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Whole blood lead levels are associated with biomarkers of joint tissue metabolism in African American and White men and women: The Johnston County Osteoarthritis Project

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Abstract

Purpose—To examine associations between biomarkers of joint tissue metabolism and whole blood lead (Pb), separately for men and women in an African American and Caucasian population, which may reflect an underlying pathology.

Methods—Participants in the Johnston County Osteoarthritis Project Metals Exposure Sub-study (329 men and 342 women) underwent assessment of whole blood Pb and biochemical biomarkers of joint tissue metabolism. Urinary cross-linked N telopeptide of type I collagen (uNTX-I) and C-

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As noted on page 5, "This study has been approved by the Institutional Review Boards of the Centers for Disease Control and Prevention and the University of North Carolina."

telopeptide fragments of type II collagen (uCTX-II), and serum cleavage neopeptide of type II collagen (C2C), serum type II procollagen synthesis C-propeptide (CPII), and serum hyaluronic acid (HA) were measured using commercially available kits; the ratio of [C2C:CPII] was calculated. Serum cartilage oligomeric matrix protein (COMP) was measured by an in-house assay. Multiple linear regression models were used to examine associations between continuous blood Pb and biomarker outcomes, adjusted for age, race, current smoking status, and body mass index. Results are reported as estimated change in biomarker level for a 5-unit change in Pb level.

Results—The median Pb level among men and women was 2.2 and 1.9 µg/dL, respectively. Correlations were noted between Pb levels and the biomarkers uNTX-I, uCTX-II, and COMP in women, and between Pb and uCTX-II, COMP, CPII, and the ratio [C2C:CPII] in men. In adjusted models among women, a 5-unit increase in blood Pb level was associated with a 28% increase in uCTX-II and a 45% increase in uNTX-I levels (uCTX-II: 1.28 [95%CI: 1.04–1.58], uNTX-I: 1.45 [95%CI: 1.21–1.74]). Among men, levels of Pb and COMP showed a borderline positive association (8% increase in COMP for a 5-unit change in Pb: 1.08 [95% CI: 1.00–1.18]); no other associations were significant after adjustment.

Conclusions—Based upon known biomarker origins, the novel associations between blood Pb and biomarkers appear to be primarily reflective of relationships to bone and calcified cartilage turnover among women and cartilage metabolism among men, suggesting a potential gender-specific effect of Pb on joint tissue metabolism that may be relevant to osteoarthritis.

Keywords

Biomarkers; blood lead; joints; osteoarthritis

1. Introduction

Approximately 95% of total body lead (Pb) burden in adults is stored in bone (Wittmers, Jr. et al. 1988), and despite decreased Pb exposure in the United States in recent years (Muntner et al. 2005; Centers for Disease Control and Prevention (CDC) 2009), people with previous exposure to this ubiquitous environmental toxicant may have accumulated significant Pb burden in bone. Bone Pb has a half-life of decades, and contributes as much as 65% to measured whole blood Pb levels (Barry and Mossman 1970). The stored bone Pb can be released into blood chronically, especially during times of increased bone turnover (Machida et al. 2009; Nie et al. 2009), making bone both a potential target tissue for Pb toxicity and a persistent endogenous source of Pb (Symanski and Hertz-Picciotto 1995; Mushak 1993; Osterloh et al. 1994; Berlin et al. 1995; Nash et al. 2004). Even mild elevations in blood Pb may have health consequences, including increased mortality, as shown by recent studies using data from the Third National Health and Nutrition Examination Survey (Schober et al. 2006) and the Study of Osteoporotic Fractures (Khalil et al. 2009).

There exist several biologically plausible ways by which long-term exposure to Pb may impact bone and other joint structures in humans. For instance, Pb is known to interfere with dietary calcium uptake and metabolism, conversion of vitamin D to 1, 25-OH vitamin D, and other regulatory aspects of bone cellular function and matrix and osteocalcin synthesis (Berlin et al. 1995; Fullmer 1997; Pounds et al. 1991). Pb exposure leads to impaired collagen synthesis by osteoblasts and impaired resorptive capacity by osteoclasts (Pounds et al. 1991). Aside from its effects on bone, recent data suggest effects of Pb on cartilage and chondrocytes as well. Zuscik and colleagues reported reversion of articular chondrocytes to a more primitive phenotype upon Pb exposure, with matrix degradation and mineralization, decreased TGF-β signaling, and chondrocyte hypertrophy (Zuscik et al. 2006). A related study revealed impairments in fracture healing in Pb-exposed mice, with associated delays in endochondral maturation (Carmouche et al. 2005). In a study of bone samples from

individuals without known bone disease or Pb exposure, Zoeger and colleagues identified marked differential accumulation of Pb in the cartilage tidemark, the transition between calcified and non-calcified cartilaginous matrix (Zoeger et al. 2006), where clefts occur as osteoarthritis develops. The affinity of Pb for joint tissues, and the role of Pb in cartilage and bone maturation and repair shown in the above studies, suggest a potential role for Pb in the osteoarthritis disease process, which affects bone, cartilage, and notably the tidemark area of cartilaginous matrix.

Associations between blood and bone Pb and bone biomarkers have been identified (Tsaih et al. 2001; Machida et al. 2009; Dowd et al. 2001), but there have been no studies assessing potential associations between whole blood Pb levels and biomarkers of joint metabolism. We examined a panel of serum and urine biomarkers, as quantitative indicators of bone and cartilage turnover (urinary cross-linked N telopeptide of type I collagen (uNTX-I), urinary C-telopeptide fragments of type II collagen (uCTX-II), serum cartilage oligomeric matrix protein (COMP), serum cleavage neopeptide of type II collagen (C2C), serum type II procollagen synthesis C-propeptide (CPII), the ratio of [C2C:CPII], and hyaluronic acid (HA)), in relationship to whole blood Pb among participants in the Johnston County Osteoarthritis Project. We hypothesized that Pb levels may be associated with both skeletal and other joint tissue metabolism, as reflected by levels of these biomarkers, which may be of potential relevance to osteoarthritis.

2. Patients and Methods

2.1. Study Population

The Johnston County Osteoarthritis Project is a population-based study in which civilian, non-institutionalized African American and White individuals, 45 years of age and older, were recruited from 1991-7 by probability sampling of six townships in Johnston County as previously described (Jordan et al. 2007). The Metals Exposure Sub-study, including whole blood Pb assessment, was designed to consist of 1700 consecutive individuals either newly enrolled in 2003-4, or returning for follow-up in 2006-8. The sample for the current analysis includes individuals who had complete blood Pb data when the biomarker assays were performed, consisting of 342 women and 212 men enrolled in 2003-4, and 117 men returning for follow-up in 2006-8. The characteristics of this subsample were similar to those of the overall metals sample (n=1635, Table 1). This study has been approved by the Institutional Review Boards of the Centers for Disease Control and Prevention and the University of North Carolina.

Demographic and clinical information was obtained by interview, race was self-reported, and current smoking status was assessed by questionnaire, coded as either a current smoker or a current nonsmoker. Participants underwent a clinical evaluation, which included measurement of height (m) without shoes and weight (kg) measured with a balance beam scale, followed by calculation of body mass index (BMI, in kg/m²). For women, menopausal status was determined by an affirmative answer to the following question: "Have you reached menopause or change of life?" For those who were not sure how to answer this question, post-menopausal status was inferred by the absence of menstrual periods for 2 years in women aged 50 and older, or by the report of surgical removal of both ovaries. Female participants were also asked if they were currently using, or had ever used hormone pills, creams, patches, or shots, not including birth control pills. These factors were included in adjusted analyses due to known effects of race, smoking, and menopausal status on biomarker and blood Pb levels.

2.2. Blood Lead (Pb) Assay

Whole blood was collected utilizing certified metal-free blood drawing equipment and vials, and stored at ~4 degrees (Wang and Peter 1985; Subramanian 1995) until shipment in batches of 50–100 to the Division of Laboratory Sciences (National Center for Environmental Health, Centers for Disease Control and Prevention, CDC) for analysis. Whole blood Pb concentrations were determined at the CDC using the PerkinElmer Inductively coupled plasma-dynamic reaction cell-mass spectrometer 6100 ELAN series DRC II, ELAN® DRC II ICP-MS (PerkinElmer SCIEX, Concord, Ontario, Canada) equipped with a Meinhard nebulizer and cyclonic spray chamber. In this multi-element analytical technique, blood samples are diluted with ≥ 18 M-ohm.cm water and with diluent containing 1% v/v tetramethyl-ammonium hydroxide, 0.5% disodium ethylenediamine tetraacetate, 10% ethyl alcohol, and 0.05% Triton X-100. Gold is added to reduce intrinsic mercury memory effects. Bismuth was added for the internal standardization of Pb. The samples were prepared with the following ratio: (Sample: Water: Diluent = 1:1:48). Pb was quantified based on the ratio of analyte signal to that of the internal standard signal in peak hopping mode. The calibration was external matrix matched, control samples were assessed along with participant samples, and accuracy was verified by the analysis of standard reference material (SRM 955 c) from the National Institute of Standards Technology. For Pb the limits of detection in $\mu\text{g/dL}$ was based upon the standardization of base blood material and was 0.25 $\mu\text{g/dL}$ (n=284). The inter-assay precisions (relative standard deviations) for lead was 3.2% at a level of 2.89 $\mu\text{g/dL}$.

2.3. Bone and joint tissue biomarker assays

Blood samples for serum biomarker determination were collected, spun and separated on site, and stored at -86°C . Second morning void urine samples were collected, spun and stored at -86°C within 8 hours of processing. Details of individual marker properties and assays are as follows:

Urine NTX-I (cross linked N telopeptide of type I collagen) is a marker of bone resorption/turnover in health and is increased in osteoporosis (Scariano et al. 1998) and in osteoarthritis (Bettica et al. 2002). Urine NTX-I was measured using the commercially available Osteomark NTX Urine kit (Wampole Laboratories, Princeton, NJ), a competitive-inhibition enzyme linked immunosorbent assay (ELISA) with reported precision of between 5–19% intra-assay and 3–5% inter-assay variability. Results are corrected for urine creatinine concentration with final values reported as nM BCE/mM creatinine.

Urine CTX-II (urinary C-telopeptide fragments of type II collagen) is associated with both the prevalence and the progression of radiographic knee and hip osteoarthritis, independent of known clinical risk factors for osteoarthritis (Christgau et al. 2001; Reijman et al. 2004; Kong et al. 2006; Meulenbelt et al. 2006), and is related to bone, cartilage, and prominently to calcified cartilage turnover (Tomiya et al. 2008; Bay-Jensen et al. 2008). This marker was measured using the commercially available Urine Cartilaps competitive ELISA (Nordic Bioscience, Herlev, Denmark), which has a reported precision of between 4.6–7.8% intra-assay and 6.9–12.2% inter-assay variability. Results are corrected for urine creatinine concentration and final values reported as ng CTX-II /mM creatinine.

Serum COMP (cartilage oligomeric matrix protein), a non-collagenous matrix protein found in cartilage, meniscus, tendon, and ligament (Garnero et al. 2000; Petersson et al. 1998), is a widely studied and validated cartilage biomarker in relation to osteoarthritis (Clark et al. 1999; Jordan et al. 2003; Sharif et al. 1995b). COMP was measured using a previously described sandwich ELISA method utilizing monoclonal antibodies 16F12

and 17C10, with a precision of between 5.8–6.6% intra-assay and 8.7–9.7% inter-assay variability (Vilim V et al. 2002). Results are reported as ng/ml COMP.

Serum C2C (collagenase-generated cleavage neopeptide of type II collagen) is indicative of cartilage collagen degradation, and is elevated in animal models of osteoarthritis (Chu et al. 2002; Matyas et al. 2004). Elevation of urine C2C (but not serum) is associated with pre-radiographic knee osteoarthritis onset (Cibere et al. 2009); in this study only serum C2C was measured, using the commercially available Collagen Type II Cleavage ELISA (Ibex Pharmaceuticals, Montreal, Quebec, Canada). This competitive ELISA has a reported precision of 9.7% intra-assay (Mazzuca et al. 2006) and <20% inter-assay variability (Ibex data sheet). Results are reported as ng/ml C2C.

Serum CPII (type II collagen c-propeptide) is cleaved from type II procollagen following release of synthesized procollagen in cartilage matrix, and is therefore indicative of type II collagen synthesis (Nelson et al. 1998); CPII was measured using the commercially available Procollagen II C-Propeptide ELISA (Ibex Pharmaceuticals, Montreal, Quebec, Canada). This competitive ELISA has a reported precision of 6.4% intra-assay (Mazzuca et al. 2006) and <25% inter-assay variability (Ibex data sheet). Results are reported as ng/ml CPII.

Serum [C2C:CPII] ratio was calculated from the values of serum C2C and CPII. This ratio is indicator of the balance between cartilage collagen degradation and synthesis which is reflective progression of knee osteoarthritis (Cahue et al. 2007).

Serum HA (hyaluronan), a widely distributed large polysaccharide, is usually regarded as an indicator of synovial inflammation in arthritis (Wells et al. 1992; Georges et al. 1997; Sharif et al. 1995a), and may be associated with total osteoarthritis burden (Kraus VB et al. 2009). We and others have reported strong and robust associations between both serum COMP and serum HA levels and the presence and severity of radiographic osteoarthritis (Clark et al. 1999; Sharif et al. 1995b). It was measured using the commercially available Hyaluronic Acid Test kit (Corgenix, Westminster, CO), an enzyme-linked binding protein assay with a reported precision of between 3.6–4.7% intra-assay and 5.7–7.0% inter-assay variability. Results are reported in ng/ml HA.

Urine Creatinine was measured using the commercially available Metra Creatinine Assay kit (Quidel, San Diego, CA), a colorimetric assay based on a modified Jaffe method with a reported precision of between 1.1–2.1% intra-assay and 2.0–6.9% inter-assay variability. Results were used to correct the urine measures (uCTX-II and uNTX-I).

2.4. Statistical Analysis

A total of 329 men and 342 women had available data for whole blood Pb and serum and urine biomarker (uNTX-I, uCTX-II, COMP, C2C, CPII, [C2C:CPII], and HA) analyses. Due to known gender differences in biomarkers of joint metabolism (Jordan et al. 2003; Kojima et al. 2008; Elliot et al. 2005; Mouritzen et al. 2003), previously shown variations in Pb level by gender, and our a priori hypotheses, all results were stratified by gender. Natural logarithm transformation was used to produce distributions closer to normality for the biomarkers in these analyses. Descriptive statistics were calculated for demographic variables, smoking status, BMI, blood Pb, and the biomarkers. Spearman correlation coefficients and 95% CIs were computed between Pb and each biomarker. Due to known effects of race and smoking both Pb and biomarker levels, the differences in mean Pb (ln-transformed f or t-tests) and biomarkers by race and smoking status were tested by separate two sample Student's t-tests.

Separate multiple linear regression models were used to examine the associations between Pb and the natural log of each of the biomarkers: uNTX-I, uCTX-II, COMP, C2C, CPII,

[C2C:CP11], and HA. The models were adjusted for variables known to impact blood Pb and/or biomarker levels: categorical race and smoking status, and continuous age and BMI. All of these covariates affected the estimates and were included in final models. Among women only, additional analyses were conducted restricted to only post-menopausal women and further adjusted for current hormone replacement therapy. The results of these models are presented as the estimated change in biomarker level for each 5-unit change in continuous blood Pb level.

A full model for each biomarker included two-way interactions between Pb and each of age, BMI, race, and smoking, as well as polynomials of Pb up to the cubic power. Backward selection was conducted to remove one interaction or the highest order polynomial term at a time until all $p < 0.1$. A p -value < 0.1 for an interaction term was considered noteworthy for potential effect modification, and supplemental analyses including the interaction term were performed as needed.

Sensitivity analyses were performed by inclusion and exclusion of the outlying observations, which were defined as those with studentized residuals >3 (Kutner M et al. 2004). One high value and one low value of COMP were excluded from the analysis of COMP among women, as the results were found to be sensitive to these two outlying values. No other biomarker analyses were sensitive to outliers (results did not change when the values were excluded), so all of the available observations were included in the remainder of the analyses.

3. Results

3.1. Women

There were 342 women with data for biomarkers and whole blood Pb; of these, one was missing menopausal status, two were missing HRT use status, 3 were missing serum samples, and 12 did not have available urine samples. This left 330 women for analyses of urine biomarkers and 339 women for serum biomarkers. Demographic and clinical characteristics are shown in Table 1. Participants had a mean age of 62 years, slightly over one-third of women were African American, 18% were current smokers, and about 1/3 had radiographic knee osteoarthritis. The mean BMI among these women was about 31 kg/m². The vast majority (96%) were post-menopausal, with 18% of those reporting current HRT usage. The median and range values for whole blood Pb and biomarkers are shown in Table 2.

The median Pb level was 1.9 µg/dL (range from 0.5–25.4 µg/dL). The mean Pb level (ln-transformed for t-tests) was noted to be higher among African American compared to Caucasian women, as were mean levels of ln uCTX-II, ln COMP, and ln C2C. Unadjusted analyses showed significant correlations between Pb and uNTX-I, uCTX-II, and COMP, but not with any of the other biomarkers (Table 3).

After adjustment for age, race, smoking status, and BMI, we identified associations between Pb and the levels of urine biomarkers uNTX-I and uCTX-II, biomarkers primarily reflective of turnover of mineralized tissues. A 5-unit increase in blood Pb level was associated with a 45% increase in uNTX-I and a 28% increase in uCTX-II levels (Table 4). These positive associations were unchanged before and after adjustment, and the results were stable when limited to postmenopausal women and further adjusted for HRT use (data not shown).

Significant interactions between age and Pb were identified for C2C among women (p interaction 0.07), indicating that age affects the Pb effect for this biomarker. For representative ages 55, 65, and 75, the expected fold changes in C2C were 1.08, 1.00, and

0.91 for each 5-fold change in Pb, respectively (Table 4). There were no significant associations between Pb and COMP, HA, CII, or the ratio [C2C:CII], among the entire sample of women (Table 4), or in analyses limited to post-menopausal women or further adjusted for HRT use ($p>0.1$ for all, data not shown).

3.2. Men

The sample consisted of 329 men, with demographic and clinical characteristics as shown in Table 1. The mean age among the men was 65 years, with a mean BMI of 30 kg/m². Of this group, 37% were African American, 23% were current smokers, and nearly 40% had radiographic knee osteoarthritis. The median and range values for whole blood Pb and biomarkers are shown in Table 2.

The median Pb level was 2.2 µg/dL (range from 0.5–25.1 µg/dL). The mean Pb level (ln-transformed for t-tests) was noted to be higher among African American compared to Caucasian men, and among current smokers compared to non-smokers. Mean levels of ln C2C were higher among African American compared to Caucasian men, while ln uNTX-I was higher in Caucasian men; other markers did not differ by race. Pb levels were significantly positively correlated with uCTX-II, COMP, and the ratio [C2C:CII], and negatively correlated with CII levels, but not correlated with uNTX-I, C2C, or HA (Table 3). There were no race or age interactions identified for Pb and any of the biomarker outcomes among men.

After adjustment for age, race, smoking status, and BMI, the only significant association was a borderline one between Pb and COMP, such that for a 5-unit increase in blood Pb, there was an 8% increase in COMP level (1.08 [95%CI: 1.00–1.18]). There were no significant associations among men between Pb and uNTX-I, uCTX-II, C2C, CII, [C2C:CII], or HA.

4. Discussion

In summary, in unadjusted analyses, significant correlations were seen between Pb level and levels of uNTX-I, uCTX-II, and COMP among women and levels of uCTX-II, COMP, CII, and [C2C:CII] among men. After adjustment, among women, we identified statistically significant positive associations between whole blood Pb level and urine NTX-I and CTX-II, markers of bone and calcified cartilage turnover, respectively. Among men, there was a statistically significant positive association between blood Pb and COMP after adjustment. Blood Pb was not significantly associated with serum C2C, CII, the ratio [C2C:CII], or HA, in adjusted analyses in men or women.

The positive association among women between blood Pb and uCTX-II level in this analysis signifies a potential association between Pb exposure and joint tissue metabolism, although we cannot determine the direction of this effect from the present cross-sectional study. It is unclear whether this finding reflects a direct effect of Pb on cartilage, or an indirect effect mediated by adverse effects of Pb on bone. Urine CTX-II is a quantitative marker of radiographic osteoarthritis (Meulenbelt et al. 2006;Reijman et al. 2004), and may be a relatively specific biomarker of calcified cartilage turnover at the junction of cartilage and subchondral bone (Tomiya et al. 2008;Bay-Jensen et al. 2008). Given the reported propensity for accumulation of Pb at the tidemark, the area of transition between calcified and more metabolically active non-calcified cartilage (Zoeger et al. 2006), and the report that CTX-II is primarily derived from sites at or near the junction of cartilage and subchondral bone (Bay-Jensen et al. 2008), uCTX-II may be a specific biomarker for the joint tissue most affected by Pb toxicity. We have identified significant associations between

blood Pb and uCTX-II among women in our cohort; confirmation of this finding in other populations will provide a better understanding of its potential implications.

The strongest association between Pb and a biomarker among women was for uNTX-I, a marker of bone turnover in osteoporosis (Scariano et al. 1998) and in osteoarthritis (Bettica et al. 2002). Consistent with prior studies of markers of bone turnover and blood Pb levels (Potula et al. 2005; Machida et al. 2009), uNTX-I was highly associated with blood Pb level in the current analysis. Despite known variations in uNTX-I by menopausal and hormone replacement status, there was no difference after adjustment for these factors in the current analysis, potentially due to small numbers of premenopausal women and of women on hormone replacement therapy. The observed association in the current analysis between elevated levels of uNTX-I and higher levels of blood Pb is likely related to increased release of Pb into blood from bone in this population of primarily post-menopausal women in a high bone turnover state, consistent with prior reports (Machida et al. 2009). Although we did not identify an association between uNTX-I and blood Pb in men, in a study of elderly men participating in the VA Normative aging study, there was evidence of increased blood Pb with increasing uNTX-I levels, although this was not the focus of the study (Nie et al. 2009). The men in that study were older (mean age 69 years) with a higher median blood Pb level (5 µg/dL) compared to the current analysis, which may at least partly explain this difference.

In contrast to the women, the only significant association among the men was between blood Pb and serum COMP. Serum COMP, one of the most studied and validated cartilage biomarkers in relation to osteoarthritis (Clark et al. 1999; Jordan et al. 2003; Sharif et al. 1995b), is not specific to cartilage, as it is also found in meniscus, tendon, and ligament (Garnero et al. 2000; Petersson et al. 1998). Among women, there was no significant association between serum COMP and blood Pb after adjustment, despite a significant Spearman correlation coefficient. Prior studies have shown gender differences in COMP (Jordan et al. 2003; Sharif et al. 1995b; Kumm et al. 2006), with generally higher levels among men.

No significant associations were identified between Pb and serum CPII (marker of collagen synthesis), serum C2C (marker of cartilage degradation), or their ratio [C2C:CPII] in adjusted analyses, despite significant correlations between Pb level and CPII and [C2C:CPII] among men. There was a significant interaction between age and C2C among women, but the association between Pb and serum C2C was not significant in either age group. A lack of association between radiographic osteoarthritis and serum C2C has been reported (Mazucca et al. 2006; Cahue et al. 2007; Cibere et al. 2009), and may represent a lack of a detectable relationship between the effects of Pb on uncalcified cartilage degradation, as reflected by serum C2C. Therefore, urine C2C is recommended for future studies, as unlike serum C2C this biomarker has revealed alterations in cartilage collagen degradation with the onset of knee osteoarthritis (Cibere et al. 2009). The lack of association between Pb and the ratio [C2C:CPII], despite evidence in the literature that such marker ratios may provide additional information beyond that from individual markers in isolation (Cahue et al. 2007; Cibere et al. 2009), may be in part due to adjustment of the models for race (associated with C2C) and smoking (associated with CPII).

The lack of association between blood Pb and serum HA, often considered a marker of synovial inflammation (Wells et al. 1992; Sharif et al. 1995a), among men or women, is not supportive of a Pb effect on synovium. Studies have shown an effect of Pb on cultured synovial cells, including decreased proliferation and protein synthesis, but only in an in vitro experiment using 10-fold higher concentrations of Pb, so it is unclear how those findings relate to Pb level and serum HA as analyzed in the current study (Goldberg et al. 1983).

There are some limitations to the current study. Whole blood Pb measurements were used as a biomarker for Pb exposure. Specialized x-ray fluorescence techniques to assess bone Pb are considered the “gold standard” for measurement of Pb storage in bone, but this procedure is expensive and not widely available (Barbosa et al. 2005; Sakai 2000). Since blood Pb levels reflect recent exposure as well as mobilization of Pb from bone (Barbosa et al. 2005; Nie et al. 2009), and blood Pb has been associated with all-cause mortality, cardiovascular disease, and renal disease (Staessen et al. 1992; Jain et al. 2007; Martin et al. 2006; Schober et al. 2006; Weisskopf et al. 2009; Khalil et al. 2009), this measure of Pb was used as a readily available and economical alternative biomarker for Pb exposure (Barbosa et al. 2005; Sakai 2000). The current study is also limited due to its cross-sectional design, although the potential for future longitudinal studies of Pb in this population exist. Bone density measurements have been collected as part of the parent study but were not interpreted or analyzed at the time of this analysis. The strengths of this study include a population-based, biracial sample and the inclusion of multiple biomarkers representing turnover of different joint tissues.

5. Conclusions

In conclusion, this is the first study evaluating potential effects of low levels of whole blood Pb on multiple biomarkers of joint tissue metabolism. Whole blood Pb levels reflect systemic Pb from both current exposure and bone turnover causing release of stored Pb. Our results suggest that systemic Pb is associated with bone turnover (uNTX-I), as well as mineralized (uCTX-II) among women, and non-mineralized (COMP) cartilage turnover among men, but as this is a cross-sectional analysis, we cannot determine whether cartilage turnover is an indicator, or consequence, of Pb release. The association between blood Pb level and cartilage-associated biomarkers, along with our recent cross-sectional analysis showing associations between blood Pb and radiographic knee osteoarthritis and knee symptoms (Nelson et al. 2011), suggests the possibility that Pb may play a role in the pathophysiology of osteoarthritis. Longitudinal studies including assessments of radiographic and symptomatic osteoarthritis, in conjunction with both blood and bone Pb, would be valuable to further evaluate the potential effects of Pb on joint symptoms, onset and progression of this highly prevalent and often debilitating disease.

Research Highlights

- We assessed whole blood lead in conjunction with osteoarthritis biomarkers.
- In women, blood lead is associated with urine biomarkers of calcified cartilage and bone turnover,
- And in men with cartilage oligomeric matrix protein, a serum marker of joint tissue metabolism,
- Suggestive of a gender-specific effect of lead on joint tissues of potential relevance in osteoarthritis.

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Abbreviations

uNTX-I	urinary cross-linked N telopeptide of type I collagen
uCTX-II	urinary C-telopeptide fragments of type II collagen
COMP	cartilage oligomeric matrix protein
C2C	cleavage neoepitope of type II collagen
CPII	type II procollagen synthesis C-propeptide
[C2C:CPII]	ratio of C2C to CPII
HA	hyaluronic acid
Pb	lead

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Table 1

Characteristics of the biomarkers and metals exposure sub-study of the Johnston County Osteoarthritis Project

Clinical Characteristics	Women (n=342)	Men (n=329)	Overall (n=1635)*
	<i>Mean (SD) or %</i>	<i>Mean (SD) or %</i>	<i>Mean (SD) or %</i>
Mean age (SD, years)	62.4 (9.4)	64.5 (10.8)	65.3 (11.0)
Mean BMI (SD, in kg/m ²)	30.8 (7.0)	29.8 (5.4)	31.2 (7.1)
African American (%)	35.1	37.4	35.6
Current smoker (%)	17.7	23.1	16.5
Knee Osteoarthritis (%) [†]	32.8	38.9	40.3

SD: standard deviation; BMI: Body Mass Index

* Overall numbers are for the entire sample with blood Pb and knee radiographs, including the biomarker subset.

[†] Knee osteoarthritis defined as a Kellgren-Lawrence grade ≥ 2 at either knee or knee replacement due to osteoarthritis.

Table 2

Whole blood lead and serum or urine biomarker levels in the biomarkers and metals exposure sub-study of the Johnston County Osteoarthritis Project, by gender

Concentration	Women (n=342) ^a		Men (n=329)	
	<i>Median</i>	<i>Range</i>	<i>Median</i>	<i>Range</i>
Whole blood Lead (µg/dL)	1.9	0.5–25.4	2.2	0.5–25.1
uNTX-I (nmol BCE/mmol creatinine)	86.5	12.8–996.4	39.5	5.9–4214.7
uCTX-II (ng/mmol creatinine)	185.2	8.09–1396.6	234.5	47.5–2705.0
COMP (ng/mL)	1597.0	472.9–4429.6	1329.0	339.3–7279.0
C2C (ng/mL)	167.7	72.0–1060.2	177.1	54.0–935.6
CP II (ng/mL)	857.6	228.0–2991.3	937.8	319.9–2352.1
[C2C:CPII] ratio	0.20	0.05–0.76	0.19	0.05–0.92
HA (ng/mL)	12.5	0.1–391.3	40.8	0.6–707.8

^a 3 missing serum and 12 missing urine data

uCTX-II- Urine C-telopeptide fragments of type II collagen, COMP-Cartilage Oligomeric matrix protein, CPII: type II collagen carboxy-propeptide, C2C: cleavage neoepitope of type II collagen, HA- Hyaluronic Acid, uNTX-I- urine N-telopeptide of type I collagen, BCE: bone collagen equivalent

Table 3

Spearman correlation coefficients (unadjusted) between individual biomarkers and blood Pb in the biomarkers and metals exposure sub-study of the Johnston County Osteoarthritis Project

Biomarker	Women		Men	
	<i>Spearman coefficient</i>	<i>95% CI</i>	<i>Spearman coefficient</i>	<i>95% CI</i>
uNTX-I	0.220	0.107, 0.314	0.005	−0.103, 0.113
uCTX-II	0.202	0.093, 0.300	0.113	0.004, 0.218
COMP	0.138	0.016, 0.226	0.231	0.125, 0.330
C2C	−0.013	−0.120, 0.093	0.038	−0.070, 0.146
CPII	0.028	−0.079, 0.134	−0.110	−0.215, −0.002
[C2C:CPII]	−0.026	−0.132, 0.081	0.144	0.037, 0.248
HA	0.067	−0.039, 0.172	−0.003	−0.110, 0.106

uNTX-I- urine N-telopeptide of type I collagen, uCTX-II- Urine C-telopeptide fragments of type II collagen, COMP-Cartilage oligomeric matrix protein, HA- Hyaluronic Acid, C2C: cleavage neoepitope of type II collagen, CPII: type II collagen carboxy-propeptide

Table 4

Adjusted estimates^a and 95% confidence intervals for the expected fold change in biomarker level for a 5-unit change in Pb level in the biomarkers and metals exposure sub-study of the Johnston County Osteoarthritis Project

	Women (n=339) ^b		Men (n=329)	
	Estimate	95% CI	Estimate	95% CI
uNTX-I	1.45	1.21–1.74	1.06	0.95–1.18
uCTX-II	1.28	1.04–1.58	1.07	0.97–1.18
COMP ^c	0.96	0.87–1.06	1.08	1.00–1.18
C2C ^c	1.08	0.98–1.20		
	55 yrs			
	65 yrs	1.00	0.92–1.08	1.00
	75 yrs	0.91	0.79–1.06	
CPII	1.09	0.97–1.22	0.99	0.93–1.05
[C2C:CPII]	0.94	0.83–1.05	1.00	0.93–1.07
HA	0.96	0.71–1.29	1.01	0.88–1.16

^aResults from general linear regression models adjusted for continuous age and BMI, and categorical race and smoking status

^bWomen: n for urine biomarkers =330, for COMP=337

^cThere was a significant interaction between age and Pb among women only for C2C (p interaction 0.07 for each), so estimates are given for the representative ages 55, 65, and 75 years.