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Toenail metal concentrations and age at menopause

A prospective study

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Background: Menopause timing is related to cancer, cardiovascular disease, and mortality. Lead has been associated with an earlier age at menopause, but no study has considered exposure to other metals or multiple metals simultaneously.

Methods: At baseline, we measured toenail concentrations of 16 metals for 903 premenopausal women in the Sister Study (2003–2009). Age at menopause was ascertained through follow-up questionnaires. Cox proportional hazard models were used to estimate hazard ratios (HRs) and 95% confidence intervals (CIs) for the associations between individual metals and age at menopause. We used quantile-g-computation to examine the association between age at menopause and the joint effect of a simultaneous increase in (1) all metals and for subgroups of metals categorized as (2) essential or (3) non-essential.

Results: For individual metals, we observed negligible associations except for an interquartile range increase in lead which was modestly associated with an earlier age at menopause (HR = 1.03, 95% CI = 1.01, 1.05). In the mixture analyses, a quartile increase in all metals was associated with a later age at menopause (HR = 0.81, 95% CI = 0.64, 1.02). The metals with the largest negative contributions (i.e., associated with a later age at menopause) were chromium and nickel. The joint effect for the essential metals remained inverse (HR = 0.83, 95% CI = 0.64, 1.07), but was attenuated for nonessential metals (HR = 0.98, 95% CI = 0.76, 1.24).

Conclusions: Although no individual metal was strongly associated with age at menopause, our joint effect analysis suggests that having low levels of essential metals could be associated with an earlier age at menopause.

Key Words: nails, menopause, chromium, nickel, female

Introduction

Earlier age at menopause is associated with reduced fertility and a higher risk of cardiovascular disease and death[1,2] and a lower risk of breast, endometrial, and ovarian cancer.[3–5] Thus, the length of the reproductive lifespan is an important factor in a woman's later-life health. Loss of ovarian function and decreases in endogenous estrogen production that occur with menopause are plausible biologic mechanisms by which timing of menopause may influence these health outcomes.[6,7] Achieving a better understanding of how environmental factors may be related to timing of menopause could provide clues for potential biologic mechanisms underlying associations between environmental chemicals and chronic disease.

Metals are one plausible group of environmental chemicals that may influence the timing of menopause. There is evidence that some metals, such as cadmium and lead, have endocrine-disrupting properties[8–10] and that lead in particular may act as a reproductive toxicant.[11] Previous epidemiologic research on metals and menopause has been focused on exposure to lead. An earlier age at menopause was observed in a small population occupationally exposed to lead from smelting compared with similar aged women without occupational exposure.[12] Higher levels of lead measured in the bone were associated with earlier menopause in a subpopulation of the Nurses' Health Study cohort[13] and similar associations were observed for blood lead in the National Health and Nutrition Examination Survey.[14]

Multiple metals can arise from similar sources (e.g., smoking, occupational exposures, dietary sources), which may partly

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What this study adds

Previous research on metals and age at menopause has focused on cross-sectional studies of lead. In this prospective study, we evaluate a large panel of toenail metals in relation to age at menopause and use a novel time-to-event extension of quantile-g-computation to evaluate joint effects. Although no individual metal strongly contributed to menopause timing, a quartile increase in the overall metal mixture was associated with a 2 year later age at menopause and appeared to be driven by essential metals. These results suggest that low levels of essential metals may be associated with an earlier age at menopause.

explain correlation of multiple metals within individuals.[15] However, previous studies have not considered the association with menopause given these coexposure patterns which could result in residual confounding. Metals also have diverse biologic functions and may be expected to have differing associations with the timing of menopause; some are essential (e.g., cobalt, copper, nickel, and chromium) and are necessary for normal physiologic function whereas others are considered nonessential and are often referred to as being "toxic" (e.g., lead, arsenic, cadmium, and mercury).[16]

The aim of this prospective study was to evaluate whether toenail metal and/or trace element concentrations (antimony, arsenic, cadmium, chromium, cobalt, copper, iron, lead, mercury, manganese, molybdenum, nickel, selenium, tin, vanadium, and zinc, to be henceforth referred to as metals), individually or in combination, were associated with age at menopause. We hypothesized that women with higher exposure to metals, specifically nonessential metals such as cadmium and lead, would tend to have an earlier age at menopause.

Methods

Study population

The Sister Study is a prospective cohort of 50,884 women designed to identify environmental and lifestyle risk factors for breast cancer.[17] Study participants were enrolled from 2003 to 2009; to be eligible, they had to be between the ages of 35 to 74, reside in the United States or Puerto Rico, have a sister who had been previously diagnosed with breast cancer and have no history of breast cancer themselves.

At the time of study enrollment, participants completed extensive questionnaires on their demographics, lifestyle factors, and medical history including information on reproductive history and menopausal status. The women completed a home visit with a trained examiner who collected biospecimens and conducted anthropometric measurements including height and weight to calculate body mass index (BMI). Women were asked to remove their nail polish and provide clippings from each toenail. Participants complete annual health updates and more extensive follow-up questionnaires to assess changes in their health including menopausal status. Response rates have remained at approximately 90% for each update.[18] Data used were from Release 6.0.

Metal concentrations were assessed for 2,617 women with toenail samples collected at baseline who were part of a subset of participants in a sister-matched case-control study of young-onset breast cancer.[15] Women were eligible for the current study of metals and age at menopause if they were premenopausal at baseline, had no history of hysterectomy or oophorectomy, and had no prior diagnosis of cancer other than nonmelanoma skin cancer (n = 907).

Exposure assessment: toenail metal concentrations

We used inductively coupled plasma mass spectrometry (ICP-MS) to assess baseline toenail concentrations for a panel of 16 trace elements (antimony, arsenic, cadmium, chromium, cobalt, copper, iron, lead, mercury, manganese, molybdenum, nickel, selenium, tin, vanadium, and zinc). As previously described,[15,19] the two big toenails were washed, air-dried, and then digested in acid (9:1 HNO_3/HCL) and diluted with deionized water. ICP-MS analyses were conducted using Agilent 8800 ICP-QQQ (Santa Clara, CA). Quality control was conducted using continuous calibration verification, duplicate and spiked samples, within- and between-batch analyses of a laboratory-prepared toenail matrix digest, and comparison with a standard reference material (Japan NIES #13 Hair). Random effect models were used to correct for sample batch. If a measured value was available despite being below the limit of quantification that value was used in the analysis. After batch correction, a small number of metal measurements (<0.1%) were negative. For some calculations that required positive values (e.g., log transformations), these values were imputed as a small positive value (0.001) but were otherwise left negative.

Outcome assessment: age at menopause

Participants provided updated information on menopause approximately annually at the time of each follow-up questionnaire. Age at menopause was defined as the self-reported age at which they had not had a menstrual cycle in the previous 12 months and were previously uncensored. Women were considered censored at the earliest occurrence of death, incident cancer (other than non-malignant melanoma), oophorectomy, hysterectomy, age 55, or the end of follow up. Age 55 is considered late age at menopause; approximately 80% of women will have undergone menopause by age 55.[20]

Statistical analysis

We examined univariate characteristics of the metals and covariates. For the metals, we examined univariate histogram distributions and calculated a Spearman correlation matrix.

To model associations between individual metal concentrations measured in toenails collected at baseline and age at menopause, we fit confounder-adjusted Cox proportional hazards models to estimate the hazard ratio for a unit increase of concentration. The timescale of interest for our study was age and the target parameters were the hazard ratios comparing the hazard of incident menopause at one unit increments of exposure or contrasting discrete levels of exposure. The origin of the time scale was age 35 but follow-up for each woman began at her study baseline age, and we considered metals exposure at that baseline age as the exposure of interest. We examined fits in the continuous model with both untransformed exposure as well as with natural-log-transformed exposure, to explore the impacts of scale changes on inferences. We allowed for the potential for nonlinearity of the hazard ratios by characterizing each exposure into categories defined by quartiles. We performed a global test of the proportional hazards assumption for the underlying fit via a global test of a time trend in Schoenfeld residuals.[21]

All exposures and covariates (except for current age and menopause status) were based on their value at baseline. Covariates were selected to comprise a minimally sufficient set of confounders based on a directed acyclic graph (eFigure 1; http://links.lww.com/EE/A94) and included baseline measurements of BMI (linear, standardized by mean-centering and dividing by the standard deviation), smoking (current/former/never), race and ethnicity (non-Hispanic White, non-Hispanic black, Hispanic, other), education status (<high school or high school degree, some college, bachelor's degree, or more than a bachelor's degree), and parity (linear, number of children). We excluded participants who were menopausal at the end of follow-up but missing information on age at menopause, metals measurement, race/ethnicity, or BMI (N = 5), resulting in a final sample size of 902 women.

Our primary interest in the analysis was in assessing the impact of the exposure "mixture" on the age at incident natural menopause. As previously described in detail,[22] quantile-g-computation is a parametric, generalized-linear-model–based approach that uses a basic implementation of g-computation[23] to estimate a mixture effect, which is defined as increasing all exposures in the mixture by a single quantile.[22] This approach provides a valid estimate of the overall mixture effect as well as weights that indicate the contribution of the individual components of the mixture to the overall estimate. For the current analysis, we used a novel application of this method for survival analysis. This was implemented by categorizing all metals in quartiles,

X_j^q, and fitting an adjusted Cox proportional hazards model $\lambda(t, \boldsymbol{X}, \boldsymbol{Z}) = \lambda_0(t)\exp(\boldsymbol{\alpha Z} + \sum_{j=1}^{p} \beta_j X_j^q)$, where $\lambda_0(t)$ represents the (unspecified) hazard at the referent level of all covariates at time t, $\boldsymbol{\alpha}$ represents log-hazard ratios for confounders $\boldsymbol{Z}$ and $\sum_{j=1}^{p} \beta_j = \psi$, which is interpreted as the change in the log-hazard of menopause for a one-unit change in the p exposures comprising the mixture of interest. If all coefficients are in the same direction, then we can express the model as $\lambda_0(t)\exp(\boldsymbol{\alpha Z} + \psi \sum_{j=1}^{p} w_j X_j^q)$, where each metal is given either a weight $w_j = \beta_j / \sum_{j=1}^{p} \beta_j$ corresponding to the proportion of the total effect due to that metal. When model coefficients for the exposures are not in the same direction, then there are two sets of weights that yield the proportion of the positive (or negative) "partial effect" of all exposures with positive (or negative) coefficients, but the interpretation of ψ is unchanged. Confidence intervals for ψ are estimated via the delta method. We used results from the quantile-g-computation fit to generate survival curves for the time to menopause using Efron's estimator of the baseline hazard.[24]

We examined three mixture groups: all metals combined, nonessential metals (antimony, lead, mercury, cadmium, arsenic, tin, vanadium), and essential metals (molybdenum, selenium, zinc, manganese, copper, nickel, iron, cobalt, chromium). The classifications for nonessential and essential groupings were selected a priori based on the presence or absence of a known or suspected physiologic role for each metal.[16] When the overall mixture effect for specific subgroups was estimated in the quantile-g-computation model, the metals not included as part of the subgroup of interest were retained in the model similar to confounders and thus overall mixture effect for the subgroup is adjusted for all of the components of the metal mixture.

Our approach to assessing nonlinearity of the mixture was to contrast 2 models: (1) all exposures are entered as both linear and quadratic terms and the overall effect is allowed to be quadratic versus (2) all exposures are entered as linear terms and the overall effect is assumed linear. An overall assessment of nonlinearity is then given by the quadratic term coefficient in the overall model. Confidence intervals for the nonlinear model were estimated via a nonparametric bootstrap with 1,000 samples. This approach can be underpowered when there are few truly nonlinear terms, but it is less subject to over-fitting and issues of multiple comparisons that would arise if assessing nonlinearity on an exposure-by-exposure basis. We assessed the impact of using quartiles to categorize the exposure by fitting additional quantile-g-computation models where the number of quantiles was varied from 2 to 20. We assessed potential sensitivity to reverse causation from measuring metals during perimenopause using a sensitivity analysis where we restricted the analysis to participants who were younger at baseline (i.e., those below the 90th or 50th percentile of age at baseline). Because iron concentrations may be influenced by menstrual cycling and thus possibly increased or decreased by perimenopausal status, we performed an additional sensitivity analysis excluding iron from the mixture of interest. We also conducted a sensitivity analysis without censoring at age 55.

Results

Based on a Kaplan-Meier estimator, the median age of natural menopause in the study population was 52.4 years. The median time from toenail sample donation to menopause was 10.3 years. We observed that 344 (38%) of the women in the study reported natural menopause before a censoring event and the Kaplan-Meier estimator of natural menopause by age 55 years was 77%. This study population was predominately non-Hispanic white (90%), most had at least a college degree (64%), and were never smokers (69%; Table 1). The average examiner-measured BMI at baseline was 26.5 kg/m^2. Toenail metal median concentrations and quartile cut-points are displayed in eTable 1; http://links.lww.com/EE/A94. Zinc had the highest median concentration (103.6 μg/g), whereas cadmium, cobalt, molybdenum, and vanadium had the lowest median concentration (0.01 μg/g). The correlation of metals concentrations varied across the metals, ranging from −0.07 to 0.72 (eFigure 2; http://links.lww.com/EE/A94). There was no statistically significant deviation from proportional hazards (P = 0.46).

When we considered each metal individually in relation to age at menopause, we observed associations that were close to null across almost all the metals (Table 2). For an interquartile range (IQR) increase in lead, we observed a modest association with an earlier age at menopause (HR = 1.03, 95% CI = 1.01, 1.05). These associations did not substantially change with adjustment for confounders or by changes in exposure scale.

Using quantile-g-computation, increasing all metals by one quartile was associated with a later age at menopause (HR = 0.81, 95% CI = 0.64, 1.02; Table 3). As expected, the metals did not have coefficients that were consistently in the same direction (Figure 1, eTable 2; http://links.lww.com/EE/A94). Cobalt, cadmium, antimony, molybdenum, and arsenic had positive weights, indicating an association with an earlier age at menopause. The metals with the largest positive weights were cobalt (0.50) and cadmium (0.22). The remaining 12 metals had negative weights, indicating associations with a later age at menopause, with the strongest negative weights for nickel (0.20), chromium (0.18), and vanadium (0.13). Lead, which was the only metal associated with age at menopause in the individual metal models, showed little evidence of an association in the overall mixture model (0.03). Individual exponentiated β coefficients for a quartile increase in each metal, holding all other metals constant, as estimated from quantile-g-computation are provided in eTable 2; http://links.lww.com/EE/A94. When we estimated the overall mixture effect for the nonessential and essential mixture groups separately, we observed that the point estimate for the essential metal mixture remained inverse (HR = 0.83, 95% CI = 0.64, 1.07), whereas the HR for the nonessential metal mixture was close to 1.0 (HR = 0.98, 95% CI = 0.76, 1.24), although there was substantial overlap in the confidence intervals. The weights (relative sizes of coefficients, among those with the same sign) for the nonessential and essential metal groups are provided in eFigures 3 and 4; http://links.lww.com/EE/A94; they have

Table 1.

Study participant characteristics at baseline (N = 902), Sister Study, 2003–2009.

	N (%)	Mean (SD)
Age at toenail collection		44.07 (4.43)
BMI		26.54 (6.12)
Parity		1.73 (1.25)
Smoking		
Never smoked	624 (69)	
Past smoker	211 (23)	
Current smoker	67 (7)	
Race/ethnicity		
Non-Hispanic White	809 (90)	
Non-Hispanic Black	39 (4)	
Hispanic	28 (3)	
Other	26 (3)	
Highest education achieved		
High school diploma or less	78 (9)	
Some college	243 (27)	
Bachelor's degree	336 (37)	
Master's or doctoral degree	245 (27)	

SD indicates standard deviation.

Table 2.

HRs and 95% CIs for the associations between individual metals and age at natural menopause.

	HR (95% CI)			
	Log transformed		IQR scaled	
	Crude	Adjusted[a]	Crude	Adjusted[a]
Antimony	0.93 (0.83, 1.05)	0.97 (0.86, 1.09)	1.00 (0.97, 1.02)	1.00 (0.98, 1.02)
Arsenic	0.99 (0.83, 1.18)	1.00 (0.84, 1.20)	0.99 (0.97, 1.02)	0.99 (0.97, 1.02)
Cadmium	1.01 (0.90, 1.13)	0.99 (0.88, 1.11)	1.01 (0.99, 1.03)	1.00 (0.98, 1.02)
Chromium	0.96 (0.88, 1.04)	0.95 (0.87, 1.03)	1.02 (0.98, 1.06)	1.01 (0.97, 1.05)
Cobalt	1.04 (0.93, 1.17)	1.03 (0.92, 1.16)	1.01 (1.00, 1.03)	1.00 (0.99, 1.02)
Copper	0.97 (0.70, 1.34)	0.96 (0.69, 1.34)	1.02 (0.96, 1.09)	1.03 (0.96, 1.10)
Iron	0.92 (0.80, 1.06)	0.93 (0.81, 1.08)	0.97 (0.93, 1.01)	0.98 (0.93, 1.02)
Lead	1.02 (0.92, 1.13)	1.01 (0.91, 1.13)	1.03 (1.00, 1.05)	1.03 (1.01, 1.05)
Mercury	0.98 (0.90, 1.06)	0.97 (0.89, 1.06)	0.98 (0.90, 1.08)	0.97 (0.88, 1.06)
Magnesium	0.98 (0.88, 1.10)	1.00 (0.89, 1.12)	1.01 (0.98, 1.04)	1.02 (0.99, 1.04)
Molybdenum	0.96 (0.84, 1.11)	0.98 (0.86, 1.13)	1.01 (0.99, 1.02)	1.01 (0.99, 1.02)
Nickel	0.96 (0.90, 1.02)	0.95 (0.89, 1.01)	1.00 (0.99, 1.00)	1.00 (0.99, 1.00)
Selenium	0.82 (0.47, 1.44)	0.96 (0.54, 1.73)	0.98 (0.88, 1.11)	1.01 (0.91, 1.13)
Tin	0.98 (0.88, 1.10)	0.98 (0.87, 1.09)	1.02 (0.98, 1.07)	1.02 (0.97, 1.06)
Vanadium	0.97 (0.90, 1.06)	0.99 (0.91, 1.08)	0.96 (0.89, 1.03)	0.97 (0.90, 1.04)
Zinc	0.97 (0.60, 1.54)	0.87 (0.54, 1.41)	0.99 (0.94, 1.06)	0.98 (0.92, 1.05)

[a]Adjusted for BMI, smoking, race, education, and parity.

Table 3.

HRs and 95% CIs for the association between increasing all of the metals by a quartile within the (1) overall metal mixture, (2) the nonessential, and (3) the essential metal mixture in relation to age at menopause

	HR (95% CI)[a]
All metals	0.81 (0.64, 1.02)
Nonessential[b]	0.98 (0.76, 1.24)
Essential[c]	0.83 (0.64, 1.07)

[a]Adjusted for BMI, smoking, race, education, and parity.

[b]Nonessential includes antimony, lead, mercury, cadmium, arsenic, tin, vanadium.

[c]Essential includes molybdenum, selenium, zinc, manganese, copper, nickel, iron, cobalt, chromium.

similar patterns to the weights provided for the overall metal mixture. The corresponding exponentiated coefficients are those provided in eTable 2; http://links.lww.com/EE/A94.

We observed little change in our overall metal mixture estimate when iron was excluded (eTable 3; http://links.lww.com/EE/A94). These results did not appear to be strongly influenced by age at baseline: excluding the oldest women (>90th percentile) in our population, those who would have been most likely to be in perimenopause at the time of sample collection did not alter our overall conclusions (eTable 4; http://links.lww.com/EE/A94). However, we did note a strong, but imprecise, positive association among the overall metal mixture and age at menopause among those women >90th percentile in age (HR = 1.62, 95% CI = 0.83, 3.15). Varying the number of quantiles used to characterize the metal concentrations also did not impact the overall mixture estimate (eTable 5; http://links.lww.com/EE/A94). Allowing for a nonlinear association between individual exposures, as well as the overall mixture, in relation to age at menopause demonstrated a similar inverse association with age at menopause, and there was very weak evidence of nonlinearity in the overall mixture effect (eTable 6; http://links.lww.com/EE/A94). The survival curves generated under the two models were nearly identical, suggesting that nonlinearity, even if present, does not change the interpretation of our results.

Results generated under the quantile-g-computation suggest that the absolute risk of menopause by age 55 is approximately 20% lower among those exposed in the highest quartile versus those exposed in the lowest and equates approximately to a 2-year delay in the age at menopause (eFigure 5; http://links.lww.com/EE/A94). Results for the overall mixture remained very similar without censoring at age 55 (HR = 0.84, 95% CI = 0.67, 1.04).

Discussion

In this prospective study of toenail metal concentrations in relation to age at natural menopause, we estimated that a simultaneous increase in all metals by a quartile was associated with a 2-years later age at menopause. The individual metals within the mixture had both inverse and positive associations with menopausal age. In the mixture analysis, chromium and nickel had the largest negative weights indicating an association with a later age at menopause, whereas cobalt and cadmium had the largest positive weights, indicating an association with an earlier age at menopause. The overall inverse association between the metal mixture and age at menopause persisted when we limited the mixture to the essential metals, but was not evident for the nonessential metals. This suggests that the observed inverse overall mixture effect may be largely driven by the essential metals, suggesting that deficiencies among them may cause early menopause. When evaluated in individual statistical models (not controlling for other metals), there was little evidence that individual metals were related to age at menopause.

Previous research on associations with metal biomarkers in relation to age at menopause has been focused on exposure to lead, due to its established reproductive toxicity.[11] Prior studies have included an occupational cohort exposed to very high levels from smelting,[12] a cross-sectional examination of blood lead levels in National Health and Nutrition Examination Survey,[14] and a study of bone lead for which measurements were largely conducted after menopause.[13] These studies all concluded that higher biomarker levels of lead were related to an earlier age at menopause. Our individual metal analysis suggested a small association between lead and earlier menopause. However, this association with lead was not evident in the mixture analysis that used exposure quantiles to reduce the influence of outliers, nor in our analysis in which lead was log-transformed, suggesting that the association was driven by women in the high lead exposure range. Because the previous studies by Mendola et al[14] and Eum et al[13] were cross-sectional, reverse-causation is possible, as measured lead levels may have been influenced by postmenopausal releases of bone lead.[25] In our sensitivity analysis that restricted to the oldest women in our sample who would have been closest to the menopause transition at baseline (>90th

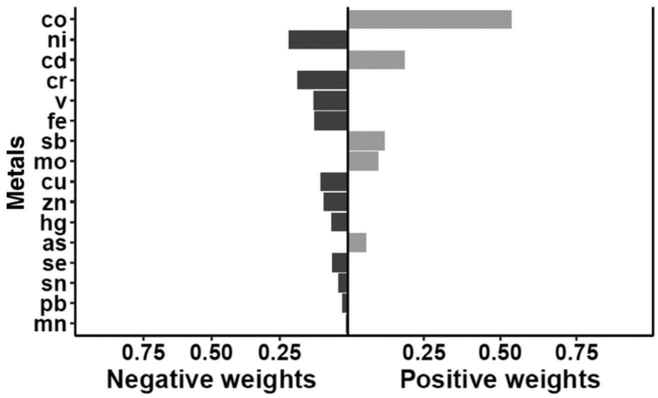

Figure 1. Weights for the metal mixture in relation to age at menopause.

percentile for age at baseline), we observed a strong association between the overall metal mixture and an earlier age at menopause. This association in our oldest premenopausal women is consistent with the cross-sectional results and suggests that reverse causality could be playing a role. We prospectively ascertained information regarding age at menopause multiple times over follow-up, requiring women to have ceased menses for a full 12 months before being considered postmenopausal. In a cross-sectional study design, age at menopause is difficult to precisely define, as women may be in early menopause and have not yet reached the 12-month threshold at the time of data collection.

The overall mixture effect appeared to be spread across a number of predominately essential metals with modest negative effects, indicating no individual metal was a strong driver of the association. Previous studies have suggested that use of vitamin and mineral supplements[26] and dietary factors including consumption of legumes[27] are related to a later age at menopause. Vitamin and mineral supplements contain numerous essential trace elements and legumes are a source of elements including nickel, iron, and zinc.[28,29] Therefore, the findings observed here in our mixture analyses suggest that intake of essential elements in the diet or via supplementation may influence the timing of menopause.

We observed a positive weights in the quantile-g-computation model for cobalt and cadmium, indicative of an associations with earlier age at menopause. These findings for cadmium are consistent with prior research on smoking,[30] a primary source of cadmium exposure.[31] However, even with adjustment for smoking status, cadmium remained positively weighted. These findings are consistent with a prior study that observed cadmium was associated with lower anti-Müllerian hormone levels, which are predictive of an earlier age at menopause.[32] Cobalt also was given a positive weight but has been less well-studied; although similar to cadmium, cobalt has endocrine-disrupting properties including binding to estrogen receptor (ER)-α.[8] However, these results for the positively-weighted metals should be interpreted with caution as the coefficients for individual metals were imprecise, and the magnitude of the positive and negative weights cannot be directly compared (though the coefficients in eTable 2; http://links.lww.com/EE/A94, on which they are based can be).

An important strength of this study was the evaluation of the joint impact of exposure to multiple metals using a novel application of quantile-g-computation in a survival analysis context. We were interested in the overall mixture effect as it is important to characterize the overall effect of combined chemical exposures, to evaluate the total body burden of environmental chemicals.[33] This method was selected for this study as our research goal was to evaluate the joint effects of exposures in a mixture, regardless of whether individual exposures are beneficial or harmful. Given the bidirectional associations observed in our study, the use of a statistical method that can simultaneously estimate both positive and negative weights for the overall mixture effect was an important strength. Other available approaches to estimate the overall mixture effect, such as weighted quantile sum (WQS), require a directional homogeneity assumption to estimate the overall mixture effect.[22] Utilizing an analytic method to account for the mixture was important, as it permitted us to observe an association that would have otherwise been missed had we simply evaluated each metal individually.

This study is the first to consider toenail metal concentrations in relation to age at menopause. Toenail metal concentrations are a promising noninvasive biomarker for metal concentrations and are estimated to reflect a 4- to 6-month window of exposure in the past 6–12 months.[34,35] These markers have been previously found to correlate well within individuals over a period of months[36] and years,[15] suggesting that they may be an adequate proxy for long-term exposure. In the Normative Aging Study, the correlation of toenail lead concentrations over time was higher than that for blood lead.[37] Additionally, we have previously shown that toenail cadmium levels correlate with smoking status,[36] a primary source of cadmium exposure.[31] However, we only had toenail metals assessed a single time point, and thus, we cannot know to what extent these measures may represent long-term exposure levels.

Women in our study who had a hysterectomy were censored from our analysis, because they were no longer at risk for natural menopause. Our analysis assumes that the timing of hysterectomy and when natural menopause would have occurred are not related through factors not included in our model. Similarly, because the natural time-scale for studying menopause is age, for women who had baseline age greater than 35 (the origin for our analysis), we also assumed that among premenopausal women age at entry was not related to the timing of menopause (e.g., via effects of birth cohort).[22,38]

It is unclear what the relevant time period is for which environmental exposures may impact when a woman undergoes menopause. Previous studies of smoking and menopause have found that current smokers have an earlier age at menopause than former smokers, suggesting that recent or ongoing exposure during the menopausal transition is relevant.[30,39] However, we cannot rule out the possibility that exposure to metals earlier in life during potentially critical biological windows may also influence age at menopause.

A strength of our study was the use of a biologic measure that represents relatively recent exposure patterns and levels (2003–2009), which is important because exposure in the general population to nonessential and potentially toxic metals such as lead has declined substantially over time.[15,40] Women in our study had lower toenail concentrations of certain nonessential metals (median As 0.05 μg/g, Pb 0.11 μg/g, Hg 0.10 μg/g) compared with those observed in the Normative Aging study (median As 0.07 μg/g, Pb 0.32 μg/g, Hg 0.25 μg/g)[37] and in a New Hampshire case-control study (mean As 0.10 μg/g).[41] The findings presented here for the overall mixture may not be generalizable to other populations with different metal concentrations and coexposure patterns.

Overall, we found little evidence that nonessential metals, such as lead, which has been previously associated with age at menopause, were related to an earlier age at menopause in our sample. In contrast, our results suggest that increasing exposure to the overall metal mixture was associated with an approximately 2 years later age at menopause, although we did not identify individual metals that were strong drivers of this association. Rather, it appeared that this association was due to small contributions across a number of essential metals. This suggests that low levels of essential metals may be related to an earlier age at menopause. Future study on the role of those essential metals and their potential impact on age at menopause is warranted.

Conflicts of interest statement

The authors declare that they have no conflicts of interest with regard to the content of this report.

References

1. Bellavia A, Wolk A, Orsini N. Differences in age at death according to smoking and age at menopause. *Menopause*. 2016;23:108–110.
2. Ley SH, Li Y, Tobias DK, et al. Duration of reproductive life span, age at menarche, and age at menopause are associated with risk of cardiovascular disease in women. *J Am Heart Assoc*. 2017;6:006713.
3. Anderson KN, Schwab RB, Martinez ME. Reproductive risk factors and breast cancer subtypes: a review of the literature. *Breast Cancer Res Treat*. 2014;144:1–10.
4. Dossus L, Allen N, Kaaks R, et al. Reproductive risk factors and endometrial cancer: the European Prospective Investigation into Cancer and Nutrition. *Int J Cancer*. 2010;127:442–451.
5. Wentzensen N, Poole EM, Trabert B, et al. Ovarian cancer risk factors by histologic subtype: an analysis from the ovarian cancer cohort consortium. *J Clin Oncol*. 2016;34:2888–2898.
6. Graaf YVD, de Kleijn MJJ, van der Schouw YT. Menopause and cardiovascular disease. *J Psychosom Obstet Gynecol*. 1997;18:113–120.
7. Kelsey JL, Gammon MD, John EM. Reproductive factors and breast cancer. *Epidemiol Rev*. 1993;15:36–47.
8. Byrne C, Divekar SD, Storchan GB, Parodi DA, Martin MB. Metals and breast cancer. *J Mammary Gland Biol Neoplasia*. 2013;18:63–73.
9. Choe S-Y, Kim S-J, Kim H-G, et al. Evaluation of estrogenicity of major heavy metals. *Sci Total Environ*. 2003;312:15–21.
10. Divekar SD, Li H-H, Parodi DA, et al. Arsenite and cadmium promote the development of mammary tumors. *Carcinogenesis*. 2019:bgz176. [Epub ahead of print]
11. Mendola P, Messer LC, Rappazzo K. Science linking environmental contaminant exposures with fertility and reproductive health impacts in the adult female. *Fertil Steril*. 2008;89(2 suppl):e81–e94.
12. Popovic M, McNeill FE, Chettle DR, Webber CE, Lee CV, Kaye WE. Impact of occupational exposure on lead levels in women. *Environ Health Perspect*. 2005;113:478–484.
13. Eum KD, Weisskopf MG, Nie LH, Hu H, Korrick SA. Cumulative lead exposure and age at menopause in the Nurses' Health Study cohort. *Environ Health Perspect*. 2014;122:229–234.
14. Mendola P, Brett K, Dibari JN, Pollack AZ, Tandon R, Shenassa ED. Menopause and lead body burden among US women aged 45-55, NHANES 1999-2010. *Environ Res*. 2013;121:110–113.
15. O'Brien KM, White AJ, Sandler DP, Jackson BP, Karagas MR, Weinberg CR. Do post-breast cancer diagnosis toenail trace element concentrations reflect prediagnostic concentrations? *Epidemiology*. 2019;30:112–119.
16. Tchounwou PB, Yedjou CG, Patlolla AK, Sutton DJ. Heavy metals toxicity and the environment. *EXS*. 2012;101:133–164.
17. Sandler DP, Hodgson ME, Deming-Halverson SL, et al; Sister Study Research Team. The sister study cohort: baseline methods and participant characteristics. *Environ Health Perspect*. 2017;125:127003.
18. Sister Study. The sister study: participation rates for annual and detailed follow-ups. 2018. Available at: https://sisterstudy.niehs.nih.gov/English/images/SIS-FU-TimelineDR6-FURR-508.pdf. Accessed September 20, 2019.
19. O'Brien KM, White AJ, Jackson BP, Karagas MR, Sandler DP, Weinberg, CR. Toenail-based metal concentrations and young-onset breast cancer. *Am J Epidemiol*. 2019;188:646–655.
20. Shadyab AH, Macera CA, Shaffer RA, et al. Ages at menarche and menopause and reproductive lifespan as predictors of exceptional longevity in women: the Women's Health Initiative. *Menopause*. 2017;24:35–44.
21. Grambsch PM, Terry MT. Proportional hazards tests and diagnostics based on weighted residuals. *Biometrika*. 1994;81:515–526.
22. Keil AP, Buckley JP, O'Brien KM, Ferguson KK, Zhao S, White AJ. A Quantile-based g-computation approach to addressing the effects of exposure mixtures. *Environ Health Perspect*. 2020;128:47004.
23. Robins J. A new approach to causal inference in mortality studies with a sustained exposure period—application to control of the healthy worker survivor effect. *Math Model*. 1986;7:1393–1512.
24. Efron B. The efficiency of Cox's likelihood function for censored data. *J Am Stat Assoc*. 1977;72:557–565.
25. Nash D, Magder LS, Sherwin R, Rubin RJ, Silbergeld EK. Bone density-related predictors of blood lead level among peri- and postmenopausal women in the United States: the Third National Health and Nutrition Examination Survey, 1988-1994. *Am J Epidemiol*. 2004;160:901–911.
26. Stepaniak U, Szafraniec K, Kubinova R, et al. Age at natural menopause in three central and eastern European urban populations: the HAPIEE study. *Maturitas*. 2013;75:87–93.
27. Dunneram Y, Greenwood DC, Burley VJ, Cade JE. Dietary intake and age at natural menopause: results from the UK Women's Cohort Study. *J Epidemiol Community Health*. 2018;72:733–740.
28. Meiners CR, Derise NL, Lau HC, Crews MG, Ritchey SJ, Murphy EW. The content of nine mineral elements in raw and cooked mature dry legumes. *J Agric Food Chem*. 1976;24:1126–1130.
29. Sharma AD. Low nickel diet in dermatology. *Indian J Dermatol*. 2013;58:240.
30. Cooper GS, Sandler DP, Bohlig M. Active and passive smoking and the occurrence of natural menopause. *Epidemiology*. 1999;10:771–773.
31. Bernhard D, Rossmann A, Wick G. Metals in cigarette smoke. *IUBMB Life*. 2005;57:805–809.
32. Lee YM, Chung HW, Jeong K, et al. Association between cadmium and anti-Mullerian hormone in premenopausal women at particular ages. *Ann Occup Environ Med*. 2018;30:44.
33. Gibson EA, Goldsmith J, Kioumourtzoglou MA. Complex mixtures, complex analyses: an emphasis on interpretable results. *Curr Environ Health Rep*. 2019;6:53–61.
34. Slotnick MJ, Nriagu JO. Validity of human nails as a biomarker of arsenic and selenium exposure: a review. *Environ Res*. 2006;102:125–139.
35. Yaemsiri S, Hou N, Slining MM, He K. Growth rate of human fingernails and toenails in healthy American young adults. *J Eur Acad Dermatol Venereol*. 2010;24:420–423.
36. White AJ, O'Brien KM, Jackson BP, Karagas MR. Urine and toenail cadmium levels in pregnant women: a reliability study. *Environ Int*. 2018;118:86–91.
37. Wu AC, Allen JG, Coull B, et al. Correlation over time of toenail metals among participants in the VA normative aging study from 1992 to 2014. *J Expo Sci Environ Epidemiol*. 2018;29:663–673.
38. Flanders WD, Klein M. Properties of 2 counterfactual effect definitions of a point exposure. *Epidemiology*. 2007;18:453–460.
39. Hyland A, Piazza K, Hovey KM, et al. Associations between lifetime tobacco exposure with infertility and age at natural menopause: the Women's Health Initiative Observational Study. *Tob Control*. 2016;25:706–714.
40. US EPA O. Report on the Environment (ROE). US EPA. Available at: https://www.epa.gov/report-environment. Published February 6, 2015. Accessed November 22, 2019.
41. Gruber JF, Karagas MR, Gilbert-Diamond D, et al. Associations between toenail arsenic concentration and dietary factors in a New Hampshire population. *Nutr J*. 2012;11:45.