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# Urinary Metal Mixtures and Longitudinal Changes in Glucose Homeostasis: The Study of Women's Health Across the Nation (SWAN)

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## Abstract

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

**Objective:** We assessed the association of exposure to metal mixtures, based on assessment of 15 urinary metals, with both baseline levels and longitudinal changes in homeostatic model assessments for insulin resistance (HOMA-IR) and β-cell function (HOMA-β).

**Methods:** We examined 1,262 women, aged 45–56 years at baseline (1999–2000), who were followed through 2015–2016, from the Study of Women's Health Across the Nation. Urinary concentrations of 15 metals (arsenic, barium, cadmium, cobalt, cesium, copper, mercury, manganese, molybdenum, nickel, lead, antimony, tin, thallium, and zinc) were determined at baseline. HOMA-IR and HOMA-β were repeatedly measured over 16 years of follow-up. A two-stage modeling was used to account for correlations in dependent and independent variables: In stage-1, linear mixed effects models were used to estimate the participant-specific baseline HOMA

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Declaration of competing interests

The authors declare they have no actual or potential competing interest.

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The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

levels from random intercepts and participant-specific rates of changes from random slopes. In stage-2, adaptive elastic-net (AENET) models were fit to identify components of metal mixtures associated with participant-specific baseline levels and rates of changes in HOMA-IR and HOMA-β, respectively. An environmental risk score (ERS) was used to integrate metal mixture effects from AENET results.

**Results:** In multivariable adjusted AENET models, urinary zinc was associated with higher HOMA-IR at baseline, whereas molybdenum was associated with lower HOMA-IR at baseline. The estimated changes in baseline HOMA-IR for one standard deviation increase in log-transformed urinary metal concentrations were 5.76% (3.05%, 8.55%) for zinc and −3.25% (−5.45%, −1.00%) for molybdenum, respectively. Urinary zinc was also associated with lower HOMA- β at baseline. Arsenic was associated with a slightly faster rate of decline in HOMA-β in the AENET model evaluating associations between metals and rate of changes. Significant associations of ERS with both HOMA-IR and HOMA-β at baseline were observed. ERS for the rate of changes was not calculated and examined in relation to rates of changes in HOMA-IR and HOMA-β because only a single metal was selected by AENET.

**Conclusion:** Exposure to metal mixtures may be exerting effects on insulin resistance and β-cell dysfunction, which might be mechanisms by which metal exposures lead to elevated diabetes risks.

### Keywords

Metals; Mixtures; Insulin resistance; β-cell dysfunction; Women

## 1. Introduction

Type 2 diabetes mellitus (T2DM) is a major global health concern and its incidence has rapidly increased over the past two decades (Magliano et al., 2019). The etiology of T2DM is not fully understood yet and the role of environmental exposures, specifically metals, in the pathogenesis of T2DM has received less attention by the medical community. Metals are widely dispersed in the environment, including soil, water, air, dust, human food chain, as well as in manufacturing products (Järup, 2003; Tchounwou et al., 2012; Wang et al., 2019a). The general population can be exposed to a myriad of metals through food, drinking water, and ambient air throughout their lifetime. Growing evidence from epidemiologic studies suggests that exposure to metals may play a role in the induction or exacerbation of diabetes (Li et al., 2017; Maull et al., 2012; Menke et al., 2015; Wang et al., 2020; Yuan et al., 2018). These findings provided an impetus to investigate the underlying mechanisms by which metal exposures may influence T2DM risk.

The etiopathogenic mechanisms underlying T2DM involve insulin resistance and β-cell dysfunction, which commonly precede the onset of diabetes by one to two decades (DeFronzo, 2004; Warram et al., 1990). Biological studies provide evidence that both essential and non-essential metals may impact these conditions. Essential metals including cobalt, copper, manganese, molybdenum, nickel, and zinc are required for various biological pathways and appropriate amounts of these metals are necessary for multiple physiological functions in humans (Zoroddu et al., 2019). For example, zinc is necessary for insulin

synthesis, storage and secretion in β-cells (Chausmer, 1998), and have a preventative role in insulin resistance, for example, zinc complexes showed insulin-like effects (Adachi et al., 2006). On the contrary, non-essential metals including arsenic, barium, cadmium, cesium, mercury, lead, antimony, tin, and thallium have no known physiological roles (Zoroddu et al., 2019). Metals such as arsenic, cadmium and lead, are well-known inducers of oxidative stress (Ercal et al., 2001). The accumulation of these metals in pancreatic islets is hypothesized to lead to impaired function and apoptotic death of β-cells via the induction of oxidative stress (Lu et al., 2011; Patra et al., 2011). These metals have also been demonstrated to disrupt glucose uptake by interfering with insulin intracellular signaling pathways in adipocytes and muscle cells (Han et al., 2003; Kim et al., 2015; Mohammed Abdul et al., 2015).

Only a few epidemiologic studies have examined the associations of metal exposures with insulin resistance and β-cell dysfunction and those studies have yielded inconsistent results (Barregard et al., 2013; Feng et al., 2015; Grau-Perez et al., 2017; He et al., 2013; Moon, 2013; Park et al., 2016; Rhee et al., 2013; Wallia et al., 2014). Most studies were cross-sectional and focused on a limited number of metals although the general population is exposed to metal mixtures (Wang et al., 2019a) This narrow focus on individual metals is partly due to statistical challenges given the complex correlations among metal exposures and the lack of well-established statistical methods to evaluate the effects of exposure to metal mixtures (Braun et al., 2016; Park et al., 2017; Wang et al., 2018, 2019b). Quantifying the health impact of exposure to metal mixtures is needed to enhance understanding of the role of environmental risk factors in the pathogenesis of metabolic diseases including T2DM.

Within this context, we evaluated the associations of 15 urinary metal concentrations with level of and longitudinal changes in homeostatic model assessments for insulin resistance (HOMA-IR) and β-cell function (HOMA-β) over 16 years of follow-up in the Study of Women's Health Across the Nation (SWAN), a multi-site, multi-ethnic prospective cohort study of midlife women. HOMA-IR is an index of insulin resistance calculated from fasting glucose and insulin based on a homeostasis model (Matthews et al., 1985). Higher levels of HOMA-IR indicate greater insulin resistance. HOMA-β is an index of β-cell function also calculated from the homeostasis model using fasting glucose and insulin levels (Matthews et al., 1985). It represents a percent of normal β-cell function and lower levels indicate worse β-cell function.

We used a two-stage modeling approach and employed a machine-learning based approach, adaptive elastic-net (AENET), which was proposed for analyzing high dimensional data while dealing with the collinearity problem (Zou and Zhang, 2009), to identify important components of metal mixtures associated with longitudinal changes in HOMA-IR and HOMA-β. We further constructed an environmental risk score (ERS) (Park et al., 2017; Wang et al., 2018, 2019b), as a summary measure of health risk of exposure to multiple metals, to assess the overall effects of exposure to metal mixtures on HOMA-IR and HOMA-β.

## 2. Material and methods

### 2.1 Study population

Participants in the current analysis were from the SWAN, an ongoing, multi-site, multi-ethnic, community-based longitudinal study designed to investigate the natural history of the menopausal transition and its effect on midlife health including risk factors for age-related chronic diseases (Sowers et al., 2000). Between 1996 and 1997, a total of 3,302 women from seven study sites, including Boston, MA; Chicago, IL; southeast Michigan, MI; Los Angeles, CA; Oakland, CA; Newark, NJ; and Pittsburgh, PA, participated. Each site enrolled White women and women from one minority group (Black women from Boston, Chicago, Southeast Michigan, and Pittsburgh; Chinese women from Oakland; Japanese women from Los Angeles; Hispanic women from Newark). Eligibility criteria for enrollment into the SWAN cohort included: age 42 to 52 years in 1996/97; having an intact uterus and at least one ovary; having at least one menstrual period and not taking hormone therapy in the past 3 months; and having self-identified with the site's designated race/ethnic groups. Participants returned for regular examinations approximately annually. Institutional Review Board approval was obtained at each study site, and all participants provided signed informed consent at each study visit.

To evaluate associations between urinary metals and longitudinal glucose outcomes, we used data from the SWAN Multi-Pollutant Substudy (MPS), which was initiated to examine the associations of multiple environmental chemicals with metabolic and reproductive health outcomes in midlife women (Ding et al., 2020; Wang et al., 2019a). A subset of 1,400 SWAN participants from the five SWAN sites (Boston, southeast Michigan, Los Angeles, Oakland and Pittsburgh) who provided urine samples to the SWAN Repository at the third SWAN follow-up visit (V3, 1999–2000, SWAN-MPS baseline) were assayed for metal concentrations. Women from Chicago and Newark were excluded because urine samples were not collected in these two sites. This subpopulation, by design, included self-identified White, Black, Chinese, and Japanese but not Hispanic women who were recruited exclusively from Newark. Among these five sites, women for whom urine samples were not available were less educated and more likely to be current smokers or obese than women with available urine. For this analysis, we excluded 39 participants who had no information on key covariates (education, household income, body mass index (BMI), physical activity, total energy intake), 46 participants with missing information on fasting glucose or insulin levels, and 53 participants who were taking antidiabetic medications at SWAN-MPS baseline, yielding 1,262 participants eligible for the present study. We censored 347 observations in subsequent follow-up visits when a participant was taking antidiabetic medications because the true untreated levels of the outcome parameters are unknown. A final sample of 1,262 women representing 9,527 observations (7.5 per person) through 2016 was used for data analysis. 804 women remained in the cohort at the last follow-up visit. When compared to those 804 women, women who lost to follow-up were more likely to be Black and had higher BMI at SWAN-MPS baseline. An overview of our analytic sample is illustrated in Figure A.1.

### 2.2 Insulin resistance and β-cell dysfunction

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

### 2.3 Urinary metals

Urinary metal concentrations were analyzed with high-resolution inductively coupled plasma-mass spectrometry (ICP-MS) (Thermo Scientific iCAP RQ, Waltham, MA) in first morning spontaneously voided urine samples collected at the SWAN-MPS baseline at the Applied Research Center of NSF International (Ann Arbor, Michigan), a part of the Michigan Children's Health Exposure Analysis Resource (M-CHEAR) Laboratory Hub. The analytic methods and quality control procedures have been described previously (Wang et al., 2019a). Urinary concentrations of the following 15 metals were used in the current analysis, including arsenic, barium, cadmium, cobalt, cesium, copper, mercury, manganese, molybdenum, nickel, lead, antimony, tin, thallium, and zinc. The limits of detection (LOD) and detection rates are presented in Table A.1. Participants with metal concentration below the limit of detection (LOD) were assigned a value equal to the LOD divided by the square root of 2 (Arunajadai and Rauh, 2012). Urinary concentrations of beryllium, chromium, platinum, uranium, vanadium and tungsten were also determined in the SWAN-MPS. However, due to the relatively low detection rates (<40%) as described previously, these metals were excluded from the current analysis (Wang et al., 2019a). Pairwise Spearman correlations among specific gravity adjusted urinary metal concentrations were calculated and presented in a heat map.

### 2.4 Covariates

Age (continuous), self-reported race/ethnicity (White, Black, Chinese, or Japanese), and education level (≤ high school, some college, or college degree/post-college) were assessed through a self-administered questionnaire at baseline. At each study visit, annual household income ($19,999 or under, $20,000-$49,999, $50,000-$99,999, or $100,000 or above), smoking (never smoked, former smoked only, or current smoking), alcohol drinking (use less than once per month, use once per month, or twice or more times per month), menopausal status (premenopausal, post-menopausal, unknown menopause status due to hormone therapy or hysterectomy), and use of exogenous hormones were self-reported. Physical activity was evaluated at each visit using a modified version of the Kaiser Physical Activity Survey (Sternfeld et al., 2000), and a total score ranged from 3 to 15 was calculated

indicating the activity levels during the previous 12 months in 3 distinct domains: active living, household/caregiving, and sports/exercise. BMI was calculated at baseline as weight in kilograms divided by the square of height in meters. Dietary intake was collected at baseline, using a detailed semi-quantitative food frequency questionnaire (FFQ) adopted from Block FFQ (Block et al., 1986). The 103-food item FFQ included 4 seafood items (fried fish/fish sandwich, tuna fish/tuna salad, shellfish, and other fish) and 1 rice item (rice/dishes made with rice). For analysis, weekly seafood intake was computed by summing the frequency of intake for the 4 fish items. Total energy intake was obtained from the FFQ based on each food intake. Urinary specific gravity was determined using a handheld digital refractometer (ATAGO model PAL-10S, Tokyo, Japan) as a marker of urine dilution at baseline. In this analysis, age, race/ethnicity, study sites, education level, BMI, dietary factors, and urinary specific gravity were time-independent, while all other covariates were modeled as time-varying covariates.

### 2.5 Statistical analysis

A two-stage modeling approach was used to evaluate the associations of metal mixtures with longitudinal HOMA measures (HOMA-IR and HOMA-β) because there is no available analytical approach that handles correlations for both dependent and independent variables. In stage 1, to account for correlations in outcome measurements within each participant, linear mixed effects models were used to capture changes in HOMA measures over the follow-up period. Given the highly skewed distributions of both HOMA-IR and HOMA-β, logarithmic transformations were applied. Time (year) was modeled using a linear term. Random intercepts and random slopes of time were included in the models to allow for the variability of HOMA levels at baseline and their rates of change between each study participant. The participant-specific baseline HOMA levels and participant-specific annualized slopes (rates of changes) were estimated and used as dependent variables in the next stage of analysis. The conditional $R^2$ of linear mixed effects models was 0.66 for HOMA-IR and 0.56 for HOMA-β.

In stage 2, which was used to avoid multicollinearity among correlated exposure variables, AENET was used to select components of metal mixtures associated with baseline levels of HOMA measures and with their rates of changes, respectively. Ordinary least squares-based variable selection methods are commonly used but prone to over-fitting and do not work well in the presence of potentially high-dimensional predictors, or when predictors are highly correlated (multicollinearity) (Tibshirani, 1996). To combat this issue, elastic-net (ENET), a shrinkage regression method, has been introduced (Zou and Hastie, 2005). ENET executes variable selection by shrinking coefficients of “unimportant” predictors towards exact zeros, and has the ability to handle the complex correlations between predictor variables (Zou and Hastie, 2005). Adaptive elastic net (AENET), as its name would suggest, is an adaptive version of ENET that not only deals with the collinearity problem over ENET but satisfies the asymptotic normality assumption that allows us to conduct statistical inference and hypothesis testing by providing large sample standard errors and p-values (Zou and Zhang, 2009). It should be noted that AENET performs variable selection by shrinking certain coefficients to zero but not based on p-values of coefficients (like forward

selection, backward elimination, and stepwise selection). In this study, AENET models were fitted as follows:

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

where $Y_i$ represents participant-specific baseline HOMA levels or participant-specific rates of changes in HOMA measures estimated from stage 1, $X_{ki}$ denotes urinary concentration of $k^{th}$ metal (we have a total of 15 metals in the initial model to be selected), and $\mathbf{Z_i}$ indicates the vector of confounders. Two AENET models were performed to select metals associated with (1) baseline levels of HOMA-IR and HOMA-β, and (2) rates of changes in HOMA-IR and HOMA-β, separately. Given the highly skewed distributions of all metal concentrations, logarithmic transformations were applied. To better compare the associations of different metals with HOMA measures, we further standardized the log-transformed urinary metal concentrations by subtracting the mean of the corresponding log-transformed concentrations divided by its standard deviation (SD). This way the association was interpreted as the percent change in baseline level/rate of change in HOMA in relation to a one SD increase in log-transformed concentrations of selected metals in AENET models. All potential confounders, including age at baseline, race/ethnicity, study site, education level, annual household income, BMI, smoking, alcohol drinking, physical activity score, menopausal status, hormone therapy, dietary intake of seafood and rice, total zinc intake from diets and supplements, total energy intake, and urinary specific gravity were always adjusted for ("forced") in the models. We decided not to include time-varying BMI in the model because of its role as a potential diabetes risk factor and the fact that it could be affected by metal exposures at baseline (Niehoff et al., 2020; Wang et al., 2018). We adjusted for seafood and rice intake because it may impact glucose homeostasis (Hosomi et al., 2012; Zuñiga et al., 2014) and has been identified as important determinants of metal mixtures in our previous study in the SWAN-MPS (Wang et al., 2019a). We adjusted for total zinc intake to better capture the potential effects of urinary zinc excretion that are independent of dietary zinc intake. For other essential metals, such as copper, no dietary intake was adjusted for due to lack of data. We used a Directed Acyclic Graph to show the hypothesis relations between metals, confounders, and HOMA measures (See Figure A.2). To better visualize the results of two separate AENET models (baseline level and rate of change), we plotted trajectories of HOMA measures from baseline to the end of follow-up using the coefficient estimates of these two AENET models for all metal concentrations fixed at their 25th, 50th, 75th and 90th percentiles, respectively, with all other covariates adjusted. AENET penalized parameters were ascertained based on 10-fold cross-validation for minimal prediction errors. The R package 'gcdnet' was used to implement AENET (Yi and Zou, 2017).

To better summarize the combined effects of exposure to metal mixtures, we constructed an ERS, as an integrative index health risk of exposure to multiple chemicals in epidemiological research (Park et al., 2017; Wang et al., 2019b, 2018). The underlying idea behind the ERS is to build a risk score as a weighted sum of the chemical concentrations from the simultaneous assessment of multiple chemicals. In this analysis, weights were determined by the magnitudes of the associations of each metal from the AENET models. In this way, ERS was computed as a weighted sum of non-zero metal predictors selected from

AENET by $ERS_i = \sum_{j=1}^{P} \hat{\beta}_j Z_i^j$, where $Z_i^j (j = 1, \ldots, p)$ is a standardized log-transformed concentration of the *j*-th metal and $\hat{\beta}_j$ is the beta coefficient (weight) of the *j*-th metal. From this equation, the ERS for participant *i* can be interpreted as the effects on insulin resistance/β-cell dysfunction corresponding to her urinary concentrations of selected non-zero metal predictors from AENET. We further categorized ERS into quartiles and fit the multiple linear regression models to examine the associations between the ERS and HOMA outcomes. To compare the magnitude of the ERS association with those for selected individual metals, we also categorized those metals into quartiles and computed effect estimates for HOMA outcomes.

For metals that were selected in the AENET models, we also included them together in Bayesian kernel machine regression (BKMR) (Bobb et al., 2015), which enabled us to further evaluate the potential interactions between those metals and non-linear relationships between metals and HOMA measures. Specifically, we examined (1) univariate exposure–response functions of each standardized log-transformed metal concentration with other metals fixed at the median; and (2) bivariate exposure-response functions of each standardized log-transformed metal concentration with the second metal fixed at selected percentiles while other metals fixed at the median, to evaluate potential interactions between metals. Gaussian kernel exposure response machine function was used to fit the model. The same covariates from AENET models were adjusted in BKMR model. The R package ‘bkmr’ was used to implement BKMR (Bobb et al., 2015).

We conducted several sensitivity analyses to evaluate the robustness of our primary findings. First, hyperglycemia has been associated with increased urinary zinc excretion (Chausmer, 1998). Because participants who had relatively high glucose levels at the SWAN-MPS baseline may also have high levels of urinary zinc excretion at that time, reverse causation may account for observed associations between urinary zinc concentration and HOMA measures. To examine the potential impact of reverse causation on our results, we excluded participants with fasting glucose level ≥ 100 mg/dL (impaired fasting glucose) or HOMA-IR ≥ 4.2 (90th percentile) at SWAN-MPS baseline. Second, because adjustment for seafood intake may not sufficiently control for the less toxic organic arsenic in evaluation of the association between arsenic and HOMA measures (Navas-Acien et al., 2011), we evaluated the association in a subpopulation with seafood intake less than 1 time/week. In addition, we adjusted BMI at baseline in the primary analysis. However, it is possible that cumulative exposures to metals could impact BMI at baseline, making it a potential intermediate (Niehoff et al., 2020; Wang et al., 2018). Therefore, as a sensitivity analysis, we excluded BMI at baseline from the model adjustment in case of over-adjustment bias. All analyses were conducted using R, version 3.5.3 (www.R-project.org).

## 3. Results

### 3.1 Descriptive statistics

Characteristics of the study population at baseline are summarized in Table 1. The mean (SD) age of the 1,262 participants was 49.7 (2.8) years. Geometric means (geometric standard deviations) of HOMA-IR and HOMA-β were 2.1 (1.7) and 154.5 (1.6),

respectively, at baseline. Women with HOMA-IR at baseline greater than the median level (1.8) were more likely to be Black, and to have lower education level, lower family income, higher BMI, and higher alcohol consumption. Women with HOMA-β at baseline less than the median level (148.9) were less likely to be Black, more likely to be Chinese or Japanese, and to have higher family income, higher BMI, and higher alcohol consumption. The distributions and detection rates of all 15 urinary metal concentrations are summarized in Table A.1. Highest mean concentration was observed for zinc (272.44 μg/L), and lowest was observed for thallium (0.08 μg/L). In general, most metals were modestly and positively correlated with each other (Figure A.3). The strongest correlation was between copper and nickel ($R$=0.54).

### 3.2 Metal mixtures and HOMA-IR

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

We constructed ERS of HOMA-IR at baseline using estimated weights for copper, molybdenum, lead, and zinc from the AENET model (Table A.2). The ERS ranged from −0.26 to 0.27 with a mean (SD) equal to 0 (0.08). In accordance with the ERS formula, higher ERS for HOMA-IR at baseline indicates higher HOMA-IR (greater insulin resistance) at baseline attributable to higher concentrations of copper, lead, zinc but lower concentration of molybdenum. After adjusting for confounders, women in the highest quartile of ERS, on average, had 15.70% (95% CI: 9.15%, 22.64%) higher HOMA-IR at baseline, compared to those in the lowest quartile (Table 3). When comparing the effect estimates between quartiles of ERS and quartiles of selected individual metals, stronger effect sizes of ERS were observed than those for individual metals (Table A.3). The ERS of rate of change in HOMA-IR was not calculated because only zinc was selected in the AENET.

We ran an BKMR model regressing HOMA-IR at baseline while including copper, molybdenum, lead, and zinc to explore the potential non-linearity and interaction between those metals. Positive linear associations of log-transformed copper and zinc concentrations

with log-transformed HOMA-IR at baseline and an inverse linear association between log-transformed molybdenum concentration and log-transformed HOMA-IR at baseline were confirmed (Figure A.4A). The association between lead and baseline HOMA-IR was slightly diminished in the BKMR model. Further, there was little evidence for interactions between the metals given associations between each metal and HOMA-IR at baseline did not differ by varying quantiles of the other three metals (Figure A.4B).

### 3.3 Metal mixtures and HOMA-β

Table 4 shows the associations of selected components of metal mixtures with baseline HOMA- β and its rate of change in the AENET models. After adjusting for all potential confounders, a one SD increase in urinary metal concentration was associated with 1.59% (95%CI: −3.63%, 0.50%) lower baseline HOMA- β level for arsenic, and 2.66% (95%CI: −5.07%, −0.30%) lower level for zinc, respectively. In contrast, a one SD increase in urinary cobalt concentration was associated with 2.22% (95%CI: −0.10%, 4.60%) higher HOMA-β at baseline. HOMA-β declined during the follow-up (−1.00% annually, 95%CI: −1.02%, −0.90%). Urinary arsenic concentration was associated with faster rate of decline in HOMA-β, such that a one SD increase in urinary arsenic concentration was associated with 0.02% more rapid decline (95%CI: −0.05%, 0%) in HOMA-β annually. Beta coefficients of selected non-zero predictors in AENET models are shown in Table A.4. Predicted HOMA-β levels over the follow-up based on coefficients in these two AENET models are shown in Figure 2.

We constructed ERS of HOMA-β at baseline using estimated weights for arsenic, cobalt, and zinc from the AENET model (Table A.4). The ERS ranged from −0.1 to 0.1 with a mean (SD) equal to 0 (0.03). Lower ERS for HOMA-β at baseline indicates lower HOMA-β (greater β-cell dysfunction) at baseline attributable to higher concentrations of arsenic and zinc but lower concentration of cobalt. After adjusting for confounders, women in the lowest quartile of ERS, on average, had 8.96% (95% CI: −13.89%, −3.77%) lower HOMA-β at baseline, compared to those in the highest quartile (Table 5). The ERS effect size was stronger than those for individual metals (Table A.5). The ERS of rate of change in HOMA-β was not calculated because only arsenic was selected in the AENET.

We ran an BKMR model regressing HOMA-β at baseline with arsenic, cobalt, and zinc included in the model. Results from BKMR showed a positive linear association between log-transformed cobalt and log-transformed HOMA-β at baseline, and an inverse association between log-transformed zinc concentration and log-transformed HOMA-β at baseline (Figure A.5A). There was a potential interaction between arsenic and zinc given stronger inverse associations between arsenic and baseline HOMA-β were observed when urinary zinc concentrations were fixed at lower values (Figure A.5B). No interactions were detected between other metals.

### 3.4 Sensitivity analysis

In the sensitivity analysis, 186 women who had a fasting glucose level ≥ 100 mg/dL or a HOMA-IR ≥ 4.2 at the SWAN-MPS baseline were excluded. After exclusion of these participants, we still observed a positive association between urinary zinc concentration and

HOMA-IR; a one SD increase in urinary zinc concentration was associated with 3.97% (95% CI: 1.59%, 6.40%) higher HOMA-IR at baseline and 0.08% (95% CI: −0.01%, 0.18%) increase in the annual rate of increase in HOMA-IR, respectively (Table A.6). Similarly, urinary zinc concentration was inversely associated with HOMA-β at baseline, a one SD increase in urinary zinc concentration was associated with 0.94% (95% CI: −3.17%, 1.34%) lower HOMA-β at baseline (Table A.7).

Urinary arsenic was associated with a lower baseline level and a faster rate of decrease in HOMA-β in the primary analysis. In the sensitivity analysis, the association between urinary arsenic and HOMA-β was evaluated in a subpopulation of 371 participants with seafood intake less than 1 time/week. In this subpopulation, similar associations were observed as in the primary analysis, however, the confidence intervals of arsenic's effects became wider (Table A.8), possibly due to a reduced statistical power in accordance with the smaller sample size in this analysis.

Coefficients of urinary copper and lead in relation to HOMA-IR at baseline were shrunk to zeros in the AENET model without adjustment for BMI at baseline (Table A.9). Similar findings were observed for rate of change in HOMA-IR, HOMA-β at baseline, and rate of change in HOMA-β in AENET models without adjustments for BMI at baseline (Table A.9 and Table A.10).

## 4. Discussion

In this study, we evaluated the associations between the urinary concentrations of 15 metals and HOMA-IR and HOMA-β in a prospective cohort of 1,262 women over 16 years of follow-up. Using a two-stage modeling approach with AENET, we found that metals primarily impacted HOMA measures at baseline—urinary zinc was associated with higher HOMA-IR and lower HOMA-β at baseline, while urinary molybdenum was associated with lower HOMA-IR at baseline. Arsenic was associated with a faster rate of decline in HOMA-β, however, the magnitude of the association was modest. Additionally, we estimated the combined effects of metal mixtures using ERS. The significant associations of ERS with both baseline levels of HOMA-IR and HOMA-β suggest the potential impacts of metal mixtures on insulin sensitivity and β-cell function.

To the best of our knowledge, this study is the first to evaluate the association of exposure to metal mixtures with insulin resistance and β-cell dysfunction. Existing epidemiologic evidence has suggested that metals with high degree of toxicity, particularly arsenic, play a role in dysregulated glucose metabolism, although the evidence is inconsistent (Grau-Perez et al., 2017; Park et al., 2016; Rhee et al., 2013). In this study, we found that in addition to arsenic, other metals including copper, cobalt, molybdenum, lead, and zinc may also play a role. Most previous studies focused only on "priority toxic metals" while other potentially important metals were not investigated. Additionally, all previous studies have not addressed exposure to metal mixtures. Given the fact that people are co-exposed to multiple metals, and given the high degree of correlations between urinary metal concentrations in SWAN participants (Wang et al., 2019a), differences between our mixture analysis and previous studies might be attributed to complex correlation structures among metals. Simultaneously

incorporating several metals as predictors in regression models is prone to over-fitting, leading to a poor model performance and variance inflation with a large number of predictors, especially when predictors are highly correlated (Tibshirani, 1996). The statistical approach we used here (AENET) has been shown to overcome these issues (Zou and Zhang, 2009) and offers the ability to identify which components of metal mixtures are potentially exerting adverse effects (Wang et al., 2018, 2019b). Our mixture analysis also accounted confounding due to co-exposure to other metal components as previous studies suggested metals may interfere with each other metabolically (López Alonso et al., 2004). Furthermore, if individual metals have relatively small effects but exposure to metal mixtures influence the body's response to insulin and/or insulin secretion, the metal components that truly disrupt these physiological functions may not be adequately captured by the conventional single-pollutant approach. The observed associations between ERS and HOMAs were also larger than those assessed individually, suggesting that combined effects of metal mixtures may be larger than each individual effect. Our findings highlight the importance of considering metal mixtures, rather than individual metals with known toxicities, in evaluation of associations between metal exposures and health outcomes in future studies.

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

Urinary zinc concentration was adversely associated with both HOMA-IR and HOMA-β. Zinc is an essential nutrient that is necessary for biochemical pathways and required by thousands of proteins for catalytic functions (Jansen et al., 2009). Humans rely on a daily intake of dietary zinc to maintain health and prevent disease, and zinc leaves the body in urine and feces (Roohani et al., 2013). Zinc intake has been associated with a lower risk of T2DM in women (Vashum et al., 2013). In our study, zinc status was assessed from both zinc intake and urinary excretion. We found a positive association between urinary zinc and HOMA-IR, and an inverse association between urinary zinc and HOMA-β, after adjustment for zinc intake from both diets and supplements, suggesting that women with excess zinc excreted in urine may be at elevated risk of insulin resistance and β-cell dysfunction regardless of the amount of dietary zinc intake. Mechanistic studies found that, in pancreatic β cells, zinc was necessary for insulin synthesis, storage and secretion, and has accounted

for the conformational integrity of insulin in its hexameric crystalline form (Jansen et al., 2009). Excessive urinary excretion of zinc was found to lead to a loss of zinc in β-cells, which accounted for a reduced insulin secretion (Jansen et al., 2009). Certain zinc complexes showed insulin-mimetic effects including reducing hyperglycemia and increasing lipogenesis in animal models (Jansen et al., 2009). Zinc has also been shown to improve glucose transportation in peripheral tissues by improving binding of insulin to its receptor through enhancing tyrosine kinase phosphorylation (Jansen et al., 2009). Additionally, zinc is a structural part of antioxidant enzymes such as superoxide dismutase that could protect insulin and β-cells from being attacked by free radicals (Jansen et al., 2009). Despite this evidence, hyperglycemia, on the other hand, was suggested to interfere the active transportation of zinc back to renal cells, leading to a loss of this mineral in the urine (Chausmer, 1998). This raised the possibility that the observed association could also be explained by the increased urinary excretion of zinc in women who already had relatively high glucose levels at baseline. However, in the sensitivity analysis after excluding women who had relatively high glucose levels at baseline, the findings of associations between urinary zinc and HOMA measures did not change, though effect estimate for HOMA-β was attenuated, diminishing the likelihood that reverse causation bias drove the observed results. Our most recent study also reported that a higher urinary zinc excretion was associated with increased risk of T2DM in SWAN (Wang et al., 2020). The results of current analysis suggest that an elevated urinary excretion of zinc may increase risk of T2DM possibly through its adverse effect on insulin resistance.

We observed a positive association between urinary lead and HOMA-IR at baseline in the AENET model. However, the association was not statistically significant in this model and was attenuated in the BKMR model. Bone lead stores accrued from cumulative environmental exposures for decades are the major endogenous source of lead. Bone lead has been considered a proxy for cumulative exposure to lead and found to be a better biomarker of lead dose than blood lead in recent studies of the relationship between lead exposure and chronic health outcomes such as cardiovascular disease (Ding et al., 2018, 2016). Urinary lead adjusted for urine dilution has been found to closely reflect lead mobilized from the bone (Tsaih et al., 1999, 2001; Wang et al., 2019c). Given the fact that midlife women experience an increased bone turnover rate compared to women of other ages (Hernandez-Avila et al., 2000; Tsaih et al., 2001), the observed association could be attributed to in part to a greater mobilization of lead from bone into the circulation. However, it should be noted that urinary lead was not associated with HOMA-IR in the AENET model without BMI adjustment in the sensitivity analysis. Given recent findings reporting a positive association between lead exposure and BMI (Niehoff et al., 2020; Wang et al., 2018), the observed association between urinary lead and HOMA-IR in our primary analysis could also possibly reflect the over-adjustment bias considering BMI a possible intermediate. Thus, the interpretation of our result in the association between lead and HOMA-IR needs to be cautious and further work is necessary to confirm this association.

The evidence of underlying biological mechanisms linking other metal exposures to insulin resistance and β-cell dysfunction is limited. We found positive but not statistically significant association of urinary copper concentration with HOMA-IR in AENET, while this association was diminished without adjustment for baseline BMI in the sensitivity

analysis. Copper is also an essential element that is needed for multiple biological functions (ATSDR, 2004). However, long-term exposure to excess copper through environmental contamination has also shown to induce oxidative damages (Gaetke and Chow, 2003). In a study of diabetic mice, the treatment of a copper chelating agent was found to reduce insulin resistance and ameliorate glucose intolerance (Tanaka et al., 2009). We found molybdenum concentration was significantly inversely associated with HOMA-IR. A potential beneficial effect of molybdenum on insulin sensitivity is supported by an study of mice which showed the molybdenum treatment improved glucose tolerance, replenished glycogen stores, and corrected lipogenic enzyme gene expression (Tanju Özcelikay et al., 1996), likely through its insulin-like actions (Fillat et al., 1992). We observed a positive but not statistically significant association between urinary cobalt concentration and HOMA-β. Limited evidence suggested that cobalt may improve the insulin secretion profiles through its antioxidative effects (Vasudevan and McNeill, 2007).

Certain urinary metals were associated with HOMA at baseline but not related to rates of changes during the follow-up. These findings suggest that metals may exert their effects on insulin sensitivity and β-cell function even before the midlife for women. Growing evidence found that already in young adults, insulin resistance and β-cell dysfunction have been associated with adverse metabolic profiles and increased risk of T2DM (Elder et al., 2012; Würtz et al., 2012). Further studies are needed to confirm our findings in populations of younger ages. On the other hand, this finding does not necessarily mean that metal exposure has no impact on insulin sensitivity or β-cell function in postmenopausal women. Some metals found in urine samples may primarily reflect recent exposures. Thus, future studies with repeated metal measurements are warranted to examine whether metal exposures could have an impact on longitudinal rate of changes in HOMA measures.

The primary strength of our study is its utilization of a large prospective cohort with repeated HOMA measures over 16 years follow-up. The prospective design also minimized the possibility of reverse causation. Furthermore, we used a two-stage model, for the first time, to evaluate the association between metal mixtures and longitudinally measured quantitative outcomes

Several limitations should be considered as well. First, we measured all metal concentrations in urine and urinary concentrations may not unanimously reflect metals of forms and various exposure sources. Second, metals included in the current analysis have very different half-lives in the human body. Urinary concentrations of metals with short half-lives such as arsenic mainly reflect recent exposures. In contrast, metals such as cadmium are not rapidly excreted and have half-lives of years to decades. Information on the temporal variability of urinary metals concentrations, especially for those with short half-lives, is needed to better characterize cumulative metal exposures in future studies. Third, urinary metal concentrations could be influenced by renal clearance. We acknowledge that information on renal function is not available in SWAN although renal clearance is considered relatively stable in this age group (Murphy et al., 2016). Fourth, arsenic metabolism data were not available in our study. One recent prospective study found that urinary monomethylarsonate concentration was associated with higher HOMA-IR when either inorganic arsenic or dimethylarsinate concentration decreased (Grau-Perez et al., 2017). Additional

measurements of arsenic metabolism will be critical to providing a better understanding of arsenic exposures and associated health risks in our future studies. Fifth, in our study, urinary zinc was adjusted for dietary intake of zinc and zinc supplements in the regression analysis to better capture renal clearance and excretion of zinc. However, the dietary intake of other essential metals was not measured, and we were unable to distinguish between the metals from dietary sources (or other external sources) and the metals from internal sources. Sixth, metal-metal interactions were not considered in AENET when important metal components were selected. Exposure to metal mixtures with complex exposure profile may have additive, synergistic or antagonistic effects on the same adverse outcome (Wang et al., 2018). Given our sample size, adding the pairwise linear interaction terms in the AENET model might lead to problems including smoothing out the magnitude of exposures' effects, missing important variables, selection of spurious interaction effects and inflation of false positive results, particularly in presence of nonlinear interactions (Narisetty et al., 2019). Finally, associations between metal mixtures and HOMA measures were modeled in two separate models (baseline level and rate of change). Currently there is no statistical approach that can be used to study the relationship between longitudinal responses and chemical mixtures while addressing statistical challenges such as complex correlations and evaluation of the overall effects. Least absolute shrinkage and selection operator (LASSO) penalized linear mixed effects model is another shrinkage regression method designed for analyzing high-dimensional longitudinal data (Groll and Tutz, 2014). However, with correlated variables as predictors, LASSO tends to randomly select only one out of these correlated variables and ignore the others (Friedman et al., 2010). More updated statistical methods for mixture analysis that can also be used in high-dimensional longitudinal data analysis are needed for future studies.

## 5. Conclusions

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

## Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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## Abbreviations:

| | |
|---|---|
| **AENET** | adaptive elastic-net |
| **BKMR** | Bayesian Kernel Machine Regression |
| **BMI** | body mass index |
| **ERS** | environmental risk score |
| **ENET** | elastic-net |
| **FFQ** | food frequency questionnaire |
| **HOMA-IR** | homeostatic model assessments for insulin resistance |
| **HOMA-β** | homeostatic model assessments for β-cell function |
| **ICP-MS** | inductively coupled plasma-mass spectrometry |
| **LASSO** | least absolute shrinkage and selection operator |
| **LOD** | limit of detection |
| **SWAN** | Study of Women's Health Across the Nation |
| **SWAN-MPS** | Study of Women's Health Across the Nation Multi-Pollutant Substudy |

| | |
|---|---|
| **T2DM** | type 2 diabetes mellitus |

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## Highlights

- Urinary zinc was positively and molybdenum was inversely associated with HOMA-IR at baseline
- Urinary zinc was inversely associated with HOMA-β at baseline
- Urinary arsenic was associated with a faster rate of decline in HOMA-β
- Metal mixtures may play a role in insulin resistance and β-cell dysfunction

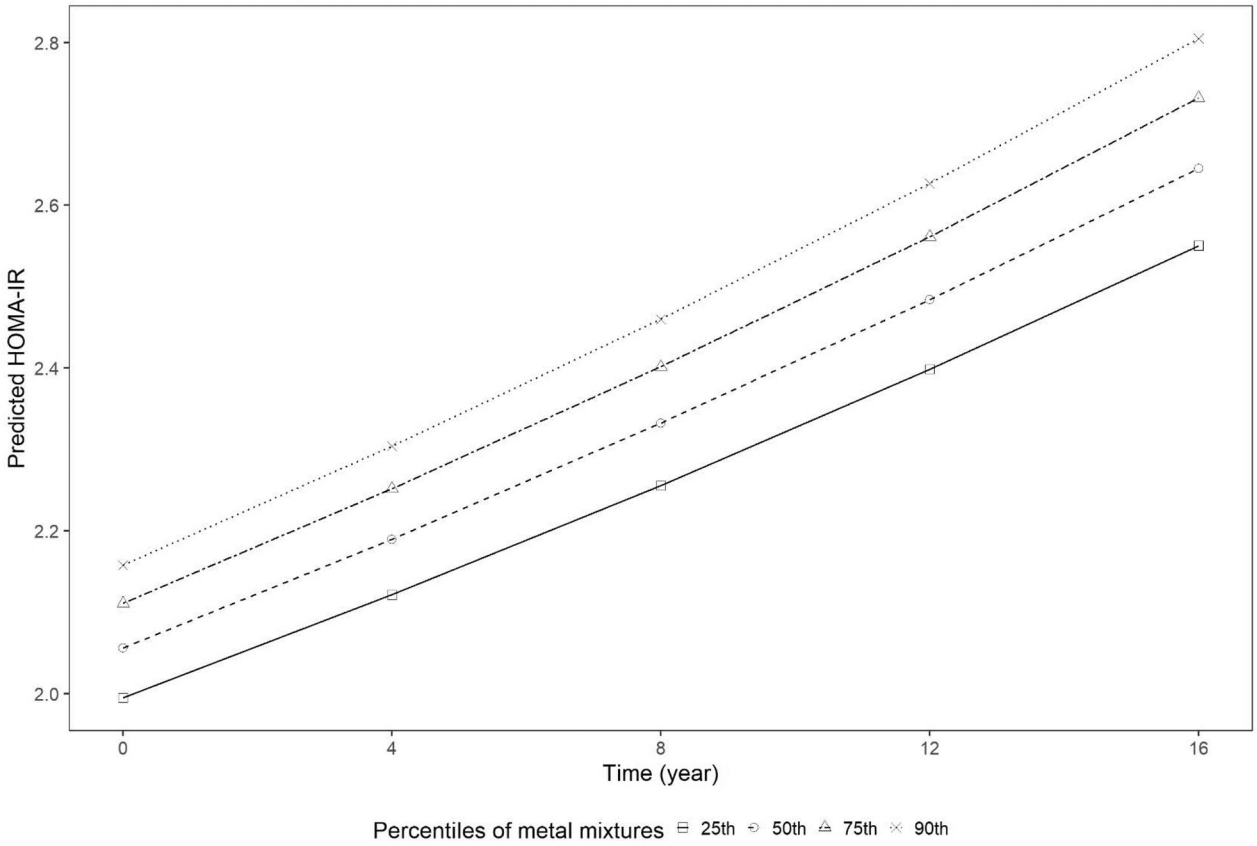


**Figure 1.**

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

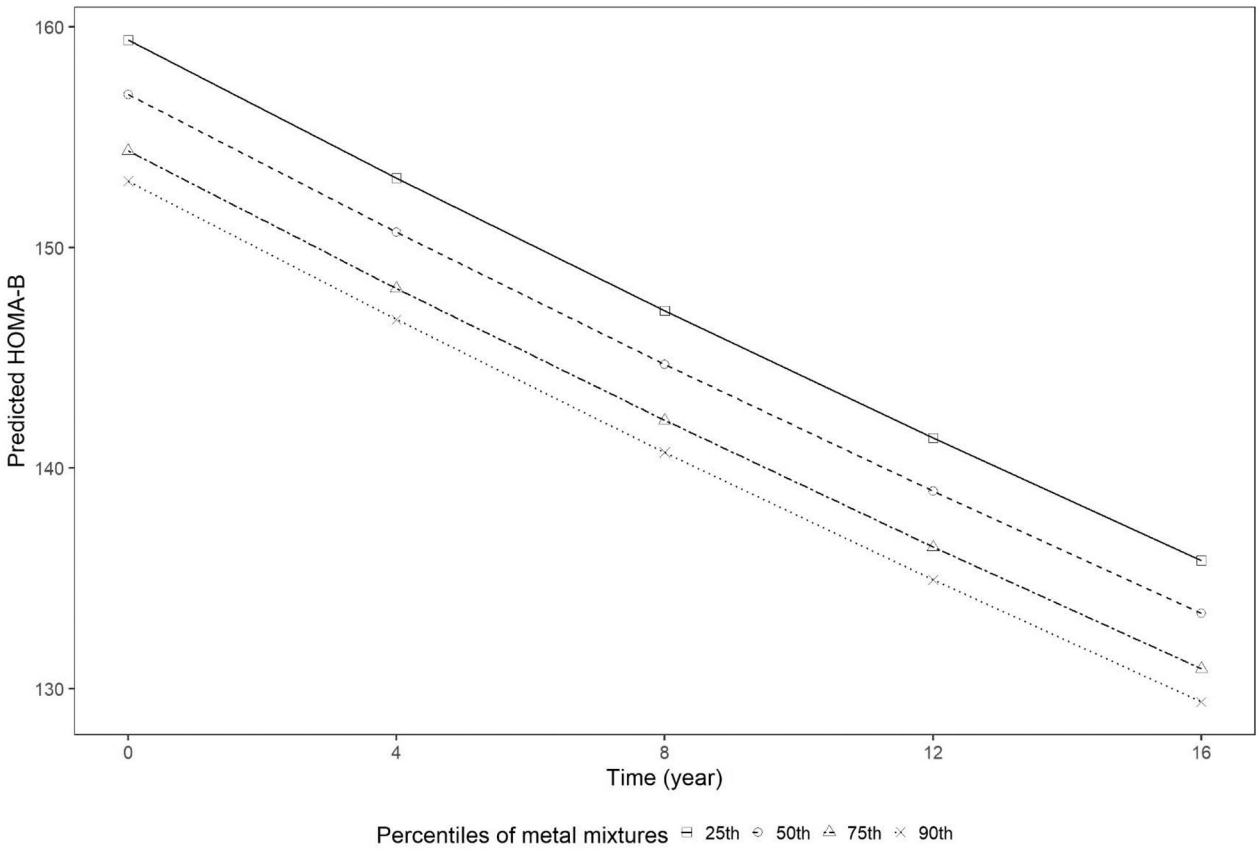


**Figure 2.**

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

**Table 1.**

Descriptive characteristics at the time of metal measurements stratified by median levels HOMA-IR and HOMA-β (n=1,262).

| Characteristics[a] | Total population (n=1,262) | HOMA-IR < median (1.8) at baseline (n=631) | HOMA-IR > median (1.8) at baseline (n=631) | HOMA-β < median (148.9) at baseline (n=631) | HOMA-β > median (148.9) at baseline (n=631) |
|---|---|---|---|---|---|
| Age, mean (SD), year | 49.7 (2.8) | 49.7 (2.7) | 49.7 (2.8) | 49.8 (2.8) | 49.6 (2.7) |
| Race/ethnicity, n (%) | | | | | |
| White | 645 (51.1) | 343 (54.4) | 302 (47.9) | 302 (47.9) | 343 (54.4) |
| Black | 254 (20.1) | 92 (14.6) | 162 (25.7) | 84 (13.3) | 170 (26.9) |
| Chinese | 166 (13.2) | 72 (11.4) | 94 (14.9) | 104 (16.5) | 62 (9.8) |
| Japanese | 197 (15.6) | 124 (19.7) | 73 (11.6) | 141 (22.3) | 56 (8.9) |
| Study site, n (%) | | | | | |
| Michigan | 218 (17.3) | 77 (12.2) | 141 (22.4) | 68 (10.8) | 150 (23.8) |
| Boston | 195 (15.5) | 105 (16.6) | 90 (14.3) | 83 (13.2) | 112 (17.8) |
| Oakland | 290 (23.0) | 125 (19.8) | 165 (26.2) | 166 (26.3) | 124 (19.6) |
| Los Angeles | 346 (27.4) | 216 (34.2) | 130 (20.6) | 209 (33.1) | 137 (21.7) |
| Pittsburgh | 213 (16.9) | 108 (17.1) | 105 (16.6) | 106 (16.6) | 108 (17.1) |
| BMI, mean (SD), kg/m$^2$ | 27.6 (7.0) | 24.4 (4.6) | 30.8 (7.6) | 25.8 (5.6) | 29.5 (7.8) |
| Education, n (%) | | | | | |
| High school or less | 214 (17.0) | 103 (16.3) | 111 (17.6) | 102 (16.2) | 112 (17.8) |
| Some College | 405 (32.1) | 171 (27.1) | 234 (37.1) | 191 (30.3) | 214 (33.9) |
| College and above | 643 (51.0) | 357 (56.6) | 286 (45.3) | 338 (53.6) | 305 (48.3) |
| Household income, n (%) | | | | | |
| Less than $19,999 | 74 (5.9) | 30 (4.8) | 44 (7.0) | 33 (5.2) | 41 (6.5) |
| $20,000 to 49,999 | 328 (26.0) | 153 (24.2) | 175 (27.7) | 143 (22.7) | 185 (29.3) |
| $50,000 to 99,999 | 528 (41.8) | 258 (40.9) | 270 (42.8) | 263 (41.7) | 265 (42.0) |
| $100,000 or more | 332 (26.3) | 190 (30.1) | 142 (22.5) | 192 (30.4) | 140 (22.2) |
| Smoking status, n (%) | | | | | |
| Never | 802 (63.6) | 414 (65.6) | 388 (61.5) | 408 (64.7) | 394 (62.4) |
| Former | 338 (26.8) | 163 (25.8) | 175 (27.7) | 170 (26.9) | 168 (26.6) |

| Characteristics[a] | Total population (n=1,262) | HOMA-IR < median (1.8) at baseline (n=631) | HOMA-IR > median (1.8) at baseline (n=631) | HOMA-β < median (148.9) at baseline (n=631) | HOMA-β > median (148.9) at baseline (n=631) |
|---|---|---|---|---|---|
| Current | 122 (9.7) | 54 (8.6) | 68 (10.8) | 53 (8.4) | 69 (10.9) |
| Alcohol drinking, n (%) | | | | | |
| Less than once per month | 655 (51.9) | 287 (45.5) | 368 (58.3) | 304 (48.2) | 351 (55.6) |
| Once per month | 298 (23.6) | 151 (23.9) | 147 (23.3) | 136 (21.5) | 162 (25.7) |
| Twice or more per month | 309 (24.5) | 193 (30.6) | 116 (18.4) | 191 (30.3) | 118 (18.7) |
| Physical activity score, mean (SD) | 7.8 (1.8) | 8.1 (1.8) | 7.5 (1.7) | 8.0 (1.7) | 7.7 (1.8) |
| Menopausal status, n (%) | | | | | |
| Pre-menopausal | 874 (69.3) | 442 (70.0) | 432 (68.5) | 462 (73.2) | 412 (65.3) |
| Post-menopausal | 191 (15.1) | 92 (14.6) | 99 (15.7) | 87 (13.8) | 104 (16.5) |
| Unknown[b] | 197 (15.6) | 97 (15.4) | 100 (15.8) | 83 (13.0) | 115 (18.2) |
| Hormone therapy, n (%) | 260 (20.6) | 133 (21.1) | 127 (20.1) | 111 (17.6) | |
| Total energy intake, mean (SD), kcal/d | 1819 (701) | 1800 (683) | 1838 (719) | 1844 (713) | 1795 (689) |
| Dietary seafood intake, mean (SD), times/week | 1.9 (1.6) | 1.9 (1.7) | 1.8 (1.6) | 2.0 (1.8) | 1.7 (1.5) |
| Dietary rice intake, mean (SD), times/week | 3.4 (3.3) | 3.5 (3.4) | 3.3 (3.3) | 3.9 (3.7) | 2.8 (2.9) |
| Total zinc intake, mean (SD), mg/day | 14.8 (10.7) | 14.6 (10.7) | 14.9 (10.8) | 14.8 (10.8) | 14.8 (10.8) |
| Fasting glucose level, GM(GSD), mg/dL | 86.8 (1.1) | 82.3 (1.1) | 91.7 (1.1) | 90.0 (1.1) | 83.5 (1.1) |
| Fasting insulin level, GM(GSD), uIU/mL | 9.5 (1.6) | 6.9 (1.2) | 13.6 (1.5) | 7.8 (1.4) | 11.8 (1.6) |
| HOMA-IR, GM(GSD) | 2.1 (1.7) | 1.3 (1.2) | 3.1 (1.6) | 1.7 (1.6) | 2.4 (1.8) |
| HOMA-β, GM(GSD) | 154.5 (1.6) | 133.0 (1.6) | 179.6 (1.6) | 106.7 (1.3) | 223.4 (1.4) |

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

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

**Table 2.**

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

| | **Selected metals in AENET**[a] | **SD for log-transformed urinary metal concentration** | **Percentage change in HOMA-IR**[b] **at baseline for 1-SD increase in log-transformed urinary metal concentration**[c] **(95% CI)** |
|---|---|---|---|
| Baseline HOMA-IR | Copper | 0.71 | 1.57% (−1.09%, 4.29%) |
| | Molybdenum | 0.83 | −3.25% (−5.45%, −1.00%) |
| | Lead | 0.85 | 0.70% (−1.59%, 3.05%) |
| | Zinc | 0.89 | 5.76% (3.05%, 8.55%) |
| Annualized rate of change in HOMA-IR[d] | **Selected metals in AENET** | | **Percentage change in annualized rate of change in HOMA-IR for 1-SD increase in log-transformed urinary metal concentration (95% CI)** |
| | Zinc | 0.89 | 0.06% (−0.03%, 0.15%) |

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

[b]HOMA-IR was log-transformed.

[c]All urinary metal concentrations were log-transformed and standardized.

[d]Average rate of change = 1.51% (95% CI: 1.41%, 1.61%).

**Table 3.**

The association of between environmental risk score (ERS) and HOMA insulin resistance (HOMA-IR) at baseline.

| ERS quartiles | Quartile 1 | Quartile 2 | Quartile 3 | Quartile 4 | P for trend |
|---|---|---|---|---|---|
| Percentage change (95% CI) in HOMA-IR[a] | Ref | 4.02% (−0.96%, 9.24%) | 6.91% (1.50%, 12.60%) | 15.70% (9.14%, 22.64%) | <0.0001 |

[a]Linear regression model was adjusted for age, race/ethnicity, study site, education level, annual household income, body mass index, smoking, alcohol drinking, physical activity score, menopausal status, hormone therapy, dietary intake of seafood and rice, total zinc intake from diets and supplements, total energy intake, and urinary specific gravity.

**Table 4.**

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

| | **Selected metals in AENET**[a] | **SD for log-transformed urinary metal concentration** | **Percentage change in HOMA-β**[b] **at baseline for 1-SD increase in log-transformed urinary metal concentration**[c] **(95% CI)** |
|---|---|---|---|
| Baseline HOMA-β | Arsenic | 1.26 | −1.59% (−3.63%, 0.50%) |
| | Cobalt | 0.82 | 2.22% (−0.10%, 4.60%) |
| | Zinc | 0.89 | −2.66% (−5.07%, −0.30%) |
| Annualized rate of change in HOMA-β[d] | **Selected metals in AENET** | | **Percentage change in annualized rate of change in HOMA-β for 1-SD increase in log-transformed urinary metal concentration (95% CI)** |
| | Arsenic | 1.26 | −0.02% (−0.05%, 0%) |

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

[b]HOMA-β was log-transformed.

[c]All urinary metal concentrations were log-transformed and standardized.

[d]Average rate of change = −1.00% (95% CI: −1.02%, −0.90%).

**Table 5.**

The association of between environmental risk score (ERS) and HOMA β-cell function (HOMA-β) at baseline.

| ERS quartiles | Quartile 1 | Quartile 2 | Quartile 3 | Quartile 4 | P for trend |
|---|---|---|---|---|---|
| Percentage change (95% CI) in HOMA-β[a] | −8.96% (−13.89%, −3.77%) | −5.99% (−10.72%, −1.00%) | −7.97% (−12.40%, −3.31%) | Ref | <0.0001 |

[a]Linear regression model was adjusted for age, race/ethnicity, study site, education level, annual household income, body mass index, smoking, alcohol drinking, physical activity score, menopausal status, hormone therapy, dietary intake of seafood and rice, total zinc intake from diets and supplements, total energy intake, and urinary specific gravity.