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Urinary Metals and Metal Mixtures and Timing of Natural Menopause in Midlife Women: the Study of Women's Health Across the Nation

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Abstract

Background: Exposure to metals and metal mixtures may influence ovarian aging. However, epidemiologic evidence of their potential impact is lacking.

Objective: We prospectively examined the associations of 15 urinary metal concentrations and their mixtures with natural menopause in the Study of Women's Health Across the Nation Multi-Pollutant Study.

Methods: The study population consisted of 1082 premenopausal women from multiple racial/ethnic groups, aged 45–56 years at baseline (1999–2000), with the median follow-up of 4.1 years. Urinary concentrations of 15 metals, including arsenic, barium, cadmium, cobalt, cesium, copper,

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Conflict of interest

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

mercury, manganese, molybdenum, nickel, lead, antimony, tin, thallium, and zinc, were measured at baseline. Natural menopause was defined as the final bleeding episode prior to at least 12 months of amenorrhea, not due to surgery or hormone therapy. Cox proportional hazards models were used to examine associations between individual metal concentrations and timing of natural menopause. The associations between metal mixtures and natural menopause were evaluated using elastic net penalized Cox regression, and an environmental risk score (ERS) was computed to represent individual risks of natural menopause related to metal mixtures.

Results: The median age at natural menopause was 53.2 years. Using the Cox proportional hazards models, the adjusted hazard ratio (HR) (and its 95% confidence interval (CI)) for natural menopause was 1.32 (1.03, 1.67) for arsenic and 1.36 (1.05, 1.76) for lead, comparing the highest with the lowest quartiles of metal concentrations. The predicted ages at natural menopause in the highest and lowest quartiles were 52.7 and 53.5 years for arsenic; and 52.9 and 53.8 years for lead. A significant association between ERS and menopause was also observed. Women in the highest vs. the lowest quartiles of ERS had an HR of 1.71 (1.36, 2.15), equivalent to a 1.6 year earlier median time to natural menopause.

Conclusion: This study suggests that arsenic, lead, and metal mixtures are associated with earlier natural menopause, a risk factor for adverse health outcomes in later life.

Keywords

Metals; mixtures; midlife women; menopause; aging

1. Introduction

According to the Endocrine Society scientific statement, an endocrine-disrupting chemical (EDC) is “a compound, either natural or synthetic, which, through environmental or inappropriate developmental exposures, alters the hormonal and homeostatic systems that enable the organism to communicate with and respond to its environment” (Diamanti-Kandarakis et al., 2009). EDCs are highly heterogenous and include chemicals, such as phthalates, phenols, polychlorinated biphenyls, perfluoroalkyl and polyfluoroalkyl substances, and metals. However, relatively little consideration has been given to the fact that many metals may disrupt the endocrine system. Metals are widely dispersed in the environment, and the general population can be exposed to metals through smoking, dietary sources, drinking water, and ambient air (Wang et al., 2019a). Substantial evidence suggests that arsenic (ATSDR, 2007a), cadmium (ATSDR, 2012), mercury (ATSDR, 1999), and particularly lead (ATSDR, 2007b; Mendola et al., 2008) alter fertility and reproductive outcomes, and have endocrine-disrupting potential.

The final menstrual period (FMP) indicates the end of a woman’s reproduction capability, or menopause, and is considered a risk marker for age-related morbidity and mortality. Earlier age at menopause has been associated with overall mortality (Jacobsen et al., 2003; Mondul et al., 2005; Ossewaarde et al., 2005), cardiovascular disease (Atsma et al., 2006; Hu et al., 1999), osteoporosis (Kritz-Silverstein and Barrett-Connor, 1993), mental disorders (de Kruif et al., 2016), and other chronic conditions (Shuster et al., 2010). Growing evidence from epidemiologic studies suggests that environmental factors may play a role in determining

age at menopause (Ding et al., 2020b; Gore et al., 2011; Grindler et al., 2015; Vabre et al., 2017). However, it is still unclear whether exposure to metals is associated with ovarian aging and the timing of menopause.

Few epidemiologic studies have examined associations between exposure to metals and age at menopause, and those studies have yielded inconsistent results (Eum et al., 2014; Mendola et al., 2013; Pan et al., 2020; Popovic et al., 2005; Yunus et al., 2014) Most studies were cross-sectional, and primarily focused on lead exposure (Mendola et al., 2013; Popovic et al., 2005). Associations between other metals and menopause have been largely unexplored. Furthermore, people are generally exposed to multiple metals as mixtures (Wang et al., 2019a), yet most studies have narrowly focused on individual metals, possibly 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 (Park et al., 2017; Wang et al., 2019b, 2018). To our knowledge, no study has considered multiple metals, except for a recent prospective cohort study, which examined 16 metals and their mixtures in toenails from 903 premenopausal women in the Sister Study (White et al., 2020). Individual metals were not associated with the timing of menopause; however, later age at menopause was linked to a quartile increase in metal mixtures (HR=0.81, 95% CI: 0.64, 1.02) (White et al., 2020).

We thus examined the associations of 15 urinary metal concentrations with the timing of natural menopause in the Study of Women's Health Across the Nation (SWAN), a multi-site, prospective cohort study of midlife women from multiple racial/ethnic groups. We hypothesized that higher lead concentration would be associated with earlier natural menopause. We also sought to determine associations between other metals and natural menopause as an initial exploratory inquiry of environmental factors related to ovarian aging. Finally, we constructed an environmental risk score (ERS) (Park et al., 2017, 2014; Wang et al., 2020, 2019b, 2018) as a summary measure of the health risk of exposure to multiple metals to evaluate the association between exposure to metal mixtures and natural menopause.

2. Materials and methods

2.1 Study design and population

SWAN is an ongoing, multi-site, community-based prospective study of a cohort of midlife women from multiple racial/ethnic groups, designed to characterize the physiological and psychosocial changes that occur during the menopausal transition and their effects on subsequent health endpoints. Study participants were enrolled between 1996 and 1997 from seven study sites; each site recruited White women and Black women were recruited at the Boston, MA, Pittsburgh, PA, southeast Michigan, MI, and Chicago, IL site; Hispanic women from the Newark, NJ site; Chinese women from the Oakland, CA site; and Japanese women from the Los Angeles, CA site (Sowers et al., 2000). To be eligible, women had to be aged 42 to 52 years, have an intact uterus and at least one ovary, have had at least one menstrual period and not taken menopausal hormone therapy (HT) in the past three months, and self-identified with the site's designated racial/ethnic groups. Participants returned for regular examinations approximately annually. The institutional review board at

each participating site approved the study protocol, and all participants provided written, signed informed consent.

The SWAN Multi-Pollutant Study (SWAN-MPS) used urine samples from the SWAN Repository collected during the third SWAN follow-up visit (1999–2000) for environmental exposure assessment (Ding et al., 2020a; Wang et al., 2019a). A subset of 1,400 SWAN participants from the five SWAN sites who provided urine samples to the SWAN Repository (Boston, MA; southeast Michigan, MI; Los Angeles, CA; Oakland, CA; and Pittsburgh, PA) were assayed for metal concentrations. Women from Chicago and Newark were excluded because urine samples were not collected at these two sites. This subpopulation, by design, included self-identified White, Black, Chinese, and Japanese women but not Hispanic women who were recruited exclusively from Newark. For these analyses, we excluded 237 women who had already reached natural menopause and 48 who had a hysterectomy or oophorectomy at the SWAN-MPS baseline, and 33 who had no information on key covariates, yielding a final analytic sample of 1,082 pre- or early perimenopausal women. These women were followed approximately annually through 2014, with a median follow-up of 4.1 (interquartile range: 2.3, 6.1) years.

2.2 Natural menopause

Our primary outcome was age at the natural FMP, based upon information provided in the approximately annual interviews. The natural FMP was defined as 12 months of amenorrhea since the last menstrual period with menstruation cessation not due to other causes (including hysterectomy, bilateral oophorectomy, or HT). Menopausal status and date of the FMP were ascertained prospectively by interviewers at each annual visit. Each annual follow-up interview asks 1) whether the woman has had a period in the last year; 2) whether she has had a period in the last three months; and 3) the date of her most recent menstrual period. The natural FMP was ascertained if a participant had a menstrual period followed by at least 12 consecutive months that were both free of HT use and bleeding. The age at the natural FMP was prospectively measured, which was critical to obtain accurate age at the natural FMP (Gold et al., 2013).

2.3 Urinary metal concentrations

High-resolution inductively-coupled plasma mass spectrometry (Thermo Scientific iCAP RQ, Waltham, MA) was used to determine concentrations of a panel of 15 metals (arsenic, barium, cadmium, cobalt, cesium, copper, mercury, manganese, molybdenum, nickel, lead, antimony, tin, thallium, and zinc) in morning spontaneously voided urine samples at SWAN-MPS baseline. As previously described, the measurements followed the CDC method 3018.3 (CDC, 2012), with modifications for the expanded metals panel, performed at the Applied Research Center of NSF International (Ann Arbor, Michigan) (Wang et al., 2019a). The limits of detection (LOD) and detection rates are presented in Table S1. Concentrations below LODs were substituted with values equal to LODs divided by the square root of 2. Urinary creatinine was determined using the Cobas Mira analyzer (Horiba ABX, Montpellier, France) at baseline as a urine dilution marker.

2.4 Other covariates

Information on age, self-reported race/ethnicity (White, Black, Chinese, or Japanese), and education level (as high school or less, some college, or college degree or higher) were obtained through a self-administered questionnaire at baseline. Body mass index (BMI) at baseline was calculated as measured weight in kilograms divided by the square of measured height in meters. We did not consider time-varying BMI in the analysis, given that it could be affected by metal exposures at baseline and its potential role as an intermediate factor (Wang et al., 2018). At each study visit, smoking status (never smoked, former smoked only, or current smoking), physical activity, and parity were self-reported. Physical activity was assessed 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. Parity was classified into nulliparous or parous based on the number of live births and stillbirths. Uniform protocols were used to collect information on these covariates across the study sites. We used a Directed Acyclic Graph to show the hypothesized relations between metals, confounders, and natural menopause (Figure S1).

2.5 Statistical analysis

Cox proportional hazards models were used to estimate the hazard ratios (HRs) and 95% confidence intervals (CIs) for natural menopause associated with individual metals. Participants contributed follow-up time from the SWAN-MPS baseline to the date of FMP. We used age, rather than time since the SWAN-MPS baseline, as the time scale (Thiébaut and Bénichou, 2004). We censored women when they reported initiating HT if no subsequent HT-free bleeding occurred, at the date of hysterectomy or bilateral oophorectomy, or at the last menstrual period at the end of data collection if it occurred before 12 months of amenorrhea, or at the date of the participants' last follow-up visits. Urinary metal concentrations were categorized into quartiles, and HRs (95% CI) of diabetes were calculated comparing the second, third, and fourth metal quartile to the first quartiles (reference group). We calculated the survival probability of natural menopause (i.e., probability of not having natural menopause) in each quartile of metal concentrations, and the median age at natural menopause was estimated as the time at which 50% had reached their natural menopause. We also modeled the associations using continuous metal concentrations. Given the highly skewed distributions of urinary metal concentrations, logarithmic transformations with base two were applied to all metal concentrations. The HRs were thus interpreted as effects per doubling increase in each urinary metal concentration. Potential confounders adjusted in regression analyses included race/ethnicity, study site, urinary creatinine (log-transformed), education, BMI at baseline, smoking, physical activity, and parity.

To summarize the effects of exposures to metal mixtures on natural menopause, we constructed an ERS as an integrative index of health risk associated with 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. Weights are determined by the magnitudes of the associations between chemicals and health

outcomes of interest from the regression model. To achieve this goal, we first used the elastic net (ENET) penalized Cox regression (Yang and Zou, 2013), a machine learning algorithm designed for analyzing high-dimensional data in survival analyses, to identify metals associated with natural menopause. The Cox proportional hazards model is the most popular survival approach for studying the relationship between survival time and predictor variables but is prone to over-fitting and does not work well in the presence of potentially high-dimensional predictors, or when predictors are highly correlated (multicollinearity). To address these issues, the ENET penalized Cox model, as one of the sparse penalized Cox models, has been introduced. All 15 log-transformed urinary metal concentrations were included in the same ENET penalized Cox model in our analysis. ENET shrinks coefficients of "unimportant" metals toward exact zeroes while handling the complex correlations. The same covariates from the Cox model in the individual metal analyses were adjusted ("forced") in these models. ENET penalized parameters were ascertained based on 10-fold cross-validation for minimal prediction errors. The R package 'glmnet' was used to implement the ENET penalized Cox model

ERS was then computed as a weighted sum of non-zero metal predictors estimated from ENET penalized Cox model by

$$\mathrm{ERS_i} = \sum_{\mathrm{j}=1}^{\mathrm{P}} \hat{\beta}_{\mathrm{j}} E_{\mathrm{i}}^{\mathrm{j}}, \tag{1}$$

where $E_i^j (j = 1, \ldots, \mathrm{p})$ is the log-transformed concentration of the *j*-th metal and $\hat{\beta}_{\mathrm{j}}$ is the beta coefficient (weight) of the *j*-th metal. We further categorized ERS into quartiles and fit the Cox model to compare the hazards of natural menopause between groups characterized by distinct risks attributed to different exposures to metal mixtures. We calculated the survival probability to natural menopause and estimated the median age at natural menopause of participants in different quartiles of ERS. We also examined the distributions of sociodemographic factors across ERS quartiles.

We conducted several sensitivity analyses to evaluate the robustness of our primary findings. First, early menopause has been associated with increased bone turnover rate (Hernandez-Avila et al., 2000; Tsaih et al., 2001). Because participants who approached menopause may have elevated urinary lead concentrations at that time due to its mobilization from bone, reverse causation may account for observed associations between urinary lead and age at natural menopause. To examine the potential impact of reverse causation on our results, we used urinary N-telopeptide (NTX) adjusted lead concentration. Second, we further adjusted for serum concentrations of n-perfluorooctane sulfonic acid (n-PFOS) (log-transformed) and branched-PFOS (log-transformed) because a previous study found PFOS was associated with an earlier age at natural menopause in SWAN (Ding et al., 2020b) and their modestly negative correlations with certain metals (Figure S2). Third, we calculated HRs of natural menopause in relation to metal concentrations using the covariate-adjusted standardization plus covariate adjustment method for accounting for urine dilution (O'Brien et al., 2016). Fourth, we administratively censored 44 observations in the follow-up visits at which women were over age 60 years because of a low chance of menopause not occurring at age>60 years (Gold, 2011). Fifth, we included all metals together in the quantile

g-computation (Keil et al., 2020), another advanced statistical approach that enabled us to evaluate the overall mixture effect. The overall mixture effect of the metals included in the quantile g-computation model was defined as the HR of menopause for a one quantile, simultaneous increase in all of the exposures in a specified mixture. The R package "qgcomp" was used to implement the quantile g-computation (Keil et al., 2020). Finally, HT or dropout from follow-up visits due to unknown reasons masked the actual FMP date for some women. The SWAN Data Coordinating Center conducted multiple imputations with chain equations for missing FMP age using IVEware. We conducted a sensitivity analysis to examine the associations between metal concentrations and natural menopause using ten sets of imputations. The pooled HRs and 95% CIs were computed using PROC MIANALYZE. All analyses were conducted using SAS, version 9.4 (SAS Institute, Inc., Cary, North Carolina), and R, version 4.0.2 (www.R-project.org).

3. Results

The mean (standard deviation, SD) age of participants at SWAN-MPS baseline was 49.0 (2.5) years (Table 1). Most women had at least some college education and had never smoked. Among these 1,082 women who were pre-menopausal at the SWAN-MPS baseline, 728 reached natural menopause during the follow-up, with a median follow-up of 4.1 years (median age at natural menopause=53.2 years). The detection rate and distributions of urinary metal concentrations are presented in Table S1. In general, most metals were modestly and positively correlated with each other (Figure S2). The strongest correlation was between cobalt and nickel (R=0.58).

After adjustment for race/ethnicity, study site, education, BMI at baseline, smoking, physical activity, parity, and urinary creatinine (log-transformed), the HR for natural menopause was 1.32 (95% CI: 1.03, 1.67) for arsenic and 1.36 (95% CI: 1.05, 1.76) for lead, comparing the highest with the lowest quartiles of metal concentrations (Table 2). The predicted ages at natural menopause in women in the lowest and highest quartiles of urinary concentrations were 53.5 years, and 52.7 years for arsenic; and 53.8 years, and 52.9 years for lead. Positive associations of natural menopause with $\log_2$-transformed arsenic and lead concentrations were also observed (Table S2). No significant association between other metals and the timing of menopause was observed.

In the ENET penalized Cox model, 11 out of 15 metals were retained (beta coefficients, which represents log(HR) per doubling increase in metal concentrations, were not shrunk to zero) (Figure S3). A total of five metals, including arsenic (β=0.024), cesium (β=0.014), mercury (β=0.028), lead (β=0.061), and antimony (β=0.008), were associated with an earlier age at natural menopause. Six metals, including barium (β=−0.003), copper (β=−0.015), manganese (β=−0.007), molybdenum (β=−0.041), thallium (β=−0.001), and zinc (β=−0.016) were associated with a later age at natural menopause. Beta coefficients of all the other four metals were shrunk to zero.

These beta coefficients were then used as weights in the calculation of ERS. The ERS ranged from −0.83 to 0.06 with a mean of −0.43. In accordance with the formula, women with higher concentrations of metals with positive weights (β), and/or with lower

concentrations of metals with negative weights (β) had higher ERS. Also, participants with higher ERS had an earlier onset of natural menopause compared to those with lower ERS (Figure 1). After adjustment, the HR for natural menopause comparing the highest to the lowest quartile of ERS was 1.71 (95% CI: 1.36, 2.15) (Table 3). The predicted median age at natural menopause in the fourth quartile of the ERS was 52.6 years compared to 54.2 years, 53.5 years, and 53.0 years for the first, second, and third quartiles, respectively. Of note, women with higher ERS were more likely to self-identify as Chinese or Japanese-Americans (Table S3).

In sensitivity analyses, using urinary NTX-adjusted lead slightly attenuated the associations (Table S4). Further adjustment for serum PFOS concentrations did not change results significantly (Table S5). Similar results were observed when the covariate-adjusted standardization plus covariate adjustment method was used in the Cox models for urine dilution adjustment, except that an association between cesium and natural menopause was additionally observed (Table S6). Further administrative censoring of 44 observations for women aged over 60 years (maximum age equals 63.3 years) did not alter the associations (Table S7). Using the quantile g-computation and adjusting for all the covariates, the HR for natural menopause associated with the simultaneous increase in all urinary metal concentrations by one quartile was 1.11 (95% CI: 0.93, 1.33). Lead showed the highest positive weight in the quantile g-computation model (Figure S4), consistent with the findings of single association and the ERS using AENET. However, the HRs calculated in the quantile g-computation and the ERS are not directly comparable. HR in the quantile g-computation represents the menopause associated with a one quartile simultaneous increase in all metal concentrations. In comparison, a higher ERS represents an earlier onset of natural menopause due to higher concentrations of metals with positive weights and/or with lower concentrations of metals with negative weights, but not necessarily higher concentrations of all metals included in the analysis. Finally, the associations between urinary metal concentrations and natural menopause were largely unchanged with 10 imputations of age at FMP (Table S8), while the association for arsenic was slightly attenuated.

4. Discussion

In this multi-site, prospective study of a cohort of 1,082 midlife women from multiple racial/ethnic groups, higher urinary arsenic and lead concentrations were associated with earlier natural menopause, after adjusting for sociodemographic variables, lifestyle factors, BMI, and parity. In the mixture analysis, an ERS was calculated to integrate the impacts of metal mixtures on natural menopause and capture an individual's risk based on her combined metal exposure. The association between ERS and natural menopause suggests that exposure to metal mixtures may play a critical role in ovarian aging, with women in the highest ERS quartile having 1.6 years earlier age at menopause than women in the lowest quartile.

Even a small advance in the age at natural menopause associated with metal exposures may have substantial public health consequences, given the widespread nature of metal exposures and the importance of health outcomes related to accelerated ovarian aging. The earlier occurrence of menopause indicates a reduction in the reproductive lifespan and a

higher risk of cardiovascular disease (Atsma et al., 2006; Cui et al., 2006; Hu et al., 1999; Løkkegaard et al., 2006), osteoporosis and fracture (Kritz-Silverstein and Barrett-Connor, 1993; Parazzini et al., 1996; Van der Voort et al., 2003), neurological disorders (de Kruif et al., 2016; Georgakis et al., 2016), and psychiatric disease (Bromberger et al., 2001) in later life. For example, for every one year decrease in age at natural menopause, the risk of coronary heart disease has been estimated to increase by 3% (Hu et al., 1999). All-cause mortality has been estimated to increase by 5% (Jacobsen et al., 2003; Mondul et al., 2005; Ossewaarde et al., 2005).

One robust finding from our analysis was that urinary arsenic was associated with an earlier natural menopause. Epidemiologic studies on arsenic exposure and natural menopause have been limited, and prior findings have been inconsistent. A cross-sectional study of 210 women in Bangladesh with high arsenic exposure reported that having an arsenic skin lesion, a marker of chronic arsenic exposure, was associated with an earlier age at menopause (Yunus et al., 2014). In a recent case-control study of 169 primary ovarian insufficiency cases and 209 healthy controls, higher urinary arsenic concentration was observed in patients compared to controls (Pan et al., 2020). In the same study, urinary arsenic was also associated with higher serum levels of follicle-stimulating hormone and lower estradiol and anti-Mullerian hormone levels, supporting the possibility that arsenic exposure has adverse reproductive effects that could affect ovarian reserve and timing of menopause (Pan et al., 2020). In contrast, in a recent prospective cohort study, no association between toenail arsenic and age at menopause was found (White et al., 2020). The association between arsenic and earlier natural menopause is biologically plausible. It is known that arsenic exposure can cause oxidative stress through reactive oxygen species (ROS) generation. Arsenic may impair ovarian folliculogenesis and disrupt normal function of female reproductive system through oxidative stress. For instance, female rats with an oral administration of 4 ppm arsenic through drinking water for 28 days had increased free-radical productions and induced apoptosis in the uterus (Chatterjee and Chatterji, 2017; Chattopadhyay and Ghosh, 2010). Ommati et al. treated parent mice with 0, 0.2, 2, and 20 ppm arsenic through drinking water; and they also found increases in ROS content, decreases in mitochondrial dehydrogenase activity, and autophagy in the ovaries of F1-female mice (Ommati et al., 2020).

We also found that urinary lead was associated with an earlier natural menopause. Previous studies on lead and menopause have been cross-sectional, and findings have been mixed. Popovic et al. observed that women who were occupationally exposed to lead from smelting had an earlier menopause than similar-aged women without occupational exposure (Popovic et al., 2005). Mendola et al. used the National Health and Nutrition Examination Survey 1999–2000 and reported a similar association between higher blood lead levels and higher odds of natural menopause (Mendola et al., 2013). In contrast, using cross-sectional data from 434 women participants in the Nurses' Health Study, no association was detected between patella or blood lead and age at menopause; whereas, higher tibia lead, as an indicator of cumulative lead exposure, were associated with younger age at menopause (Eum et al., 2014). Lead has been found to disrupt the hypothalamic-pituitary axis. A study of rats found that lead treatment upregulated the production of gonadotropin-releasing hormone, which further triggers the secretion of follicle stimulating hormone, a key

indicator of ovarian reserve (Sokol et al., 2002). Lead may also interfere with follicular function. In an *in vitro* study of human ovarian granulosa cells, lead treatment led to reduced levels of both mRNA and protein levels of cytochrome p450 and estrogen receptor beta (Taupeau et al., 2003). Further, a study of mice also showed that the accumulation of lead in the ovary led to altered folliculogenesis (Taupeau et al., 2001).

In this study, inverse associations of essential metals including copper, molybdenum, and zinc with natural menopause were detected in analyses of individual metals, though the associations were not statistically significant. The inverse associations were also retained in the mixture analyses. Existing evidence directly examining the associations between essential metals and age at natural menopause is sparse. In a case-control study of primary ovarian insufficiency, higher serum zinc concentration was associated with lower odds of primary ovarian insufficiency and higher follicle stimulating hormone levels (Kebapcilar et al., 2013). In a United Kingdom cohort study of women aged 40–65 years, dietary intake of legumes, which is one of the primary sources of molybdenum for the general population, was associated with later age at menopause (Dunneram et al., 2018). Another large cross-sectional study in central and eastern Europe also reported that the use of vitamin and mineral supplements, which contain numerous essential trace elements, was associated with later age at menopause (Stepaniak et al., 2013). These findings, which warrant further investigation, raise interesting questions regarding the role of essential metals in determining the timing of menopause which should be pursued in future work.

To our knowledge, the current investigation is among the first studies of metal mixtures and their effects on the occurrence of natural menopause in a prospective cohort of multi-racial/ethnic, midlife women. The statistical approaches we used here acknowledge the reality that people are exposed to multiple metals and not individual metal in isolation (Wang et al., 2019a), account for the high degree of correlation between metal concentrations that may lead to the collinearity problem if all metals were included simultaneously in the same Cox model. Further, we observed a strong association between metal mixtures, assessed by an integrated ERS, and natural menopause. This finding highlights the importance of considering metal mixtures to evaluate associations of metal exposures with health outcomes in epidemiologic studies.

Our findings of individual metals and metal mixtures are not in concordance with White *et al.*, 2020, the only other published study to our knowledge that has explored the associations of toenail metals and their mixtures and natural menopause. White *et al.*, 2020 included 907 premenopausal women with the median age of 52.4 years, recruited from the Sister Study. The Sister Study enrolled predominantly White women from the United States and Puerto Rico between 2003 and 2009. Toenail concentrations of 16 metals were measured at baseline. Information on age at natural menopause was collected retrospectively for older women or prospectively during the follow-up (Steiner et al., 2010). They found no association between individual metals and timing of menopause and a borderline significant association between exposure to metal mixtures and later age at menopause. The main distinction between the Sister Study and ours was the biomarkers of metal exposures used. Toenail concentration provide a potentially adequate proxy for long-term exposure to certain metals (Yaemsiri et al., 2010). Another major difference is that the Sister Study did not

collect information on HT or contraceptive use, which might mask signs of approaching menopause (Gold, 2011). Except for White *et al.*, 2020, no previous studies have explored the effects of these metals on timing of menopause.

In the SWAN-MPS study women with higher ERS were more likely to be Asians, including both Chinese and Japanese women. Racial/ethnic disparities in metal exposures, specifically unusually high concentrations of toxic metals found in Chinese and Japanese women, independent of other sociodemographic factors, such as geographic locations and socioeconomic status, have been reported in SWAN previously (Wang et al., 2019a). These findings contextualize racial/ethnic disparities in metal exposures in women across the United States, which may further contribute to alterations in age at natural menopause. It highlights the need to identify significant exposure routes that are driving racial disparities in metal concentrations, such as food intake including seafood and rice (Wang et al., 2019a). Ultimately, racial/ethnic-specific recommendations for reducing metals' primary sources showing reproductive toxicities are warranted to help mitigate toxic metals' body burden and, subsequently, health risks.

The primary strength of our study was its use of a large prospective cohort of community-based midlife women from four racial/ethnic groups with longitudinal determination of menopause events. Additionally, we examined a panel of 15 metals, which allowed us to investigate a large number of associations as an initial exploratory inquiry of environmental factors affecting natural menopause. Furthermore, we implemented a mixture analysis approach, ERS, to evaluate the associations between exposure to metal mixtures and age at natural menopause while accounting for statistical challenges, such as complex correlations underlying multiple metals.

Several limitations should be considered as well when interpreting the findings. First, we measured all metal concentrations in urine, and urinary concentrations may not reflect total body burden from all forms of metals and exposure sources. Second, metals included in the current analysis have 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 metal concentrations, especially for metals with short half-lives, is needed to characterize better cumulative metal exposures in future studies. Third, only total arsenic was measured in urine samples, but information on its methylated metabolites, which play an important role in its toxicity, was not available (Drobna et al., 2009). Additional arsenic metabolism measurements will improve our understanding of its potential effects on reproductive health outcomes in future studies. Fourth, the coefficient estimates from ENET penalized Cox regression were conservatively biased because of shrinkage estimation provided by the ENET, and standard errors and test statistics are difficult to compute (Yang and Zou, 2013). Fifth, we used cross-validation to minimize prediction errors of the ENET penalized Cox model, and ERS was further constructed, but these findings may not be generalizable to other populations. Though other prospective studies are needed to validate the effects of individual metals and the ERS, our findings from a large, multi-racial/ethnic sample are nonetheless important in indicating that exposure to metals and their mixtures may affect the timing of natural menopause,

which in turn may affect subsequent disease risk. Lastly, women at the SWAN-MPS baseline were between age 45 and 56 years and had menstruation, thus women who experienced menopause before baseline, especially those with premature menopause (before age 40 years) or early menopause (before age 45 years), were not included in the analysis. Thus, the effects of metals on natural menopause could be underestimated.

5. Conclusions

This prospective study demonstrated that metals, including arsenic and lead were associated with earlier natural menopause. The results of this study provide evidence that exposure to metal mixtures may affect age at natural menopause. Women in the highest ERS quartile, on average, had 1.6 years earlier age at menopause than women in the lowest quartile. This estimate was roughly equivalent to or even larger than an effect estimate of 1.1 years comparing current smokers versus never smokers in the SWAN-MPS (Ding et al., 2020b). Due to the widespread nature of metal exposures and the importance of health outcomes related to accelerated ovarian aging, the potential adverse health effects of metals should be a public health concern.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Abbreviations:

EDC	endocrine-disrupting chemical
ENET	elastic net
ERS	environmental risk score
FMP	final menstrual period
HT	hormone therapy
LOD	limit of detection
NTX	N-telopeptide
PFOS	perfluorooctane sulfonic acid
ROS	reactive oxygen species
SWAN	Study of Women's Health Across the Nation
SWAN-MPS	Study of Women's Health Across the Nation Multi-Pollutant Substudy

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Highlights

- Urinary arsenic was associated with earlier age at natural menopause.
- Urinary lead was associated with earlier age at natural menopause.
- Metal mixtures may play a role in accelerated ovarian aging.

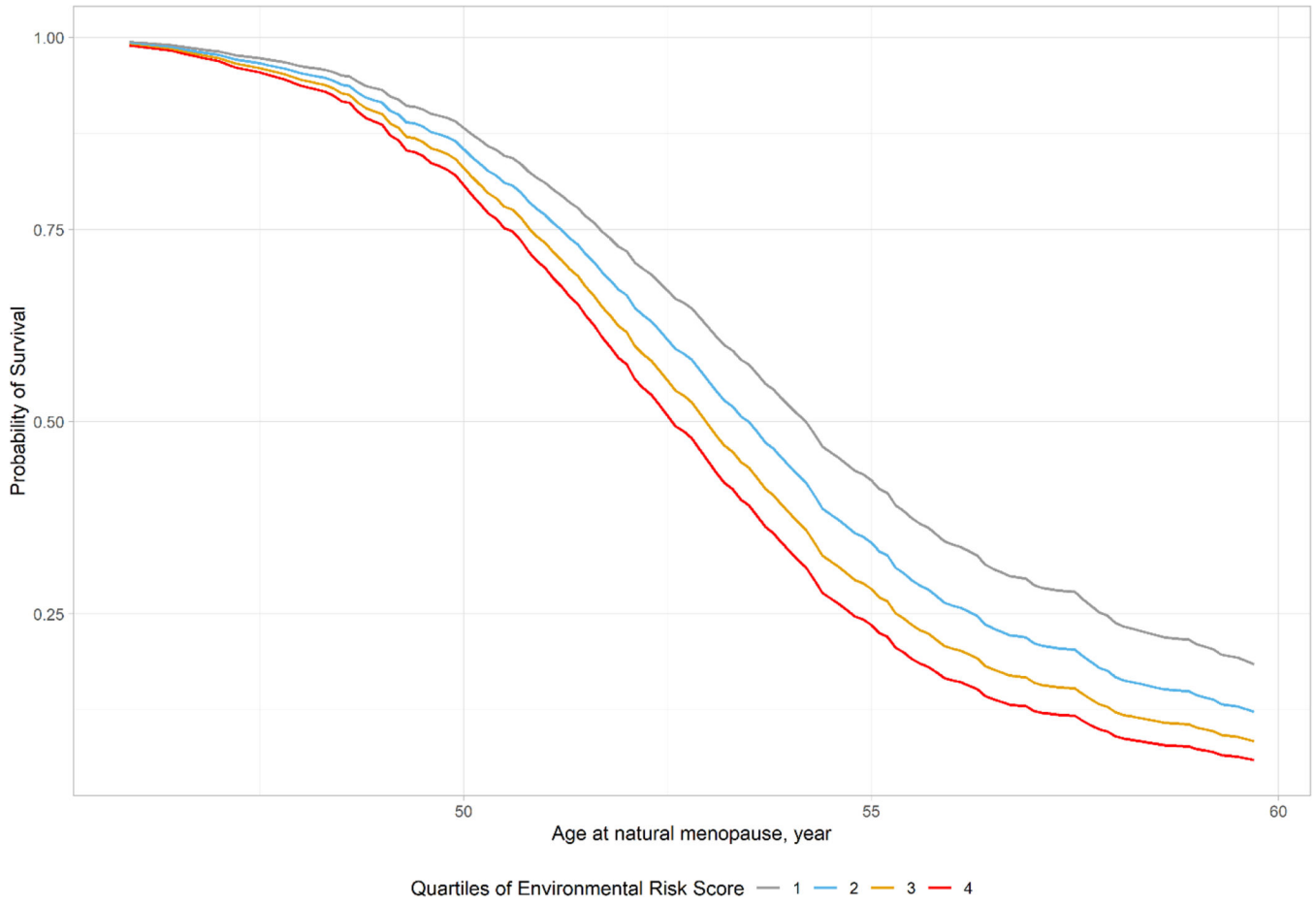

Figure 1.

Adjusted survival curves for natural menopause by quartiles of environment risk score (ERS). The model was adjusted for race/ethnicity, study sites, urinary creatinine (log-transformed), education, body mass index at baseline, smoking, physical activity, and parity. The predicted median age at natural menopause was 54.2, 53.5, 53.0, and 52.6 years for women in first, second, third, and fourth quartile of ERS, respectively.

Table 1.

Descriptive characteristics at the SWAN-MPS baseline.

Characteristics	Mean (SD) or n (%)
Age, mean (SD), year	49.0 (2.5)
Race/ethnicity, n (%)	
White	562 (51.9)
Black	221 (20.4)
Chinese	140 (12.9)
Japanese	159 (14.7)
Study site, n (%)	
Michigan	188 (17.4)
Boston	177 (16.4)
Oakland	238 (22.0)
Los Angeles	289 (26.7)
Pittsburgh	190 (17.6)
Education, n (%)	
High school or less	191 (17.7)
Some College	333 (30.8)
College	269 (24.9)
Post-college	289 (26.7)
Smoking status, n (%)	
Never	698 (64.5)
Former	281 (26.0)
Current	103 (9.5)
Parity, n (%)	
Nulliparous	209 (19.3)
Parous	873 (80.7)
Physical activity score, mean (SD)	7.8 (1.7)
Body mass index, mean (SD), kg/m^2	27.8 (7.1)

Note: SD: standard deviation.

Table 2.

Hazard ratios (HR) (95% confidence intervals, 95% CI) for natural menopause in relation to quartiles of urinary metal concentrations.

Metal concentration	Quartile 1	Quartile 2	Quartile 3	Quartile 4
Lead				
Range, μg/L	<LOD, 0.45	0.45, 0.76	0.76, 1.18	1.18, 43.59
HR (95% CI)	Ref	1.25 (1.01, 1.55)	1.11 (0.87, 1.41)	1.36 (1.05, 1.76)
Median age at menopause	53.8	53.1	53.5	52.9
Arsenic				
Range, μg/L	0.73, 6.94	6.97, 14.82	14.88, 40.27	40.36, 2983.79
HR (95% CI)[a]	Ref	1.14 (0.91, 1.42)	0.86 (0.68, 1.09)	1.32 (1.03, 1.67)
Median age at menopause	53.5	53.1	53.9	52.7
Barium				
Range, μg/L	<LOD, 0.99	0.99, 1.81	1.82, 3.01	3.01, 51.03
HR (95% CI)	Ref	0.97 (0.78, 1.19)	0.93 (0.74, 1.15)	1.00 (0.80, 1.26)
Median age at menopause	53.2	53.3	53.4	53.2
Cadmium				
Range, μg/L	<LOD, 0.22	0.22, 0.43	0.43, 0.77	0.77, 23.97
HR (95% CI)	Ref	1.08 (0.86, 1.35)	1.24 (0.97, 1.58)	1.17 (0.90, 1.53)
Median age at menopause	53.6	53.4	53.0	53.1
Cobalt				
Range, μg/L	<LOD, 0.40	0.40, 0.65	0.65, 1.07	1.07, 8.32
HR (95% CI)	Ref	1.04 (0.83, 1.31)	1.02 (0.80, 1.28)	0.99 (0.77, 1.28)
Median age at menopause	53.3	53.2	53.3	53.3
Cesium				
Range, μg/L	0.20, 3.01	3.01, 4.81	4.81, 7.43	7.43, 104.43
HR (95% CI)	Ref	1.07 (0.85, 1.34)	1.18 (0.91, 1.51)	1.26 (0.93, 1.69)
Median age at menopause	53.6	53.4	53.1	53.0
Copper				
Range, μg/L	<LOD, 6.29	6.30, 9.77	9.78, 14.56	14.57, 1889.40

Metal concentration	Quartile 1	Quartile 2	Quartile 3	Quartile 4
HR (95% CI)	Ref	0.98 (0.78, 1.24)	0.98 (0.76, 1.28)	0.91 (0.68, 1.23)
Median age at menopause	53.2	53.2	53.2	53.5
Mercury				
Range, μg/L	<LOD, 0.66	0.67, 1.23	1.23, 2.36	2.36, 32.37
HR (95% CI)	Ref	0.98 (0.79, 1.22)	1.18 (0.94, 1.47)	1.17 (0.92, 1.48)
Median age at menopause	53.5	53.6	53.0	53.1
Manganese				
Range, μg/L	<LOD, 0.62	0.62, 0.92	0.93, 1.52	1.53, 41.97
HR (95% CI)	Ref	0.98 (0.78, 1.22)	0.97 (0.77, 1.21)	0.94 (0.74, 1.18)
Median age at menopause	53.2	53.2	53.3	53.4
Molybdenum				
Range, μg/L	2.48, 24.92	24.93, 44.10	44.19, 72.82	72.93, 694.53
HR (95% CI)	Ref	0.97 (0.78, 1.21)	1.00 (0.79, 1.27)	0.90 (0.69, 1.17)
Median age at menopause	53.2	53.3	53.2	53.5
Nickel				
Range, μg/L	<LOD, 2.39	2.40, 3.82	3.82, 5.87	5.90, 73.60
HR (95% CI)	Ref	0.90 (0.72, 1.13)	0.99 (0.78, 1.26)	1.00 (0.76, 1.31)
Median age at menopause	53.2	53.5	53.2	53.2
Antimony				
Range, μg/L	<LOD, 0.05	0.05, 0.08	0.08, 0.13	0.13, 2.82
HR (95% CI)	Ref	1.07 (0.86, 1.33)	1.11 (0.88, 1.39)	1.07 (0.84, 1.38)
Median age at menopause	53.5	53.3	53.1	53.2
Tin				
Range, μg/L	<LOD, 0.49	0.49, 0.93	0.93, 1.74	1.74, 61.11
HR (95% CI)	Ref	0.95 (0.76, 1.18)	0.99 (0.78, 1.24)	1.08 (0.85, 1.38)
Median age at menopause	53.3	53.5	53.3	53.0
Thallium				
Range, μg/L	<LOD, 0.08	0.09, 0.15	0.15, 0.24	0.24, 15.73
HR (95% CI)	Ref	0.94 (0.76, 1.18)	0.95 (0.75, 1.20)	1.04 (0.80, 1.34)

Metal concentration	Quartile 1	Quartile 2	Quartile 3	Quartile 4
Median age at menopause	53.2	53.4	53.4	53.1
Zinc				
Range, μg/L	7, 168	168, 310	311, 530	531, 3225
HR (95% CI)	Ref	0.92 (0.74, 1.15)	0.92 (0.72, 1.19)	0.83 (0.62, 1.11)
Median age at menopause	53.0	53.3	53.2	53.6

[a]All models were adjusted for race/ethnicity, study sites, and urinary creatinine (log-transformed), education, body mass index at baseline, smoking, physical activity, and parity.

Table 3.

Hazard ratios (HR) (95% confidence intervals, 95% CI) for natural menopause by quartiles of environmental risk score (ERS).

	HR (95% CI)			
ERS quartiles	**Quartile 1**	**Quartile 2**	**Quartile 3**	**Quartile 4**
Base model[a]	1.00	1.25 (1.01, 1.56)	1.50 (1.20, 1.87)	1.72 (1.37, 2.16)
Full model[b]	1.00	1.25 (1.01, 1.56)	1.49 (1.19, 1.86)	1.71 (1.36, 2.15)

[a]Base model: adjustment for race/ethnicity, study sites, and urinary creatinine (log-transformed).

[b]Full model: base model with additional adjustment for education, body mass index at baseline, smoking, physical activity, and parity.