0.71-1.39)	0.82 (0.67-1.01)	0.73 (0.59-0.89)	0.95 (0.77-1.17)	0.66 (0.56-0.78)
p_{trend}	0.063	0.673	0.069	0.002	0.324	<0.001
Blood selenium (µg/L)						
Q1 (120.1-180.6)	1.00 (ref.)	1.00 (ref.)	1.00 (ref.)	1.00 (ref.)	1.00 (ref.)	1.00 (ref.)
Q2 (180.7-194.9)	1.20 (0.98-1.46)	0.86 (0.65-1.14)	1.29 (1.02-1.63)	1.08 (0.89-1.31)	1.11 (0.97-1.27)	1.10 (0.93-1.29)
Q3 (195.0-210.0)	1.24 (0.99-1.55)	1.01 (0.77-1.32)	1.27 (0.99-1.62)	1.12 (0.94-1.33)	1.00 (0.85-1.18)	1.02 (0.85-1.22)
Q4 (210.1-356.0)	1.31 (1.05-1.63)	1.12 (0.89-1.42)	1.44 (1.13-1.83)	1.21 (1.02-1.44)	1.11 (0.95-1.30)	1.19 (1.00-1.42)
p_{trend}	0.013	0.161	0.007	0.023	0.379	0.111

APPENDIX B (CONTINUED)

TABLE XXXIV. (CONTINUED)

SENSITIVITY ANALYSIS: PREVALENCE RATIOS FOR METABOLIC SYNDROME AND COMPONENTS BY METAL BIOMARKERS WITH ADDITIONAL ADJUSTMENT FOR SERUM COTININE (N=1,088)

Metal biomarker	Metabolic syndrome[a] PR (95% CI)	High blood pressure[a] PR (95% CI)	High triglycerides[a] PR (95% CI)	Low HDL[a] PR (95% CI)	High glucose[a] PR (95% CI)	Abdominal obesity[b] PR (95% CI)
Serum zinc (µg/L)						
Q1 (49.1-78.6)	1.00 (ref.)	1.00 (ref.)	1.00 (ref.)	1.00 (ref.)	1.00 (ref.)	1.00 (ref.)
Q2 (78.7-86.7)	1.09 (0.90-1.32)	1.00 (0.80-1.25)	1.15 (0.95-1.41)	1.04 (0.87-1.23)	1.04 (0.86-1.26)	0.99 (0.83-1.17)
Q3 (86.8-96.3)	1.12 (0.92-1.35)	0.98 (0.79-1.23)	1.27 (1.06-1.54)	1.06 (0.90-1.25)	1.04 (0.91-1.19)	1.07 (0.93-1.23)
Q4 (96.4-232.5)	1.32 (1.11-1.57)	1.13 (0.89-1.43)	1.43 (1.24-1.66)	1.14 (1.02-1.28)	1.16 (1.02-1.32)	0.92 (0.78-1.08)
p_{trend}	0.004	0.393	<0.001	0.031	0.023	0.559

[a] Adjusted for age (continuous, years), gender (male or female), race/ethnicity (non-Hispanic white, non-Hispanic black, Hispanic, or other including multi-racial), family income: poverty ratio (continuous), total caloric intake (continuous, kcal/day), educational attainment (less than high school, high school diploma or GED, or at least some college), smoking status (current, former, or never), average number of drinks per day in past year, physical activity status (met the 2008 physical activity guidelines or did not), survey cycle (2011-2012 or 2013-2014), body mass index (continuous, kg/m^2), and serum cotinine (continuous, ng/mL)

[b] Includes all variables listed above except for body mass index

[c] Calibrated to remove the contribution of organic arsenic from recent seafood consumption

APPENDIX B (CONTINUED)

TABLE XXXV.

SENSITIVITY ANALYSIS: PREVALENCE RATIOS FOR METABOLIC SYNDROME AND COMPONENTS BY BLOOD LEAD CONCENTRATIONS WITH ADDITIONAL ADJUSTMENT FOR FEMORAL NECK BONE MINERAL DENSITY (N=338)[a]

	Metabolic syndrome[b]	**High blood pressure**[b]	**High triglycerides**[b]	**Low HDL**[b]	**High glucose**[b]	**Abdominal obesity**[c]
Blood lead (µg/dL)	**PR (95% CI)**	**PR (95% CI)**	**PR (95% CI)**	**PR (95% CI)**	**PR (95% CI)**	**PR (95% CI)**
Q1 (0.18-0.70)	1.00 (ref.)	1.00 (ref.)	1.00 (ref.)	1.00 (ref.)	1.00 (ref.)	1.00 (ref.)
Q2 (0.71-1.05)	0.98 (0.71-1.34)	1.00 (0.64-1.56)	0.86 (0.69-1.08)	0.97 (0.68-1.37)	1.05 (0.85-1.31)	1.04 (0.82-1.32)
Q3 (1.06-1.63)	0.82 (0.58-1.17)	0.89 (0.62-1.27)	0.76 (0.57-1.01)	0.69 (0.46-1.03)	0.87 (0.65-1.17)	0.98 (0.74-1.31)
Q4 (1.64-15.98)	0.75 (0.50-1.14)	0.94 (0.54-1.66)	0.64 (0.44-0.94)	0.60 (0.35-1.02)	0.90 (0.64-1.25)	0.77 (0.57-1.03)
p_{trend}	0.090	0.720	0.028	0.032	0.294	0.047

[a] Dual energy x-ray absorptiometry was only performed during the 2013-2014 survey cycle; of the569 individuals from that cycle who were eligible for analysis, 231 were missing data on femoral neck bone mineral density

[b] Models include age (continuous, years), gender (male or female), race/ethnicity (non-Hispanic white, non-Hispanic black, Hispanic, or other including multi-racial), and family income: poverty ratio (continuous), total caloric intake (continuous, kcal/day), educational attainment (less than high school, high school diploma or GED, or at least some college), smoking status (current, former, or never), average number of drinks per day in past year, physical activity status (met the 2008 physical activity guidelines or did not), body mass index (continuous, kg/m^2), and femoral bone mineral density (continuous, gm/cm^2)

[c] Includes all variables listed above except for body mass index

APPENDIX B (CONTINUED)

TABLE XXXVI.

SENSITIVITY ANALYSIS: PREVALENCE RATIOS FOR METABOLIC SYNDROME AND COMPONENTS BY BLOOD METHYLMERCURY CONCENTRATIONS WITH ADDITIONAL ADJUSTMENT FOR RECENT SEAFOOD CONSUMPTION (N=1,087)[b]

Blood methylmercury (µg/L)	Metabolic syndrome[b] PR (95% CI)	High blood pressure[b] PR (95% CI)	High triglycerides[b] PR (95% CI)	Low HDL[b] PR (95% CI)	High glucose[b] PR (95% CI)	Abdominal obesity[c] PR (95% CI)
Q1 (0.08-0.24)	1.00 (ref.)	1.00 (ref.)	1.00 (ref.)	1.00 (ref.)	1.00 (ref.)	1.00 (ref.)
Q2 (0.25-0.59)	0.97 (0.80-1.17)	0.87 (0.67-1.15)	1.06 (0.87-1.30)	0.96 (0.82-1.13)	0.92 (0.77-1.10)	0.95 (0.83-1.10)
Q3 (0.60-1.37)	0.94 (0.79-1.12)	0.95 (0.72-1.25)	1.07 (0.91-1.27)	0.87 (0.71-1.06)	0.97 (0.82-1.15)	0.90 (0.79-1.02)
Q4 (1.38-25.89)	1.10 (0.89-1.37)	1.03 (0.78-1.36)	1.18 (0.96-1.46)	1.02 (0.84-1.25)	1.01 (0.82-1.24)	0.84 (0.70-1.02)
p_{trend}	0.464	0.696	0.119	0.818	0.828	0.056

[a] One individual from the analytic sample of 1,088 was missing data on recent seafood consumption

[b] Models include age (continuous, years), gender (male or female), race/ethnicity (non-Hispanic white, non-Hispanic black, Hispanic, or other including multi-racial), and family income: poverty ratio (continuous), total caloric intake (continuous, kcal/day), educational attainment (less than high school, high school diploma or GED, or at least some college), smoking status (current, former, or never), average number of drinks per day in past year, physical activity status (met the 2008 physical activity guidelines or did not), survey cycle (2011-2012 or 2013-2014), body mass index (continuous, kg/m^2), and frequency of seafood meals in the past 30 days (continuous)

[c] Includes all variables listed above except for body mass index

APPENDIX C

TABLE XXXVII.
RELATION OF BASELINE BLOOD BIOMARKER CONCENTRATIONS WITH ADJUSTED ANNUAL CHANGES IN SYSTOLIC BLOOD PRESSURE OVER 6 YEARS OF FOLLOW-UP

Biomarker	Quartile 1 mmHg/year (95% CI)	Quartile 2 mmHg/year (95% CI)	Quartile 3 mmHg/year (95% CI)	Quartile 4 mmHg/year (95% CI)	p_{trend}
Lead					
Model 1[a]	Ref.	0.32 (-0.61, 1.25)	0.17 (-0.76, 1.10)	0.94 (0.00, 1.88)	0.081
Model 2[b]	Ref.	0.48 (-0.47, 1.43)	0.43 (-0.51, 1.38)	1.12 (0.16, 2.08)	0.040
Model 3[c]	Ref.	0.51 (-0.43, 1.45)	0.41 (-0.53, 1.34)	1.16 (0.21, 2.11)	0.037
Manganese					
Model 1[a]	Ref.	-1.55 (-2.48, -0.63)	-0.51 (-1.44, 0.43)	-0.58 (-1.52, 0.35)	0.662
Model 2[b]	Ref.	-1.53 (-2.46, -0.61)	-0.55 (-1.49, 0.38)	-0.65 (-1.59, 0.29)	0.582
Model 3[c]	Ref.	-1.64 (-2.56, -0.72)	-0.65 (-1.58, 0.28)	-0.75 (-1.69, 0.18)	0.448
Selenium					
Model 1[a]	Ref.	0.03 (-0.90, 0.96)	0.29 (-0.65, 1.23)	-0.60 (-1.54, 0.33)	0.316
Model 2[b]	Ref.	-0.08 (-1.00, 0.84)	0.17 (-0.76, 1.11)	-0.87 (-1.83, 0.09)	0.145
Model 3[c]	Ref.	-0.10 (-1.01, 0.82)	0.15 (-0.77, 1.08)	-0.99 (-1.95, -0.04)	0.090

[a] Single biomarker models, adjusted for age and sex.
[b] Mutual adjustment for baseline blood lead, manganese, and selenium, in addition to age and sex.
[c] Mutual adjustment for baseline blood lead, manganese, and selenium, in addition to age, sex, site, smoking status, educational duration, creatinine-corrected urinary arsenic concentration, diabetes, body mass index, and antihypertensive use.

APPENDIX C (CONTINUED)

TABLE XXXVIII.

RELATION OF BASELINE BLOOD BIOMARKER CONCENTRATIONS WITH ADJUSTED ANNUAL CHANGES IN DIASTOLIC BLOOD PRESSURE OVER 6 YEARS OF FOLLOW-UP

Biomarker	Quartile 1 mmHg/year (95% CI)	Quartile 2 mmHg/year (95% CI)	Quartile 3 mmHg/year (95% CI)	Quartile 4 mmHg/year (95% CI)	p_{trend}
Lead					
Model 1[a]	Ref.	-0.08 (-0.70, 0.53)	-0.23 (-0.70, 0.53)	0.33 (-0.29, 0.95)	0.415
Model 2[b]	Ref.	0.01 (-0.62, 0.63)	-0.05 (-0.67, 0.57)	0.49 (-0.16, 1.10)	0.240
Model 3[c]	Ref.	0.00 (-0.63, 0.62)	-0.04 (-0.66, 0.58)	0.53 (-0.10, 1.16)	0.169
Manganese					
Model 1[a]	Ref.	-0.83 (-1.44, -0.22)	-0.56 (-1.17, 0.06)	-0.22 (-0.84, 0.40)	0.711
Model 2[b]	Ref.	-0.82 (-1.43, -0.21)	-0.62 (-1.24, -0.01)	-0.30 (-0.92, 0.33)	0.642
Model 3[c]	Ref.	-0.88 (-1.48, -0.27)	-0.70 (-1.31, -0.08)	-0.39 (-1.01, 0.23)	0.446
Selenium					
Model 1[a]	Ref.	-0.16 (-0.77, 0.44)	0.18 (-0.44, 0.79)	-0.57 (-1.18, 0.04)	0.174
Model 2[b]	Ref.	-0.19 (-0.80, 0.42)	0.15 (-0.46, 0.77)	-0.65 (-1.28, -0.02)	0.109
Model 3[c]	Ref.	-0.20 (-0.80, 0.41)	0.13 (-0.48, 0.74)	-0.73 (-1.36, -0.10)	0.065

[a] Single biomarker models, adjusted for age and sex.
[b] Mutual adjustment for baseline blood lead, manganese, and selenium, in addition to age and sex.
[c] Mutual adjustment for baseline blood lead, manganese, and selenium, in addition to age, sex, site, smoking status, educational duration, creatinine-corrected urinary arsenic concentration, diabetes, body mass index, and antihypertensive use.

APPENDIX C (CONTINUED)

TABLE XXXIX.

RELATION OF BASELINE BLOOD BIOMARKER CONCENTRATIONS WITH ADJUSTED ANNUAL CHANGES IN PULSE PRESSURE OVER 6 YEARS OF FOLLOW-UP

Biomarker	Quartile 1 mmHg/year (95% CI)	Quartile 2 mmHg/year (95% CI)	Quartile 3 mmHg/year (95% CI)	Quartile 4 mmHg/year (95% CI)	p_{trend}
Lead					
Model 1[a]	Ref.	0.41 (-0.28, 1.09)	0.40 (-0.29, 1.08)	0.62 (-0.08, 1.31)	0.099
Model 2[b]	Ref.	0.47 (-0.23, 1.18)	0.48 -0.21, 1.18)	0.65 (-0.06, 1.37)	0.082
Model 3[c]	Ref.	0.50 (-0.20, 1.20)	0.43 (-0.27, 1.12)	0.63 (-0.08, 1.34)	0.113
Manganese					
Model 1[a]	Ref.	-0.72 (-1.40, -0.03)	0.07 (-0.62, 0.76)	-0.35 (-1.04, 0.35)	0.833
Model 2[b]	Ref.	-0.71 (-1.39, -0.02)	0.08 (-0.62, 0.77)	-0.34 (-1.04, -0.36)	0.776
Model 3[c]	Ref.	-0.74 (-1.42, -0.06)	0.04 (-0.65, 0.73)	-0.36 (-1.06, 0.34)	0.728
Selenium					
Model 1[a]	Ref.	-0.19 (-0.49, 0.87)	0.12 (-0.57, 0.81)	-0.02 (-0.71, 0.67)	0.911
Model 2[b]	Ref.	0.11 (-0.58, 0.79)	0.03 (-0.66, 0.72)	-0.20 (-0.91, 0.51)	0.609
Model 3[c]	Ref.	0.10 (-0.58, 0.78)	0.03 (-0.66, 0.72)	-0.25 (-0.96, 0.46)	0.544

[a] Single biomarker models, adjusted for age and sex.
[b] Mutual adjustment for baseline blood lead, manganese, and selenium, in addition to age and sex.
[c] Mutual adjustment for baseline blood lead, manganese, and selenium, in addition to age, sex, site, smoking status, educational duration, creatinine-corrected urinary arsenic concentration, diabetes, body mass index, and antihypertensive use.

APPENDIX C (CONTINUED)

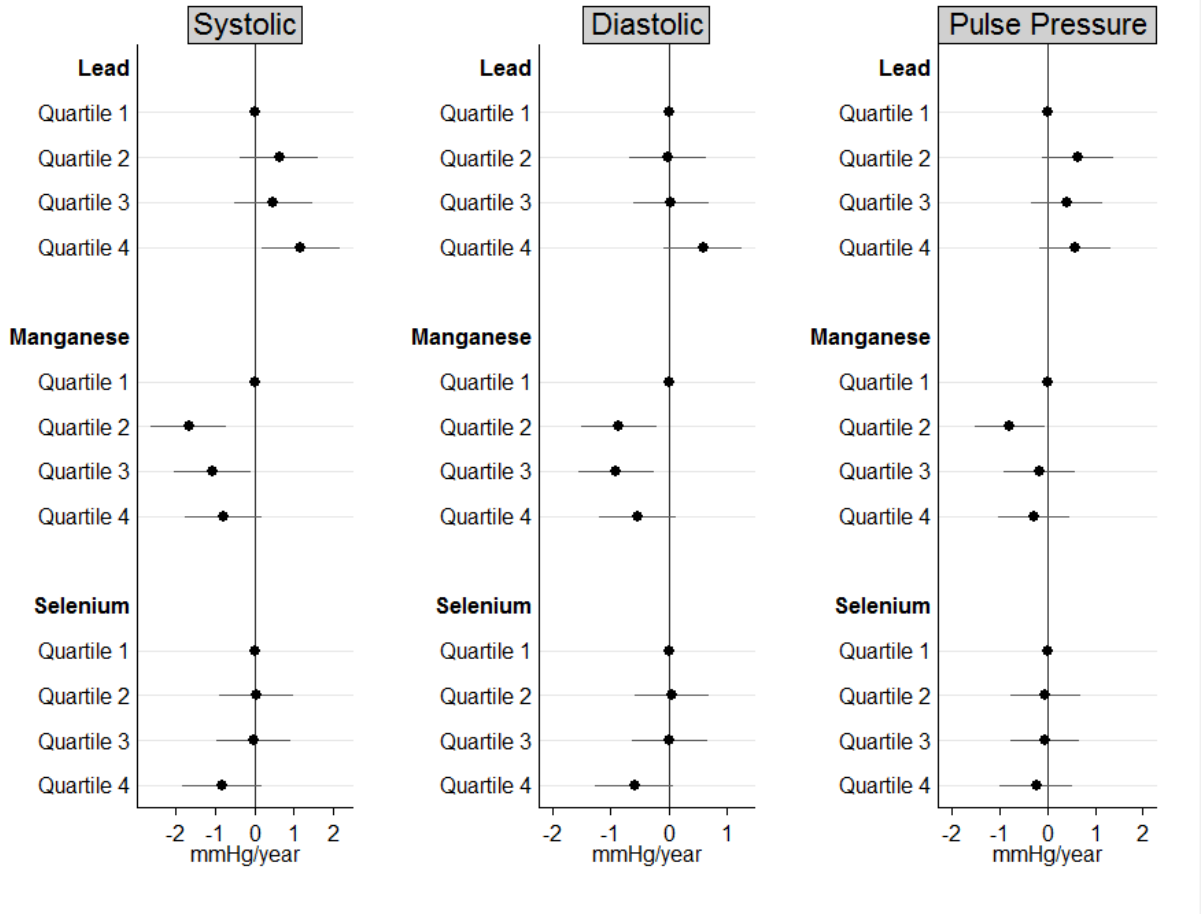
TABLE XL.
HAZARD RATIOS FOR INCIDENT HYPERTENSION BY BASELINE BLOOD BIOMARKER CONCENTRATION

Biomarker	Quartile 1 HR (95% CI)	Quartile 2 HR (95% CI)	Quartile 3 HR (95% CI)	Quartile 4 HR (95% CI)	P_{trend}
Lead					
Model 1[a]	Ref.	0.93 (0.37-2.32)	0.85 (0.32-2.23)	1.74 (0.72-4.20)	0.230
Model 2[b]	Ref.	0.97 (0.37-2.55)	0.97 (0.36-2.61)	1.48 (0.58-3.79)	0.374
Model 3[c]	Ref.	0.72 (0.21-2.43)	0.71 (0.15-3.27)	0.75 (0.16-3.55)	0.957
Manganese					
Model 1[a]	Ref.	0.20 (0.05-0.70)	0.88 (0.38-2.08)	1.70 (0.73-3.96)	0.119
Model 2[b]	Ref.	0.21 (0.06-0.77)	0.82 (0.33-2.01)	1.57 (0.65-3.79)	0.171
Model 3[c]	Ref.	0.21 (0.06-0.81)	1.00 (0.38-2.65)	1.63 (0.61-4.37)	0.191
Selenium					
Model 1[a]	Ref.	1.31 (0.52-3.29)	1.68 (0.70-4.02)	1.33 (0.53-3.36)	0.432
Model 2[b]	Ref.	1.17 (0.46-3.01)	1.35 (0.55-3.23)	1.11 (0.42-2.91)	0.593
Model 3[c]	Ref.	0.72 (0.25-2.08)	0.91 (0.32-2.55)	0.73 (0.23-2.30)	0.641

[a] Single biomarker models, adjusted for age and sex.
[b] Mutual adjustment for baseline blood lead, manganese, and selenium, in addition to age and sex.
[c] Mutual adjustment for baseline blood lead, manganese, and selenium, in addition to age, sex, site, smoking status, educational duration, creatinine-corrected urinary arsenic concentration, diabetes, and body mass index.

APPENDIX C (CONTINUED)

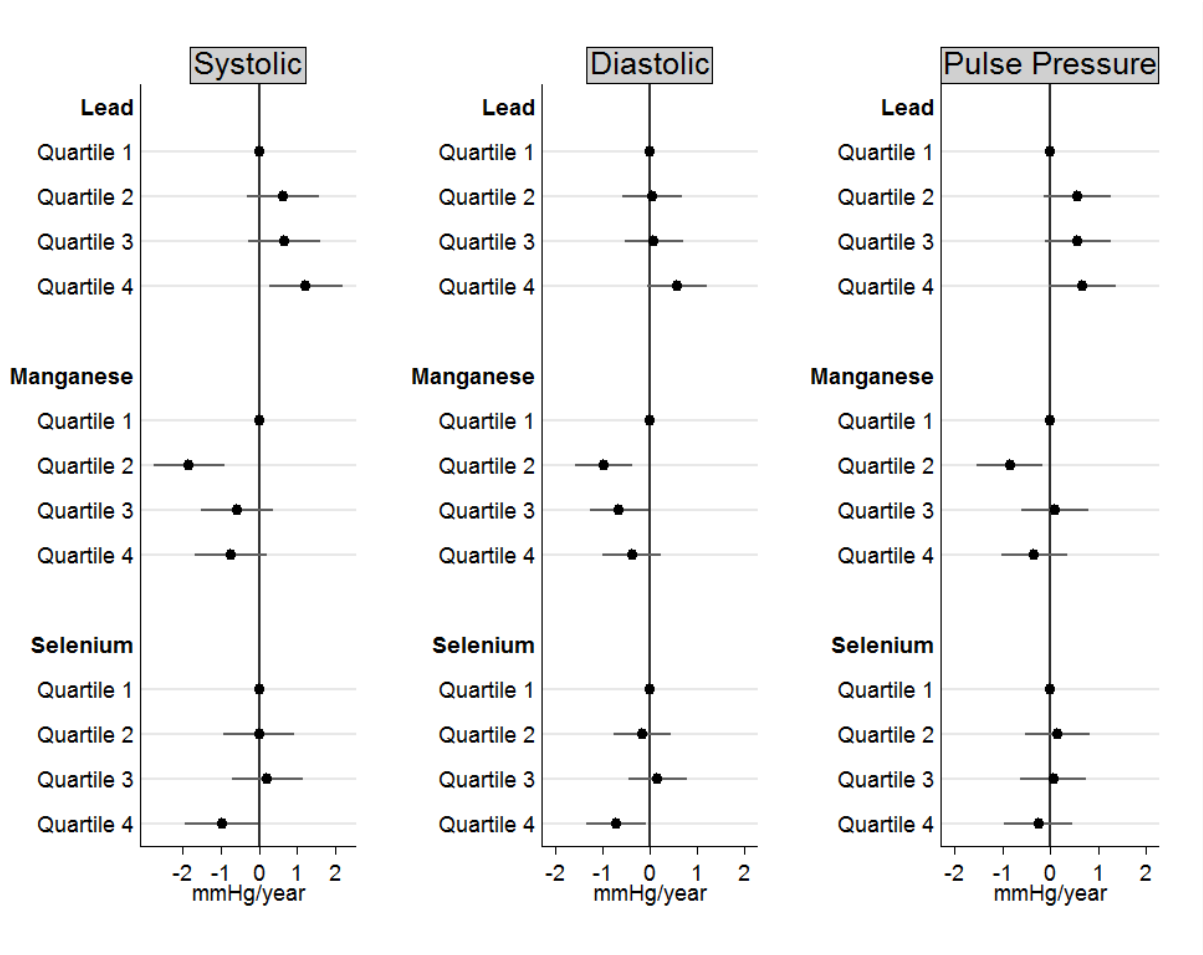
Figure 15. Adjusted annual changes in blood pressure among participants not taking antihypertensive medications[a]



[a] Mutually adjusted for baseline blood lead, manganese, and selenium, in addition to age, sex, site, smoking status, educational duration, creatinine-corrected urinary arsenic concentration, diabetes, and body mass index

APPENDIX C (CONTINUED)

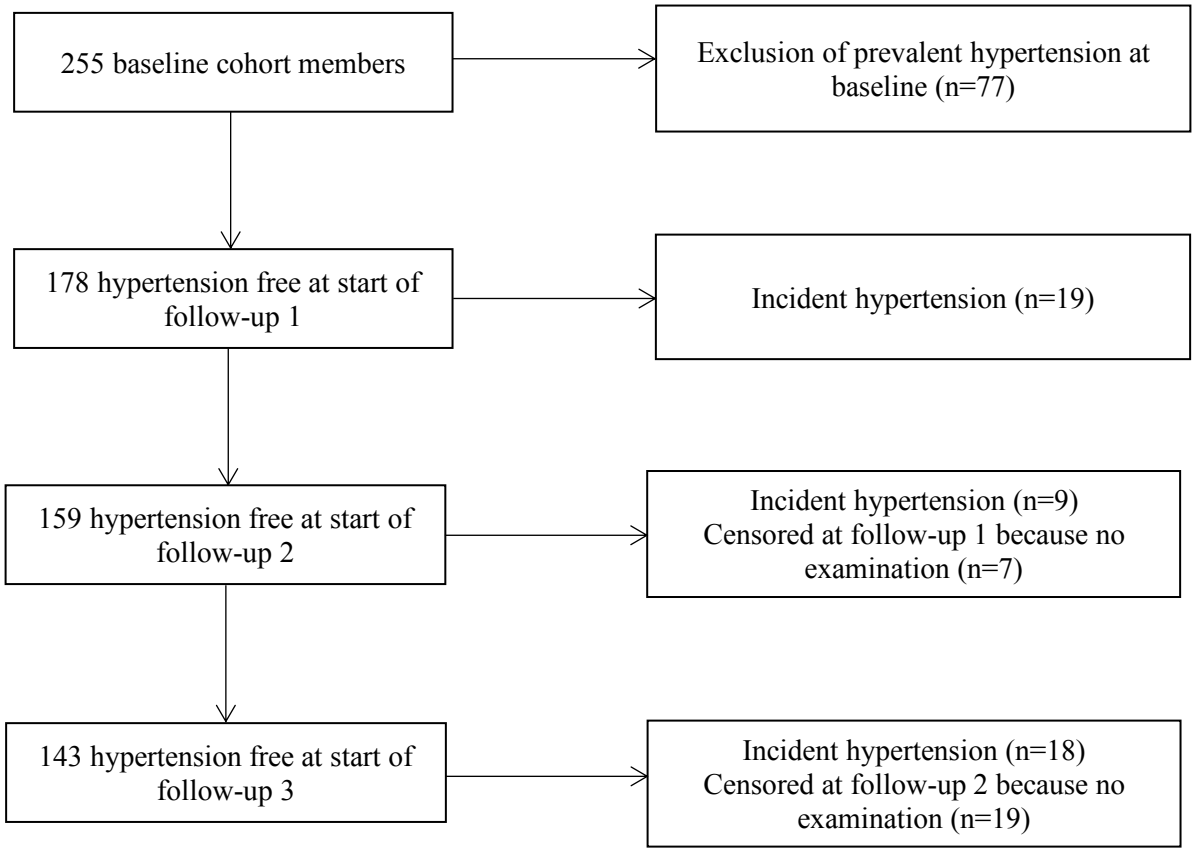
Figure 16. Adjusted annual changes in blood pressure using added constants for participants taking antihypertensive medications[a]



[a] Mutually adjusted for baseline blood lead, manganese, and selenium, in addition to age, sex, site, smoking status, educational duration, creatinine-corrected urinary arsenic concentration, diabetes, and body mass index

APPENDIX C (CONTINUED)

Figure 17. Flow diagram for discrete-time hazard models of hypertension status.



APPENDIX D

UNIVERSITY OF ILLINOIS
AT CHICAGO

Office for the Protection of Research Subjects (OPRS)
Office of the Vice Chancellor for Research (MC 672)
203 Administrative Office Building
1737 West Polk Street
Chicago, Illinois 60612-7227

Notice of Determination of Human Subject Research

June 2, 2017

20170575-104935-1

Catherine Bulka, MPH
Epidemiology and Biostatistics
1819 W Polk St
Chicago, IL 60612
Phone: (312) 355-0473

RE: **Protocol # 2017-0575**
Essential Metals and Metabolic Syndrome: Results from the Hispanic Community Health Study/Study of Latinos (HCHS/SOL)

Sponsor(s): None

Dear Catherine Bulka:

The UIC Office for the Protection of Research Subjects received your "Determination of Whether an Activity Represents Human Subjects Research" application, and has determined that this activity **DOES <u>NOT</u> meet the definition of human subject research** as defined by 45 CFR 46.102(f).

Specifically, data collection has already occurred. Participants were asked to attend in-person examinations in which they completed surveys about their demographics and medical history. They underwent clinical exams that included anthropometric measurements (e.g., waist circumference), blood pressure readings, and routine laboratory tests (e.g., cholesterol, triglycerides, glucose). The data have been processed at the University of North Carolina which serves as the Coordinating Center for the HCHS/SOL, where all identifying information was removed from the dataset.

You may conduct your activity without further submission to the IRB.

If this activity is used in conjunction with any other research involving human subjects or if it is modified in any way, it must be re-reviewed by OPRS staff.

APPENDIX E

UNIVERSITY OF ILLINOIS
AT CHICAGO

Office for the Protection of Research Subjects (OPRS)
Office of the Vice Chancellor for Research (MC 672)
203 Administrative Office Building
1737 West Polk Street
Chicago, Illinois 60612-7227

Notice of Determination of Human Subject Research

June 2, 2017

20170577-104937-1

Catherine Bulka, MPH
Epidemiology and Biostatistics
1819 W Polk St
Chicago, IL 60612
Phone: (312) 355-0473

RE: **Protocol # 2017-0577**
The role of dietary and environmental metals in metabolic syndrome: A cross-sectional analysis

Sponsor(s): None

Dear Catherine Bulka:

The UIC Office for the Protection of Research Subjects received your "Determination of Whether an Activity Represents Human Subjects Research" application, and has determined that this activity **DOES NOT meet the definition of human subject research** as defined by 45 CFR 46.102(f).

Specifically, this research will involve a secondary analysis of de-identified data from the National Health and Nutrition Examination Survey (NHANES), to evaluate associations of arsenic, cadmium, copper, lead, mercury, manganese, selenium, and zinc biomarkers with prevalent metabolic syndrome, as assessed through physical examinations in the mobile examination center.

You may conduct your activity without further submission to the IRB.

If this activity is used in conjunction with any other research involving human subjects or if it is modified in any way, it must be re-reviewed by OPRS staff.

APPENDIX F

UNIVERSITY OF ILLINOIS
AT CHICAGO

Office for the Protection of Research Subjects (OPRS)
Office of the Vice Chancellor for Research (MC 672)
203 Administrative Office Building
1737 West Polk Street
Chicago, Illinois 60612-7227

Approval Notice
Amendment to Research Protocol– Expedited Review
UIC Amendment #3

June 13, 2017

Maria Argos, PhD
Epidemiology and Biostatistics
1603 W Taylor Street
Office 878A, M/C 923
Chicago, IL 60612
Phone: (312) 355-1584 / Fax: (312) 996-0064

RE: Protocol # 2015-0536
"Bangladesh Vitamin E and Selenium Trial"

Dear Dr. Argos:

Members of Institutional Review Board (IRB) #1 have reviewed this amendment to your research under expedited procedures for minor changes to previously approved research allowed by Federal regulations [45 CFR 46.110(b)(2)]. The amendment to your research was determined to be acceptable and may now be implemented.

Please note the following information about your approved amendment:

Amendment Approval Date: June 7, 2017
Amendment:

Summary: UIC Amendment #3, dated and received by OPRS on May 30, 2017, is an investigator-initiated amendment adding a hypothesis for the secondary analysis of the prospective associations of environmental contaminants, "Subjects with lesser or greater manganese exposure (as opposed to mid-range exposures), lesser or greater selenium exposure (as opposed to mid-range exposures), greater lead exposure, and greater arsenic exposure at baseline will have an increased incidence of diabetes as assessed by HbA1c biomarker in blood, and will suffer from accelerations in age-related increases in blood pressures (Initial Review Application, Version 3, 6/9/2017)

Research Protocol(s):

a) Bangladesh Vitamin E and Selenium Trial; Version 3, 13 June 2017

Please note the Review History of this submission:

Receipt Date	Submission Type	Review Process	Review Date	Review Action
05/30/2017	Amendment	Expedited	06/07/2017	Approved

APPENDIX F (CONTINUED)

Please be sure to:

→ Use your research protocol number (2015-0536) on any documents or correspondence with the IRB concerning your research protocol.

→ Review and comply with all requirements on the enclosure,
"UIC Investigator Responsibilities, Protection of Human Research Subjects"
(http://tigger.uic.edu/depts/ovcr/research/protocolreview/irb/policies/0924.pdf)

Please note that the UIC IRB #1 has the right to ask further questions, seek additional information, or monitor the conduct of your research and the consent process.

Please be aware that if the scope of work in the grant/project changes, the protocol must be amended and approved by the UIC IRB before the initiation of the change.

We wish you the best as you conduct your research. If you have any questions or need further help, please contact the OPRS at (312) 996-1711 or me at (312) 413-9680. Please send any correspondence about this protocol to OPRS at 203 AOB, M/C 672.

Sincerely,

Jovana Ljuboje
Assistant Director, IRB #1
Office for the Protection of Research Subjects

cc: Ronald C. Hershow, Epidemiology and Biostatistics, M/C 923

VITA

EDUCATION

2014 – Present	PhD Candidate, University of Illinois at Chicago, School of Public Health, Epidemiology
2010 – 2012	MPH, Emory University, Rollins School of Public Health, Global Epidemiology
2006 – 2010	BA, Emory University, College of Arts & Sciences, Biology and Global Health

RESEARCH EXPERIENCE

2015 – Present	Pre-Doctoral Trainee, Cardiovascular Disease Epidemiology & Related Chronic Diseases in Minority Populations, Institute for Minority Health Research, University of Illinois at Chicago (NHLBI T32HL125294o1)
2014-2015	Pre-Doctoral Trainee, Occupational Safety and Health Education and Research Center, University of Illinois at Chicago (NIOSH T42OH008672)
2012 – 2014	Research Analyst, Vanderbilt University Medical Center, Department of Anesthesiology
2012 – 2014	Research Consultant, Emory University, Winship Cancer Institute, Lymphoma Program
2010 – 2012	Intern, Centers for Disease Control and Prevention, Influenza Coordination Unit
2011	Intern, Centers for Disease Control and Prevention, Office of Surveillance, Epidemiology, and Laboratory Services, Laboratory Science, Policy, and Practice Program Office

HONORS AND AWARDS

2017	University of Illinois at Chicago School of Public Health Annual Research and Practice Day Doctoral Student Poster Award
2017	Mary Hanna Memorial Journalism Award from the Journal of PeriAnesthesia Nursing, Research Category: Second Place
2015	Illinois Public Health Association Graduate Study in Public Health Scholarship
2013	Certificate and Honor Coin In Appreciation of Outstanding Commitment to the Centers for Disease Control and Prevention Response to the 2009 H1N1 Influenza A Pandemic

TEACHING EXPERIENCE

2015, '16, '17	Smoothers, Fractional Polynomials, and Splines Guest Lecturer, University of Illinois at Chicago, School of Public Health EPID 594: Applied Methods for the Analysis of Epidemiologic Data
2015	Introduction to Spatial Analysis: GIS, Clustering, and Spatial Regression Modeling Guest Lecturer, University of Illinois at Chicago, School of Public Health

EPID 594: Applied Methods for the Analysis of Epidemiologic Data

Professional Affiliations

2015 – Present Society for Epidemiologic Research

2015— Present American Heart Association

JOURNAL REVIEWS AND REFEREEINGE

Reviewer

2015— Present Abstract reviewer, Society for Epidemiologic Research Annual Meeting

Ad Hoc Reviewer

Environmental Health Perspectives
Environmental Research
Journal of Adolescent and Young Adult Oncology
Preventing Chronic Disease

Invited Lectures and Presentations

2016 Occupational Exposures and Metabolic Syndrome among Hispanics/Latinos. Oral presentation at the Hispanic Community Health Study/Study of Latinos Chicago Investigators Meeting, Chicago, IL

2017 Association of Occupational Exposures with Cardiovascular Disease among Hispanics/Latinos: Results from the Hispanic Community Health Study/Study of Latinos (HCHS/SOL). Oral presentation at the National Heart, Lung, and Blood Institute Cardiovascular Epidemiology, Biostatistics and Prevention Trainee Session at the American Heart Association EPI|Lifestyle Scientific Sessions, Portland, OR

2017 Association of Occupational Exposures with Cardiovascular Disease among Hispanics/Latinos. Oral presentation for the Hispanic Community Health Study/Study of Latinos Career Development Seminar, Chicago, IL

2017 Role of Dietary and Environmental Metal Exposures in Metabolic Syndrome. Oral presentation at the Hispanic Community Health Study/Study of Latinos Chicago Investigators Meeting, Chicago, IL

2018 Dietary Minerals and Metabolic Syndrome: Results from the Hispanic Community Health Study/Study of Latinos (HCHS/SOL). Moderated poster presentation at the National Heart, Lung, and Blood Institute Cardiovascular Epidemiology, Biostatistics and Prevention Trainee Session at the American Heart Association EPI|Lifestyle Scientific Sessions, New Orleans, LA

PROFESSIONAL PRESENTATIONS

1. **Bulka CM**, Sotres-Alvarez D, Daviglus ML, Persky VW, Durazo-Arvizu RA, Mossavar-Rahmani Y, Argos M (2018). Dietary Minerals and Metabolic Syndrome: Results from the Hispanic Community Health Study/Study of Latinos (HCHS/SOL). Poster presentation at the American Heart Association EPI|Lifestyle Scientific Sessions, New Orleans, LA.
2. **Bulka CM**, Scannell Bryan M, Durazo-Arvizu R, Slavkovich V, Graziano JH, Islam T, Yunus M, Ahsan H, Argos M (2017). Metal Exposures and Blood Pressure Changes in a Bangladeshi Cohort. Poster presentation at the Society for Epidemiologic Research Annual Meeting, Seattle, WA.
3. **Bulka CM**, Daviglus ML, Persky VW, Durazo-Arvizu RA, Elfassy T, Lash JP, Lee DJ, Ramos AR, Tarraf W, Argos M (2017). Association of Occupational Exposures with Cardiovascular Disease among Hispanics/Latinos: Results from the Hispanic Community Health Study/Study of Latinos (HCHS/SOL). Poster presentation at the American Heart Association EPI|Lifestyle Scientific Sessions, Portland, OR.
4. **Bulka CM**, Mabila S, Turyk ME, Argos M (2016). Creatinine Collider Bias: A Case Study of Urinary Inorganic Arsenic and Obesity. Poster presentation at the Epidemiology Congress of the Americas, Miami, FL.
5. **Bulka CM**, Mabila S, Turyk ME, Argos M (2016). Inverse Associations Between Inorganic Arsenic and Obesity: Findings From the National Health and Nutrition Examination Survey. Poster presentation at the American Heart Association EPI|Lifestyle Scientific Sessions, Phoenix, AZ.
6. **Bulka CM**, Jones RM, Turyk ME, Stayner LT, Argos M (2015). Arsenic in drinking water and prostate cancer in Illinois counties. Poster presentation at the Society for Epidemiologic Research Annual Meeting, Denver, CO.
7. **Bulka CM**, Nastoupil LJ, Koff J, Bernal L, Ward K, Bayakly R, Switchenko J, Waller L, Flowers CR (2013). Relationship Between Residential Proximity to Environmental Protection Agency (EPA) Designated Toxic Release Sites and the Risk of Diffuse Large B-Cell Lymphoma (DLBCL). Poster presentation at the American Society of Hematology Annual Meeting, New Orleans, LA.
8. **Bulka CM**, Nastoupil LJ, Switchenko J, Ward, K, Bayakly R, Waller L, Flowers CR (2013). Spatial Epidemiology of Chronic Lymphocytic Leukemia and Small Lymphocytic Lymphoma in the State of Georgia. Poster presentation at the American Society of Hematology Annual Meeting, New Orleans, LA.
9. **Bulka CM**, Selig SC, Martin BJ, Dmochowski RR, Hayes RM, Ehrenfeld JM (2013). Risk Factors for Postoperative Pneumonia: A Retrospective Analysis. Poster presentation at the American Society of Anesthesiologists Annual Meeting, San Francisco, CA.
10. **Bulka CM**, Nastoupil LJ, McClellan W, Ambinder A, Phillips A, Ward K, Switchenko JM, Bayakly R, Waller L, Flowers CR (2012). Residence Proximity to Benzene Release Sites is Associated with Increased Incidence of Non-Hodgkin Lymphoma. Poster presentation at the American Society of Hematology Annual Meeting, Atlanta, GA.
11. **Bulka CM**, McClellan W, Ward K, Bayakly R, Waller L, Flowers CR (2012). Residence proximity to benzene toxic release sites in Georgia and non-Hodgkin's lymphoma. Poster presentation at the International Lymphoma Epidemiology Consortium, Bethesda, MD.

Original Articles

1. **Bulka CM**, Daviglus ML, Persky VW, Durazo-Arvizu RA, Aviles-Santa ML, Gallo LC, Hosgood HD, Singer RH, Talavera GA, Thyagarajan B, Zeng D, Argos M (2017). Occupational Exposures and Metabolic Syndrome among Hispanics/Latinos: Cross-Sectional Results from the Hispanic Community Health Study/Study of Latinos (HCHS/SOL). Journal of Occupational and Environmental Medicine 59(11):1047-1055.
2. Shen MS, Dodd AC, Lakomkin N, Mousavi I, **Bulka C**, Jahangir AA, Sethi MK. Open Treatment of Ankle Fracture as Inpatient Increases Risk of Complication. Journal of Orthopaedics and Traumatology. Epub ahead of print.
3. Kresovich JK, **Bulka CM**, Joyce BT, Vokonas PS, Schwartz J, Baccarelli AA, Hibler EA, Hou L (2017). The Inflammatory Potential of Dietary Manganese in a Cohort of Elderly Men. Biological Trace Element Research. Epub ahead of print.
4. Whiting PS, Rice CD, Avilucea FR, **Bulka CM**, Shen MS, Obremskey WT, Sethi MK (2017). Patients at Increased Risk of Major Adverse Events Following Operative Treatment of Distal Radius Fractures: Inpatient versus Outpatient. *Journal of Wrist Surgery* 6(3):220-226.
5. **Bulka CM**, Davis MA, Karagas MR, Ahsan H, Argos M (2017). The Unintended Consequences of a Gluten-Free Diet. *Epidemiology* 28(3): e24-e25.
6. **Bulka CM**, Ehrenfeld JM (2017). In Reply. *Anesthesiology* 127(1): 198.
7. Chona D, Lakomkin N, **Bulka CM,** Mousavi I, Kothari P, Dodd AC, Shen MS, Obremskey WT, Sethi MK (2017). Predicing the Post-operative Length of stay for the Orthopaedic Trauma Patient. *International Orthopaedics.* Epub ahead of print.
8. **Bulka CM**, Nastoupil LJ, Koff JL, Bernal-Mizrachi L, Ward KC, Williams JN, Bayakly AR, Switchenko JM, Waller LA, Flowers CR (2016). Relations Between Residential Proximity to EPA-Designated Toxic Release Sites and Diffuse Large B-Cell Lymphoma Incidence. *Southern Medical Journal* 109(10): 606-614.
9. **Bulka CM,** Jones RM, Turyk ME, Stayner LT, Argos M (2016). Arsenic in Drinking Water and Prostate Cancer in Illinois Counties. *Environmental Research* 148: 450-456.
10. Dodd AC, Lakomkin N, **Bulka CM**, Thakore R, Collinge CA, Sethi MK (2016). Geographic Variations in Orthopedic Trauma Billing and Reimbursements for Hip and Pelvis Fractures in the Medicare Population. *Journal of Orthopaedics 25; 13(4): 264-267.*
11. **Bulka CM**, Terekhov MA, Martin BJ, Dmochowski RR, Hayes RM, Ehrenfeld JM (2016). Nondepolarizing Neuromuscular Blocking Agents, Reversal, and Risk of Postoperative Pneumonia. *Anesthesiology* 125(4): 647-655.
12. Dodd AC, **Bulka CM**, Jahangir A, Mir HR, Obremskey WT, Sethi MK (2016). Predictors of 30-day Mortality Following Hip/Pelvis Fractures. *Orthopaedics & Traumatology, Surgery & Research* 102(6): 707-710.
13. Dodd AC, Lakomkin N, Attum B, **Bulka CM**, Karhade AV, Douleh DG, Mir H, Jahangir AA, Obremskey WT, Sethi MK (2016). Predictors of Adverse Events for Ankle Fractures: An Analysis of 6800 Patients. *Journal of Foot and Ankle Surgery* 55: 762-766.
14. Switchenko JM, **Bulka CM,** Ward KC, Koff JL, Bayakly AR, Ryan PB, Waller LA, Flowers CR (2016). Resolving uncertainty in the spatial relationships between passive benzene exposure and risk of non-Hodgkin lymphoma. *Cancer Epidemiology* 41: 139-151.
15. Beazley B, **Bulka CM**, Landsman IS, Ehrenfeld JM (2016). Demographic Predictors of NPO Violations in Elective Pediatric Surgery. *Journal of Perianesthesia Nursing* 31(1): 36-40.
16. **Bulka CM,** Shotwell MS, Ehrenfeld JM (2015). Reply to Dr Bhatia. *Regional Anesthesia and Pain Medicine* 40(1): 86-87.

17. Thakore RV, Greenberg SE, **Bulka CM,** Ehrenfeld JM, Obremskey WT, Sethi MK (2015). Geographic Variations in Hospital Charges and Medicare Payments for Major Joint Replacement. *The Journal of Arthroplasty* 30(5): 728-732.
18. **Bulka CM,** Wanderer JP, Ehrenfeld JM (2014). Anesthesia technique and outcomes after hip fracture surgery. *Journal of the American Medical Association* 312(17): 1801.
19. **Bulka CM,** Shotwell MS, Gupta RK, Sandberg WS, Ehrenfeld JM (2014). Regional Anesthesia, Time to Hospital Discharge, and In-Hospital Mortality: A Propensity Score Matched Analysis. *Regional Anesthesia and Pain Medicine* 39(5): 381-386.
20. McDonald MR, Thakore R, Obremskey W, **Bulka CM**, Ehrenfeld JM, Jahangir A, Sethi MK (2014). Ankle Radiographs in the Early Postoperative Period: Do They Matter? *Journal of Orthopaedic Trauma* 28(9): 538-541.
21. Corey J, **Bulka CM**, Ehrenfeld JM (2014). Is Regional Anesthesia Associated with Reduced PACU Length of Stay? A Retrospective Analysis from a Tertiary Hospital. *Clinical Orthopaedics and Related Research* 472(5): 1427-1433.
22. **Bulka CM**, Cassedy EA, Sandberg WS, Ehrenfeld JM (2013). A Survey of Modified Rapid Sequence Induction and Intubation in Canadian Academic Centers. *Journal of Clinical Anesthesia* 25(6): 515-516.
23. **Bulka CM**, Nastoupil LJ, McClellan W, Ambinder AJ, Phillips A, Ward KC, Bayakly R, Switchenko JM, Waller L, Flowers CR (2013). Residence proximity to benzene release sites is associated with increased incidence of non Hodgkin lymphoma. *Cancer* 119(18): 3309-3317.

Manuscripts under Review

1. Karagas MR, Punshon T, Davis MA, Gossai A, **Bulka CM**, Slaughter F, Argos M, Ahsan H. Rice intake, and emerging concerns on the potential impacts of arsenic in rice: a review of the human evidence and methodologic challenges. *The Journal of Nutrition.*
2. **Bulka CM**, Scannell Bryan M, Persky VW, Daviglus ML, Durazo-Arvizu RA, Sotres-Alvarez DS, Slavkovich V, Graziano JH, Islam T, Baron JA, Ahsan H, Argos M. Changes in Blood Pressure Associated with Lead, Manganese, and Selenium in a Bangladeshi Cohort. *Environmental International.*
3. **Bulka CM**, Daviglus ML, Persky VW, Durazo-Arvizu RA, Elfassy T, Lash JP, Lee DJ, Ramos AR, Tarraf W, Argos M. Association of Occupational Exposures with Cardiovascular Disease among Hispanics/Latinos: Results from the Hispanic Community Health Study/Study of Latinos (HCHS/SOL). *Heart.*

Manuscripts in Preparation

1. Burroughs Peña MS, Swett K, **Bulka CM**, Daviglus ML, Perreira KM, Kansal MM, Loop MS, Rodriguez CJ. Occupational Environmental Exposures and Cardiac Structure and Function: The Echocardiographic Study of Latinos (Echo-SOL).

Conference Proceedings

1. **Bulka CM**, Daviglus ML, Persky VW, Durazo-Arvizu RA, Elfassy T, Lash JP, Lee DJ, Ramos AR, Tarraf W, Argos M (2017). Association of Occupational Exposures with Cardiovascular Disease among Hispanics/Latinos: Results from the Hispanic Community Health Study/Study of Latinos (HCHS/SOL). *Circulation* 135(Suppl 1): 072.

2. **Bulka CM**, Mabila S, Turyk ME, Argos M (2016). Inverse Associations Between Inorganic Arsenic and Obesity: Findings from the National Health and Nutrition Examination Survey. *Circulation* 133(Suppl 1): 064.
3. **Bulka CM**, Nastoupil LJ, Koff J, Bernal L, Ward K, Bayakly R, Switchenko J, Waller L, Flowers CR (2013). Relationship Between Residential Proximity to Environmental Protection Agency (EPA) Designated Toxic Release Sites and the Risk of Diffuse Large B-Cell Lymphoma (DLBCL). *Blood* 122(21): 1684.
4. **Bulka CM**, Nastoupil LJ, Switchenko J, Ward, K, Bayakly R, Waller L, Flowers CR (2013). Spatial Epidemiology of Chronic Lymphocytic Leukemia and Small Lymphocytic Lymphoma in the State of Georgia. *Blood* 122(21): 1683.
5. **Bulka CM**, Nastoupil LJ, McClellan W, Ambinder A, Phillips A, Ward K, Switchenko JM, Bayakly R, Waller L, Flowers CR (2012). Residence Proximity to Benzene Release Sites is Associated with Increased Incidence of Non-Hodgkin Lymphoma. *Blood* 120(21): 2710.