THE ASSOCIATIONS OF VITAMIN D AND METAL EXPOSURES WITH INFLAMMATION, AUTOIMMUNITY, AND BLOOD PRESSURE

by

Rachel D. Zamoiski, MPH

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Abstract

$1,25(OH)_2D$, the biologically active form of vitamin D, is not commonly measured, as it is tightly regulated and does not change with supplementation or sun exposure unlike 25(OH)D. Prior studies suggest that exposure to metals may inhibit production of $1,25(OH)_2D$ from 25(OH)D. Epidemiological studies have linked higher 25(OH)D with lower inflammation, while not measuring $1,25(OH)_2D$. In addition, while many studies suggest opposing effects of lead (Pb) and 25(OH)D on blood pressure, no study has looked at them together. Finally, while several metals are thought to increase inflammation, only mercury (Hg) has been studied as a risk factor for autoimmunity.

The goal of this dissertation was to expand upon previous studies suggesting inhibition of production of $1,25(OH)_2D$ with exposure to metals, to expand upon previous research suggesting an inverse association between 25(OH)D and inflammation by including $1,25(OH)_2D$; to see if 25(OH)D would attenuate the association between Pb and increased blood pressure; and to see if metals other than Hg increase risk for autoimmunity.

We first conducted an analysis of metals with 25(OH)D and $1,25(OH)_2D$. The study population was a cohort of adolescents aged 12-15 residing in Torreon, a city in Mexico home to a large Pb smelter. We found no evidence that any metal was

inhibiting production of $1,25(OH)_2D$, and instead found that higher exposures to uranium (U) and arsenic (As) were associated with increased levels of $1,25(OH)_2D$.

Second, we described the associations of $25(OH)D$ and $1,25(OH)_2D$ with inflammation, using the study population from Torreon. $25(OH)D$ was positively associated with IL-1β and P-selectin, and inversely associated with ICAM and VCAM. $1,25(OH)_2D$ was positively associated with ICAM, VCAM, and TNF-α.

Third, we used logistic regression to analyze metals as a risk factor for autoimmunity measured by anti-nuclear antibodies (ANA). We found no significant associations between ANA and any of the eight metals measured, suggesting that increased risk of autoimmunity may be unique to Hg.

Finally, we used the NHANES dataset to assess $25(OH)D$ as an effect modifier of Pb and blood pressure. While we did find a significant interaction of $25(OH)D$ and Pb, the interaction term was no longer significant after adjusting for socioeconomic status and smoking.

Dissertation Readers

Ellen K. Silbergeld, PhD (Advisor)

Mary Fox, PhD (alternate)

Eliseo Guallar, MD DrPH

Ana Navas-Acien, MD PhD

Noel R. Rose, MD PhD

Virginia Weaver, MD MPH (alternate)

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CHAPTER 1: Introduction

The goal of this research is to test the hypotheses that metals or arsenic inhibit the production of the active form of vitamin D, that vitamin D status is an effect modifier for the relationship between lead and cardiovascular health, that several metabolites of vitamin D are all inversely associated with biomarkers of inflammation, and that exposure to metals other than Hg will increase risk for autoimmunity. The hypotheses will be tested by using biomarkers of exposure and biomarkers of vitamin D status as independent variables. Outcome variables will include biomarkers of inflammation and autoimmunity, and functional assessments of cardiovascular health. While there is a large amount of information on the association between metal exposures and cardiovascular health, and there is also a large amount of information on the association between vitamin D and cardiovascular health, no study to date looks at metals and vitamin D together. There are also many studies suggesting an inverse association between 25(OH)D and inflammation, but no epidemiological studies on $1,25(OH)_2D$ and inflammation. In addition, there is a large amount of information on mercury and autoimmunity but no epidemiological studies on other metals.

1.1 Goals and Objectives

The goal of this project is to test the hypotheses that metals and arsenic inhibit the production of the active form of vitamin D, that both the inactive and active form of

vitamin D are inversely associated with inflammation, that exposure to metals will increase risk for autoimmunity, and that optimal vitamin D status will attenuate the relationship between lead exposure and increasing blood pressure.

1.2 Specific Aims

1. To evaluate the inhibitory effect of lead, cadmium, arsenic, and uranium on the production of the active form of vitamin D, $1,25(OH)_2D$, from $25(OH)D$.
2. To evaluate the associations between both $25(OH)D$ and $1,25(OH)_2D$ and biomarkers of inflammation.
3. To determine whether metals besides mercury increase risk for autoimmunity.
4. To evaluate the role of vitamin D in the context of exposure to lead in influencing blood pressure.

1.3 Hypotheses

1. Metal dose is associated with lower production of $1,25(OH)_2D$, the active form of vitamin D, from $25(OH)D$ in the kidney, due to the effect of metals on the enzyme 1α-hydroxylase in the renal proximal tubule.
2. Both $25(OH)D$ and $1,25(OH)_2D$ will be inversely associated with inflammatory biomarkers.
3. Exposure to metals known to influence inflammation will be associated with increased risk of autoimmunity, as measured by anti-nuclear antibodies (ANA).

4. Higher 25(OH)D will attenuate the association between lead and increasing blood pressure.

1.4 Background

1.4.1 Vitamin D

Vitamin D is a hormone that is produced in the skin upon exposure to UVB radiation. UVB stimulates the conversion of 7-dehydrocholesterol to vitamin D, which is then hydroxylated to 25(OH)D in the liver, and then further hydroxylated to $1,25(OH)_2D$ by the enzyme 1α-hydroxylase in the renal proximal tubule(Holick, 2007). Although $1,25(OH)_2D$ is the biologically active form of vitamin D, and the form that binds with highest affinity to the vitamin D receptor, it is not commonly measured clinically or in epidemiological studies. Instead, 25(OH)D is more typically measured.

In the US, levels of 25(OH)D below the recommended level are highly prevalent, likely due to large amounts of time spent indoors, and sun avoidance behaviors. According to NHANES data, the average serum 25(OH)D level for white people is 26 ng/mL, while the recommended optimal level is above 30 ng/mL (Holick, 2007). Average 25(OH)D levels in darker-skinned people are even lower. Black people have the lowest levels of 25(OH)D, with an average of 16 ng/mL in NHANES, and Hispanics average 21 ng/mL. This is thought to be due to differences in the efficiency of transdermal absorption of UVB. Although many foods are fortified with

vitamin D, only about 10-20% of vitamin D in humans comes from dietary sources, with the major source being sunlight exposure (Pilz et al., 2011). Data suggest that subjects exposed to UVB light for only a few weeks have measurable increases in 25(OH)D (Pilz, Tomaschitz, Ritz, & Pieber, 2009).

1.4.2 Vitamin D and blood pressure

25(OH)D is inversely associated with cardiovascular disease risk, including hypertension and cardiovascular mortality (Ganji, Zhang, Shaikh, & Tangpricha, 2011). Studies on cell cultures suggest that vitamin D directly inhibits expression of the renin gene, leading to an overall decrease in expression of the renin-angiotensin system, and therefore a decrease in blood pressure (Li et al., 2004). Lower levels of vitamin D are also associated with an upregulation of the renin-angiotensin system and increases in blood pressure (J. H. Lee, O'Keefe, Bell, Hensrud, & Holick, 2008; Li et al., 2004). Indirect evidence comes from a study of exposure to UVB light for six minutes, three times a week, for six weeks that was shown to decrease systolic and diastolic blood pressure in participants receiving UVB radiation compared to participants receiving UVA radiation, which does not stimulate production of vitamin D (Krause, Bühring, Hopfenmüller, Holick, & Sharma, 1998). Administration of vitamin D supplements has also been associated with a decrease in blood pressure (Judd, Raiser, Kumari, & Tangpricha, 2010). In adults, the typical age-associated increase in blood pressure that is part of normal aging appears to be attenuated in people with higher vitamin D (Judd, Nanes, Ziegler, Wilson, &

Tangpricha, 2008). The relationship between cardiovascular health and vitamin D is also seen in adolescents, with lower levels of vitamin D associated with higher blood pressure, as well as abdominal obesity and hyperglycemia, even after adjusting for potential confounders including age, race/ethnicity, socioeconomic status, and physical activity (Reis, Muhlen, Miller, Michos, & Appel, 2009).

1.4.3 Vitamin D and inflammation

Vitamin D is inversely associated with serum markers of inflammation, and $1,25(OH)_2D$ has been shown to decrease the production of inflammatory cytokines (Guillot, Semerano, Saidenberg-Kermanac'h, Falgarone, & Boissier, 2010). Data from epidemiological studies suggest that individuals categorized as vitamin D deficient (defined as <25 nmol/L or <10 ng/mL 25(OH)D) have higher levels of the inflammatory cytokines IL-6 and C-reactive protein (CRP) (Laird et al., 2014; Shepherd et al., 2014). One randomized controlled trial of vitamin D supplementation found no significant changes in either CRP, IL-6, IL-10, or TNF-α (Chandler et al., 2014), suggesting that the inverse relationships seen in cross-sectional epidemiological studies of 25(OH)D and inflammation may not be due to 25(OH)D causing a reduction in inflammation. Other explanations for the associations seen between 25(OH)D and inflammation could be that higher levels of inflammation cause a decrease in 25(OH)D, or an unmeasured confounder could be influencing both 25(OH)D and inflammation. Another randomized controlled trial of vitamin D supplementation found no effect of supplementation on level of CRP,

although supplementation did decrease fasting glucose levels, insulin, HOMA-IR, and LDL, all important cardiovascular biomarkers (Asemi, Hashemi, Karamali, Samimi, & Esmaillzadeh, 2013). This study suggests that while vitamin D may certainly play a causal role in some of the positive health effects associated with it, there is no evidence to suggest that inflammation is one of those health effects causally related to vitamin D.

In addition, individuals who are deficient in vitamin D are at higher risk for developing certain autoimmune diseases, including type 1 diabetes, multiple sclerosis, rheumatoid arthritis, and systemic lupus erythematosus, suggesting a role for vitamin D as a regulator of the immune system (Guillot et al., 2010). Consistent with the role of vitamin D as an immune system regulator, vitamin D supplementation has been associated with a significant decrease in risk of type 1 diabetes (Hyppönen, Läärä, Reunanen, Järvelin, & Virtanen, 2001).

Inflammation has clinical significance in the general population as a risk factor for cardiovascular disease. Atherosclerosis is now understood to be a disease greatly influenced by inflammation, and not simply an accumulation of lipids in the endothelium (Libby, 2002). Inflammation aids in the binding of white blood cells to the endothelium, and leukocytes are found in atherosclerotic lesions (Libby, 2002). Inflammation also facilitates the formation of thrombi, which are the cause of the majority of acute complications due to atherosclerosis, such as myocardial infarction or stroke (Libby, 2002). In addition, inflammatory processes in

atherosclerotic plaques can weaken the protective cap of a plaque, making it more likely to rupture (Libby, 2002). Evidence suggests that C-reactive protein, a biomarker of inflammation, is a stronger predictor of cardiovascular events than LDL cholesterol (Ridker, Rifai, Rose, Buring, & Cook, 2002). C-reactive protein is not correlated with LDL, and it remains highly predictive even after adjusting for traditional Framingham risk factors (S. Lee et al., 2011b; Madjid & Willerson, 2011; Ridker et al., 2002; Ridker & Silvertown, 2008).

1.4.4 Effects of metals on vitamin D metabolism

Several studies involving human or animal data suggest that metals may interfere with the metabolism of vitamin D by inhibiting production of 1,25(OH)2D. While the data on effects of metals on vitamin D metabolism is very limited, exposures to some metals known to affect the renal proximal tubule have been associated with decreased production of $1,25(OH)_2D$ in several studies. In one study of children with exposure to Pb, the children with higher blood Pb levels were shown to have reduced levels of $1,25(OH)_2D$ (Rosen, Chesney, Hamstra, DeLuca, & Mahaffey, 1980). In that study, the participants were divided into three groups depending on their level of Pb. In the lowest group (n=15), the mean blood Pb was 18 μg/dL, the middle group (n=18) had mean blood Pb 47 μg/dL, and the highest group (n=12) had a mean blood Pb of 74 μg/dL. The authors measured 25(OH)D and 1,25(OH)2D. The mean 25(OH)D for groups 1, 2, and 3 were 27 ng/mL, 28 ng/mL, and 20 ng/mL, respectively, and the mean $1,25(OH)_2D$ was 33 pg/mL, 20 pg/mL, and 14 pg/mL,

respectively. Groups 1 and 2 differ on $1,25(OH)_2D$ levels even with similar levels of 25(OH)D, and group 3 had the lowest level of 1,25(OH)2D. Lower $1,25(OH)_2D$ in participants with higher Pb levels is consistent with the hypothesis of decreased production of $1,25(OH)_2D$ as a result of inhibition of the 1α-hydroxylase enzyme in the kidney.

Cadmium (Cd), another metal known to be nephrotoxic (Kazantzis, 1979; J. Liu, Liu, Habeebu, Waalkes, & Klaassen, 2000; Madden & Fowler, 2000), has also been associated with lower levels of $1,25(OH)_2D$ (Nogawa et al., 1990; 1987). Among individuals exposed to Cd, those with evidence of greater renal toxicity have lower levels of $1,25(OH)_2D$ (Tsuritani et al., 1992). Itai-itai disease, which is seen in individuals with occupational exposure to Cd and is characterized by bone pain, is consistent with typical symptoms of vitamin D deficiency (Nogawa et al., 1987). One study on individuals exposed to Cd and patients with itai-itai disease reported mean $1,25(OH)_2D$ levels of 47.6 pg/mL in women exposed to cadmium, 43.5 pg/mL in men exposed to Cd, and 45.2 pg/mL in itai-itai disease patients. In comparison, unexposed women had a mean $1,25(OH)_2D$ of 53.3 pg/mL and unexposed men had a mean $1,25(OH)_2D$ 55.4 pg/mL (Nogawa et al., 1987).

Studies in rats of U, another nephrotoxic metal, have reported that U inhibits the activity of 1α-hydroxylase and reduces levels of $1,25(OH)_2D$ (Minfen Yan et al., 2011; Tissandie et al., 2007). In one study, control rats (n=10) had a mean 25(OH)D of 72.0 nmol/L and a mean $1,25(OH)_2D$ of 55.4 pmol/L, while rats exposed to U

(n=10) had a mean 25(OH)D of 67.5 nmol/L, and their $1,25(OH)_2D$ dropped to 24.3 pmol/L (Tissandie et al., 2007).

The reduction in $1,25(OH)_2D$ could be due to the fact that 1α-hydroxylase is produced in the renal proximal tubule, where these metals also have toxic effects. These data lend support to the hypothesis that toxic effects by metals in the kidney will be associated with lower production of $1,25(OH)_2D$. In addition, 1α-hydroxylase is a part of the cytochrome p450 family of enzymes, which are known to be susceptible to the effects of toxic metals due to effects on heme biosynthesis (Sakaki et al., 2011). This lends further biological plausibility to metals inhibiting the production of $1,25(OH)_2D$.

1.4.5 Metals as risk factors for hypertension

Several metals have been implicated as risk factors for hypertension. Lead exposure has been causally associated with increased systolic blood pressure (Glenn et al., 2006; Weaver et al., 2008) and higher mortality due to cardiovascular disease (Menke, 2006). Cd is also associated with increased risk for hypertension (M.-S. Lee, Park, Hu, & Lee, 2011a). As is also associated with increased blood pressure (Mordukhovich et al., 2011) and increased risk of death from cardiovascular disease (Cheng, Ke, & Guo, 2010). U exposure has also been associated with increased blood pressure, both systolic and diastolic (KURTTIO et al., 2006).

1.4.6 Opposing effects of vitamin D and metals

As reviewed in this introduction, there are many studies suggesting that various metals increase blood pressure and increase risk for hypertension. Similarly, there are numerous studies suggesting that vitamin D decreases blood pressure. While there are numerous studies examining the effects of metals, and of vitamin D, by themselves, there are none, to our knowledge, that look at these exposures together to determine if they attenuate each other.

1.4.7 Metals and autoimmunity

Epidemiological studies of Hg-exposed populations suggest a role for Hg in increasing biomarkers of autoimmunity and risk of autoimmune disease. Populations of gold miners who are highly exposed to Hg through the mining process have been shown to have positive ANA levels in over 50% of the population, whereas non-exposed populations typically have less than 10% of the population with detectable ANA (Gardner et al., 2010b; Silva et al., 2004). In addition to increasing risk for positive ANA, exposure to Hg also is associated with inflammatory cytokines, including IL-1β and TNF-α (Gardner, Nyland, & Silbergeld, 2010a). Occupational exposure to Hg has also been associated with increased risk for systemic lupus erythematosus, an autoimmune disease (Cooper et al., 2004).

Animal models also support the hypothesis of exposure to Hg increasing autoimmunity. Mice administered Hg have increased autoimmunity (Havarinasab & Hultman, 2006; Pollard et al., 2001), and also show a worsening of animal models of lupus and autoimmune cardiomyopathy (Nyland, Fillion, et al., 2011a; Via et al., 2003).

However, while numerous studies suggest a link between Hg exposure and autoimmunity, there are no epidemiological studies on exposure to other metals and risk of autoimmunity. Like Hg, other metals including Pb, Cd, and As increase inflammation. Exposure to Pb in humans has been associated with higher levels of IFN- γ.(Mishra et al., 2003) *In vitro* studies suggest that Pb and Cd exposure may upregulate inflammatory cytokines. (Flohé, Brüggemann, Herder, Goebel, & Kolb, 2002; Krocova, Macela, Kroca, & Hernychova, 2000) One study of Cd exposure in mice suggested that exposure to Cd may increase risk of autoimmunity (Ogawa et al., 2012). Studies of As in both mice and humans suggest that As may upregulate both IL-6 and TNF-α (Das, Santra, Lahiri, & Guha Mazumder, 2005; Wu, Chiou, Ho, Chen, & Lee, 2008).

1.4.8 Torreon, Mexico

Torreon, Mexico is home to the Met-Mex Penoles smelter complex, which is the largest Pb-Zn smelter in Latin America (García-Vargas et al., 2014; Vargas et al., 2001). The smelter is located near the city and is surrounded by residential

neighborhoods (Albalak et al., 2003). As a result of living in close proximity to a large smelter, the residents of Torreon exhibit high levels of exposure to metals including Pb and Cd (Benin, Sargent, Dalton, & Roda, 1999; Calderon-Salinas, Valdez-Anaya, Mazuniga-Charles, & Albores-Medina, 1996; García-Vargas et al., 2014; Vargas et al., 2001). Children in Torreon have been reported to have elevated blood Pb levels and increased risk for Pb poisoning (Albalak et al., 2003). Since the late 1990s, emission controls and interventions, coupled with increased surveillance of BPb levels in Torreon children, have led to significantly decreased Pb levels, although exposure remains a significant threat to public health (García-Vargas et al., 2014). Torreon also has high levels of As in soil and in water (Benin et al., 1999; García-Vargas et al., 2014; Rosado et al., 2007).

1.4.9 National Health and Nutrition Examination Survey

The National Health and Nutrition Examination Survey (NHANES) is a complex, multi-stage survey of the health of the general, non-institutionalized US population. It is administered by the National Center for Health Statistics, Centers for Disease Control and Prevention. Every year, approximately 5000 individuals are enrolled as participants and undergo interviews, physical examinations, and laboratory testing (National Center for Health Statistics, n.d.). The data are publicly available online. The complex, multi-stage survey design of NHANES requires the use of survey weights when analyzing NHANES data. In order to obtain a representative sample, some groups are over-sampled relative to other groups. Analyzing NHANES data as

if it were a random sample of the US population would result in too-small error estimates, so survey weights are provided with the dataset to assign each participant a weight that is used during the analysis, with the over-sampled groups assigned a lower weight.

1.5 Summary and research outline

Data from prior studies suggest that exposure to Pb or Cd may disrupt vitamin D metabolism, resulting in lower levels of $1,25(OH)_2D$ among exposed individuals. Prior studies also suggest that Hg may increase risk for autoimmunity, as miners highly exposed to Hg have significantly elevated prevalence and titers of positive ANA as compared to non-exposed populations. 25(OH)D has been inversely associated with inflammation in epidemiological studies, and while $1,25(OH)_2D$ has been shown to decrease inflammation in laboratory-based studies, its association with inflammation has not been described in an epidemiological study. 25(OH)D has also been shown to be inversely associated with blood pressure, while Pb has been shown to be positively associated with blood pressure, although 25(OH)D and Pb have not been studied together to describe their joint roles on blood pressure, and if one may attenuate the effect of the other.

In this study, several hypotheses will be tested: that metals inhibit production of $1,25(OH)_2D$, that both 25(OH)D and $1,25(OH)_2D$ are inversely associated with

inflammation, that metals will increase risk of autoimmunity, and that higher 25(OH)D will attenuate the association between Pb and blood pressure.

The first three hypotheses will be tested using research participants from the study based in Torreon, Mexico. The fourth hypothesis will be tested using the publicly available NHANES dataset.

CHAPTER 2: Association of Arsenic and Metals with 25-Hydroxyvitamin D and 1,25-dihydroxyvitamin D Levels among Adolescents in Torreon, Mexico

Rachel D. Zamoiski,[1] Eliseo Guallar,[2,3] Gonzalo G. Garcia-Vargas,[4,5] Stephen J. Rothenberg,[6] Carol Resnick,[1] Marisela Rubio-Andrade,[4] Amy J. Steuerwald,[7,8] Patrick J. Parsons,[7,8] Virginia Weaver,[1,3,9] Ana Navas-Acien,[1,2] Ellen K. Silbergeld.[1]

1. Department of Environmental Health Sciences, Johns Hopkins Bloomberg School of Public Health, Johns Hopkins University, Baltimore, MD, USA

2. Department of Epidemiology, Johns Hopkins Bloomberg School of Public Health, Johns Hopkins University, Baltimore, MD, USA

3. Welch Center for Prevention, Epidemiology, and Clinical Research, Johns Hopkins Bloomberg School of Public Health, Johns Hopkins University, Baltimore, MD, USA.

4. Facultad de Medicina, Universidad Juárez del Estado de Durango, Gómez Palacio Durango, México.

5. Secretaría de Salud del Estado de Coahuila, Coahuila, Mexico.

6. Instituto Nacional de Salud Pública, Centro de Investigación en Salud Poblacional, Cuernavaca, Morelos, Mexico.

7. Laboratory of Inorganic and Nuclear Chemistry, Wadsworth Center, New York State Department of Health, Albany, NY, USA.

8. Department of Environmental Health Sciences, School of Public Health, University at Albany, Albany, NY, USA.

9. Johns Hopkins University School of Medicine, Baltimore, MD.

Corresponding author: Ellen K. Silbergeld, Johns Hopkins Bloomberg School of Public Health, 615 N. Wolfe St, E6644, Baltimore MD 21205, 410.955.8678, esilberg@jhsph.edu

Running title: Metal exposure and vitamin D levels.

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2.1 Abstract

Background: Limited data suggest that lead (Pb), cadmium (Cd) and uranium (U) may disrupt vitamin D metabolism and inhibit production of 1,25-dihydroxyvitamin D ($1,25(OH)_2D$), the active vitamin D metabolite, from 25-hydroxyvitamin D ($25(OH)D$) in the kidney.

Objectives: To evaluate the association between blood lead (BPb) and urine arsenic (As), Cd, molybdenum (Mo), thallium (Tl) and U with markers of vitamin D metabolism ($25(OH)D$ and $1,25(OH)_2D$).

Methods: Cross-sectional study of 512 adolescents in Torreon, a smelter town in Mexico. BPb was measured using atomic absorption spectrometry. Urine As, Cd, Mo,

Tl, and U were measured using inductively coupled plasma mass spectrometry. Serum 25(OH)D and $1,25(OH)_2D$ were measured using a chemiluminescent immunoassay and a radioimmunoassay, respectively. Multivariable linear models with vitamin D markers as the outcome were used to estimate the association of As and metals levels with vitamin D levels, controlling for age, sex, adiposity, smoking, SES, and time outdoors.

Results: 25(OH)D was positively associated with Mo and Tl (increase of 1.5 (95% CI 0.4, 2.6) and 1.2 (95% CI 0.3, 2.1) ng/mL per doubling of metal levels, respectively). $1,25(OH)_2D$ was positively associated with As and U (increase of 3.4 (95% CI 0.9, 5.9) and 2.2 (95% CI 0.7, 3.7) pg/mL per doubling of metal levels, respectively), even after controlling for 25(OH)D (increase of 3.3 (95% CI 0.9, 5.8) and 2.1 (95% CI 0.6, 3.6) pg/mL per doubling of metal levels, respectively). Pb and Cd were not associated with 25(OH)D or $1,25(OH)_2D$ levels.

Conclusions: We found no evidence that As or metals inhibit production of $1,25(OH)_2D$. The positive association of U and As with $1,25(OH)_2D$ may be in response to proximal tubule damage, or it could be due to chance. Additional research is needed to confirm these findings and to identify underlying mechanisms.

2.2 Introduction

Vitamin D is a steroid hormone produced in the skin upon exposure to ultraviolet B (UVB) radiation. UVB stimulates the conversion of 7-dehydrocholesterol into vitamin D, which is then hydroxylated in the liver to 25(OH)D and further hydroxylated in the kidney to $1,25(OH)_2D$, the active form of vitamin D (DeLuca, 2004; Holick, 2007). Vitamin D deficiency may play a role in susceptibility to several chronic diseases, including atherosclerosis, hypertension, asthma, certain cancers, and certain autoimmune diseases (Brøndum-Jacobsen, Benn, Jensen, & Nordestgaard, 2012; Giovannucci, Liu, Hollis, & Rimm, 2008; Holick, 2004; 2007; Institute of Medicine (US) Committee to Review Dietary Reference Intakes for Vitamin D and Calcium, Ross, Taylor, Yaktine, & Del Valle, 2011; Ponsonby, McMichael, & van der Mei, 2002). Vitamin D status is influenced by many factors, including sun exposure, adiposity, genetics, skin complexion, geographic latitude, time of year, and age (Holick, 2004; 2007). There is also interest in identifying environmental factors that may influence vitamin D status.

A limited number of studies suggest that exposure to toxic metals may influence vitamin D status. Higher blood lead levels in children were associated with higher 25(OH)D in one study (Kemp et al., 2006) and with lower $1,25(OH)_2D$ in a different study, leading to the hypothesis that Pb could inhibit the production of $1,25(OH)_2D$ in the kidney (Rosen et al., 1980). Cadmium (Cd) exposure has also been associated with lower $1,25(OH)_2D$ concentrations (Nogawa et al., 1987; 1990) but similar

25(OH)D levels compared to unexposed subjects (Nogawa et al., 1990). In addition, experimental studies in rats reported that uranium (U) exposure decreased $1,25(OH)_2D$ levels (Tissandie et al., 2007; 2006) with no change in 25(OH)D levels (Tissandie et al., 2007).

In the present study, we evaluated the association of biomarkers of exposure to arsenic (As) and metals with 25(OH)D and $1,25(OH)_2D$ levels in a population of adolescent boys and girls from a community in Northern Mexico exposed to lead and cadmium emitted by a lead/zinc smelter complex (Albalak et al., 2003) as well as to arsenic and other metals *via* drinking water (Del Razo et al., 2002). We hypothesized that higher levels of BPb and urine Cd, and potentially other trace elements, would be associated with lower levels of $1,25(OH)_2D$.

2.3 Methods

Subjects were recruited in the city of Torreon in northern Mexico. Torreon is the home of the Met-Mex Penoles smelter complex, located in the southern area of the city and surrounded by residential areas (Albalak et al., 2003; Benin et al., 1999; García-Vargas et al., 2014). In addition, the groundwater used for drinking in much of the area contains elevated concentrations of As, up to 50 μg/L or higher (Camacho, Gutiérrez, Alarcón-Herrera, de Lourdes Villalba, & Deng, 2011). Study subjects were sampled among participants in an earlier census for biomonitoring BPb levels conducted by the State of Coahuila Department of Health since 1999. The

study population consisted of boys and girls 12-15 years of age at the time of recruitment (October 2009 to June 2010) who had participated in this earlier census and for whom there was at least one BPb measurement prior to 2004. Census participants were divided into five strata according to prior BPb levels, and subjects were randomly chosen from each stratum until the pre-specified sample size of 512 participants was reached.

The study was approved by the Institutional Review Boards of Juarez University of Durango State, the Johns Hopkins Bloomberg School of Public Health, and the New York State Department of Health. All subjects, as well as their parents or legal guardians, gave signed, informed consent.

Study data were collected by trained personnel in two home visits and a visit to the study clinic. Questionnaire data included information on family structure and income, diet, physical activity, academic history, exposure history of family members to Pb, and household smoking history. As a marker of adiposity, we measured percent fat mass using impedance as measured on a Tanita scale (Model BC-418, Tokyo, Japan). We also obtained a fasting blood specimen and a spot urine specimen from each participant. Blood was collected in EDTA purple top tubes (Becton Dickinson, Franklin Lakes, NJ). Urine was collected in plastic containers that were washed with 10% HNO_3 overnight and rinsed with deionized water. Blood and urine specimens were refrigerated immediately and taken on the same day to the Laboratory of Toxicology at the Juarez University of Durango State where they were

aliquoted and frozen at -80˚C. More details of these methods have been published elsewhere (García-Vargas et al., 2014).

Pb was measured in whole blood using a graphite furnace atomic absorption spectrometer equipped with Zeeman background correction at the Environmental Toxicology Laboratory at Juarez University of Durango State. All samples with a coefficient of variation greater than 5% were re-analyzed. The limit of detection was 0.7 μg/dL and the average CV was 3.9%. Bovine blood was used as a reference standard.

Urine Cd, As, U, molybdenum (Mo), and thallium (Tl) were measured at the Trace Elements Section at the Laboratory of Inorganic and Nuclear Chemistry, Wadsworth Center, New York State Department of Health in Albany, New York. The analyses were carried out using a Perkin Elmer ELAN DRC II inductively coupled plasma-mass spectrometer (ICP-MS) equipped with Dynamic Reaction Cell (DRC-) technology (PerkinElmer Life and Analytical Sciences, Shelton, Connecticut, USA). The limits of detection for As, Cd, Mo, Tl, and U were 1.1, 0.02, 1.0, 0.02, and 0.001 μg/L, respectively. Urine Cd values were corrected for the polyatomic interference from Mo using a procedure previously validated and based on each subject's Mo value. The details of these laboratory methods have been described elsewhere (Pollack et al 2013). Arsenic was not speciated and is reported as total arsenic. More details have been published elsewhere (García-Vargas et al., 2014).

Urine arsenic and metal concentrations were corrected for urine creatinine concentrations to account for variability in dilution in spot urine specimens. Urine creatinine concentrations were measured using a Dimension clinical chemistry system using a Flex reagent cartridge in an enzymatic assay (Siemens Dimension Vista 1500; Siemens Medical Solutions USA, Inc., Malvern, PA, United States).

There were several reasons for choosing the exposures to measure in this study. We wanted to expand upon prior research on vitamin D and Pb, Cd, and U. Although we did not identify prior research on vitamin D and As, we chose to include As in this study because of the high exposure to As through drinking water. Finally, the method of analysis generates the data for all of these metals at the same time, so we included them as an exploratory analysis since they were available.

Vitamin D metabolites were measured in serum at Heartland Assays, a commercial vitamin D laboratory located in Ames, Iowa. Total 25(OH)D was measured using a chemiluminescent immunoassay on a Diasorin Liaison analyzer. $1,25(OH)_2D$ was measured using radioimmunoassay. The details of these assays have been published elsewhere (Hollis & Horst, 2007). The coefficient of variation for a random sample of aliquots used as blind duplicates (n=20) was 8.0% for 25(OH)D and 16.3% for $1,25(OH)_2D$.

Statistical analyses were conducted using Stata 12 (Stata Corp, College Station, TX). As and metal concentrations were log base 2-transformed to correct for observed

skewed distributions. Multiple linear regression models were performed to assess the relationship of each vitamin D metabolite (outcome) with As and metals modeled either as log-transformed continuous variables or as quartiles (predictors). We ran three models with progressive adjustment factors. First we ran a model adjusted for age (continuous) and sex. Then, we ran a second model that added season (January-March, April-May, and October-December), household income ($\geq$ 3000 pesos/month, < 3000 pesos/month, and unknown), smoking (never smokers, former smokers, and current smokers), percent fat mass (continuous), and time spent walking outside (none, < 30 minutes/week, 30 minutes – 2 hours/week, 2-4 hours/week, 4-6 hours/week, and > 6 hours/week). An additional model used $1,25(OH)_2D$ as the outcome variable with 25(OH)D as a predictor, since 25(OH)D is a precursor to $1,25(OH)_2D$. For exploratory purposes we also conducted stratified analyses by sex. In the models using quartiles, instead of individual p-values for each quartile, p-trend was calculated by modeling the quartiles as a continuous variable.

As a sensitivity analysis, we reanalyzed the data after log-transforming 25(OH)D and $1,25(OH)_2D$ since the levels of vitamin D biomarkers were slightly right skewed (data not shown). In addition, we repeated the analyses for As after excluding participants who reported eating fish in the previous week to eliminate the influence of arsenobetaine and other organic arsenic compunds present in fish (Navas-Acien, Francesconi, Silbergeld, & Guallar, 2011). We also adjusted for milk consumption, since milk is fortified with vitamin D (data not shown). We also repeated the analyses using urine measurements corrected for osmolality instead of

creatinine (data not shown). Finally, to test for influential outliers, we used added-variable plots to identify outliers and then reanalyzed the data excluding these outliers (data not shown). The results of all these sensitivity analyses were similar to the original analyses.

2.4 Results

The mean (standard deviation) age of study participants was 14.0 (1.2) years. 262 subjects (51.2%) were male (Table 1). Mean (standard deviation) 25(OH)D concentration was 24.9 (8.2) ng/mL and mean (standard deviation) $1,25(OH)_2D$ concentration was 58.6 (18.3) pg/mL. The Spearman correlation coefficient between both vitamin D metabolites was 0.21 ($p < 0.001$). Higher 25(OH)D concentrations were associated with male sex, lower adiposity, and more time spent outside. Higher $1,25(OH)_2D$ concentrations were associated with male sex and lower adiposity, but not with time spent outside (data not shown). Of all the trace elements measured, As and U were the most highly correlated ($r=0.56$, $p<0.01$) (see Appendix, Table 5).

There was no evidence for an association between BPb or urine levels of Cd with 25(OH)D or $1,25(OH)_2D$, either using log-metal concentrations as continuous variables or categorized in quartiles (Tables 2-4). Urine Tl and Mo levels were positively associated with 25(OH)D but not with $1,25(OH)_2D$, while urine As was positively associated with $1,25(OH)_2D$ but not with 25(OH)D. Urine U was the only

element associated with both vitamin D metabolites, although the association between U and 25(OH)D was not significant after adjusting for season. In multivariable-adjusted models, each doubling of U levels was associated with an increase of 2.2 pg/mL $1,25(OH)_2D$ ($p = 0.01$). Urine U remained significantly associated with $1,25(OH)_2D$ after controlling for 25(OH)D, with each doubling of U levels associated with an increase of 2.1 pg/mL $1,25(OH)_2D$ ($p = 0.01$).

After excluding the 63 participants who reported eating fish in the past week, the models were repeated for arsenic, with no change in the associations seen (see Appendix, Table 8). There was no significant association with 25(OH)D, while each doubling of As was associated with an increase of 4.0 pg/mL $1,25(OH)_2D$ ($p=0.01$).

As the source of both U and As is likely groundwater contamination (Li 2005), these elements were highly correlated (Spearman correlation coefficient 0.59, $p <0.001$). When U and As were included simultaneously as predictors of vitamin D metabolites (Table 5), both were marginally associated with $1,25(OH)_2D$. A doubling of U levels was associated with an increase of 1.6 pg/mL of $1,25(OH)_2D$ ($p = 0.05$) and a doubling of As levels was associated with a 2.3 pg/mL increase in $1,25(OH)_2D$ ($p = 0.09$). When 25(OH)D was added as a covariate to the model predicting $1,25(OH)_2D$, the associations did not change (Table 5).

When stratifying by sex, the associations for As and U remained in girls and were weaker and non-significant in boys (see Appendix, Tables 6 and 7), while the

association between Tl and 25(OH)D remained in boys and was weaker and non-significant in girls. The association between 25(OH)D and molybdenum was similar in girls and boys (data not shown). However, none of the interaction terms between sex and As or metal levels were statistically significant.

2.5 Discussion

In this cross-sectional study of 512 adolescents from a smelter town in Mexico, higher urine concentrations of Mo and Tl were associated with higher serum levels of 25(OH)D and higher urine concentrations of U and As were associated with higher serum levels of $1,25(OH)_2D$. We also found that the associations of BPb and urine Cd with vitamin D metabolites were small and not statistically significant.

Although this is a cross-sectional study and the biomarkers of exposure used do not represent chronic exposure, we can infer from the subjects' residential history that they have been chronically exposed, although at varying levels throughout their lives.

Our results were not consistent with prior studies on the association of BPb and urine Cd with vitamin D. BPb has previously been positively associated with 25(OH)D in a study of 142 African American and Hispanic children in New Jersey (Kemp et al., 2006). The mean BPb in this study (4.92 μg/dL) was comparable to the mean BPb in our study. Kemp et al. collected samples across different seasons and

found that both BPb and 25(OH)D levels were higher in the Summer compared to other seasons. It is thus possible that seasonal changes in 25(OH)D levels in New Jersey were associated with other factors related to Pb exposure in children, such as outside activities (Kemp et al., 2006). Torreón, on the other hand, has a year-round sunny climate with less seasonal variation, making confounding by seasonality less likely, though still possible, as seen with U. BPb was also negatively associated with $1,25(OH)_2D$ in a study of 45 African American and Puerto Rican children in New York exposed to high levels of Pb in the 1970s (Rosen et al., 1980). In this study, mean BPb levels in the three Pb exposure groups were 18 (n=15), 47 (n=18) and 74 (n=12) μg/dL, respectively (Rosen et al., 1980). In contrast, the 90th percentile for blood lead level in our study was 7.7 μg/dL. While still above the current CDC BPb reference value for children aged 1-5 years (5 μg/dL), BPb levels in our study may not have been high enough to affect vitamin D metabolism.

Also in contrast to our results, a previous study reported that Cd exposure was inversely associated with $1,25(OH)_2D$ (Nogawa et al., 1987). In this study, Japanese men and women 59 years of age or older who had either itai-itai disease (n=5) or known cadmium exposure and renal disease from living near a zinc mine (n=36) had lower levels of $1,25(OH)_2D$ compared to healthy, unexposed control participants (n=17). The mean urine Cd in exposed participants ranged from 12-16 μg/g creatinine in exposed participants, which is 60-80 times higher than the mean Cd concentrations in our study (0.2 μg/g creatinine). It is possible that $1,25(OH)_2D$ levels were decreased in Cd exposed cases because of Cd-induced kidney disease, as

the hydroxylation of 25(OH)D to 1,25$(OH)_2$D takes place in the kidney (Jones & Prosser, 2011). As with BPb, Cd levels in our study may have not been high enough to affect vitamin D metabolism either directly or via affectation of kidney function.

We found a significant positive association between urine U levels and 1,25$(OH)_2$D. In one prior study, rats that were administered very high doses of uranium (1 mg/rat/day, twice the highest naturally-occurring exposure on earth) had 1,25$(OH)_2$D decreased by 56% compared to control rats, while 25(OH)D was the same in both groups (Tissandie et al., 2007). The high doses of U exposure in this experiment and between species variability make it difficult to compare these findings with the results of our study. We also found a significant positive association between urine As and 1,25$(OH)_2$D. We could not identify prior research in this area.

One possible explanation for the positive association between U or As with 1,25$(OH)_2$D is that these trace elements may induce a sub-clinical Fanconi syndrome. Fanconi syndrome is characterized by damage to the renal proximal tubule that results in increased urinary excretion of various minerals including phosphorus, resulting in upregulation of 1,25$(OH)_2$D to increase phosphorus absorption. Indeed, ingestion of a high amount of U has been implicated in the development of a partial Fanconi syndrome (Pavlakis, Pollock, McLean, & Bartrop, 1996). Lead exposure at higher levels than in our study has also been associated with the development of a Fanconi-like syndrome with proximal tubular

dysfunction (Loghman-Adham, 1998). Future studies with measurements of calcium or phosphorus levels are needed to confirm this hypothesis. However, while the kidney is the major source of 1,25$(OH)_2$D, extra-renal synthesis does occur and we cannot exclude the possibility of other organs playing a role in the associations seen in this study.

We also found significant positive associations between urine Mo and Tl with 25(OH)D. We could not find any prior studies on these associations and there is no clear mechanistic explanation linking metal exposure to increased levels of 25(OH)D. Since 25(OH)D is greatly influenced by sun exposure, metal levels may reflect environmental exposures that occur during outside activities and may thus be associated with increased 25(OH)D. In our study, we attempted to adjust for time spent outside by asking participants about how much time they spent engaging in physical activity. None of the outdoor sports registered, including soccer, volleyball, bicycle riding, tennis, baseball, running, or swimming, predicted 25(OH)D levels. The only predictor of 25(OH)D was the amount of time spent walking. When we adjusted for time spent walking, the associations between metal exposures and 25(OH)D did not change appreciably, but time spent walking may be a limited marker of sunlight exposure.

The strengths of this study include the use of high-quality, sensitive measurements of urinary trace elements and vitamin D metabolites, the availability of 1,25$(OH)_2$D, not frequently measured in human epidemiological studies, and the availability of

information on a potential number of confounders. Although $1,25(OH)_2D$ is not frequently measured and 25(OH)D is considered the standard biomarker for vitamin D status, $1,25(OH)_2D$ is the active form of vitamin D and is responsible for its biological activity. Future research is needed to determine if the change in $1,25(OH)_2D$ we observed in this study could have clinical implications.

Some limitations need to be considered in the interpretation of our findings. Since this is the first report for several of these associations and there is limited information on potential mechanisms, it is necessary to confirm these results in independent studies. We also cannot exclude the possibility that the associations seen in this study are due to chance. Another limitation is that 25(OH)D and $1,25(OH)_2D$ have very different half-lives, with 25(OH)D being several weeks and $1,25(OH)_2D$ being several hours. It is possible that the differing half-lives affected our findings, by limiting the number of associations seen with $1,25(OH)_2D$. Unfortunately, in a cross-sectional study, we cannot be sure. The cross-sectional design of this study also limits our ability to establish causality and the directionality of the effects. It is also possible that unmeasured confounders, including genetics, which determine both trace element levels and vitamin D status could explain the associations. The absence of speciated arsenic measurements is another limitation of this study. In addition, smoking history was collected by self-report, and biomarkers of smoking exposure were unavailable. Self-reporting of smoking, particularly in adolescents, may be inaccurate, which is a limitation. Finally, we studied a very narrow age range of participants in a smelter city, and it is

unclear if our findings can be generalized to other ages or to populations in areas with different levels of environmental metal exposures.

In this study, we found that urine U, Mo, and Tl were positively associated with 25(OH)D and urine U and As were positively associated with $1,25(OH)_2D$. From these results, we conclude that some non-essential trace elements may affect vitamin D metabolism, and this relationship deserves further study. Future studies should employ longitudinal designs and include measurements of calcium and phosphorus metabolism to better understand the implications of early trace element exposure on vitamin D and mineral metabolism.

Table 2.1: Descriptive characteristics of study participants (N = 512).

Characteristic	Number or Mean	% or SD	Median	25th %ile	75th %ile
Age (years)	14.0	±1.2			
Male	262	51.2%			
Monthly family income below 3000 pesos (230)	311	60.7%			
Never smoker	308	60.2%			
Past smoker	149	29.1%			
Current smoker	55	10.7%			
Fat mass (%)	26.4	±8.8			
25(OH)D (ng/mL)	24.8	±8.2			
$1,25(OH)_2D$ (pg/mL)	58.6	±18.3			
BPb (μg/dL)			4.0	3.0	5.6
Cd (μg/g creatinine)			0.22	0.15	0.33
As (μg/g creatinine)			36.6	26.5	47.7
U (μg/g creatinine)			0.04	0.03	0.06
Mo (μg/g creatinine)			63.0	48.0	83.3
Tl (μg/g creatinine)			0.27	0.19	0.38

Table 2.2 Difference in vitamin D levels associated with a doubling of BPb and urine As and metal levels.

	25(OH)D (ng/mL)			$1,25(OH)_2D$ (pg/mL)		
	β	95% CI	p-value	β	95% CI	p-value
BPb						
Model 1	-0.2	(-1.2, 0.7)	0.67	-0.4	(-2.5, 1.7)	0.70
Model 2	-0.5	(-1.4, 0.4)	0.32	-0.3	(-2.4, 1.9)	0.82
Model 3	-			0.0	(-2.2, 2.1)	0.98
As						
Model 1	0.7	(-0.4, 1.8)	0.22	3.6	(1.2, 6.0)	<0.01
Model 2	0.1	(-0.9, 1.1)	0.87	3.4	(0.9, 5.9)	0.01
Model 3	-			3.3	(0.9, 5.8)	0.01
Cd						
Model 1	0.1	(-0.6, 0.8)	0.75	-0.4	(-2.0, 1.1)	0.58
Model 2	0.3	(-0.4, 0.9)	0.40	0.0	(-1.6, 1.6)	0.99
Model 3	-			-0.1	(-1.7, 1.5)	0.87
Mo						
Model 1	2.4	(1.2, 3.5)	<0.01	1.8	(-0.8, 4.4)	0.18
Model 2	1.5	(0.4, 2.6)	0.01	1.5	(-1.2, 4.1)	0.28
Model 3	-			0.8	(-1.9, 3.4)	0.57
Tl						
Model 1	1.2	(0.2, 2.2)	0.02	-1.4	(-3.5, 0.8)	0.21
Model 2	1.2	(0.3, 2.1)	0.01	-0.6	(-2.8, 1.6)	0.61
Model 3	-			-1.2	(-3.4, 1.0)	0.30
U						
Model 1	0.7	(0.1, 1.4)	0.04	2.0	(0.5, 3.4)	0.01
Model 2	0.2	(-0,4, 0.8)	0.50	2.2	(0.7, 3.7)	0.01
Model 3	-			2.1	(0.6, 3.6)	0.01

Model 1: Adjusted for age and sex.
Model 2: Adjusted for age, sex, season, SES (family income <3000 pesos/month, >= 3000 pesos/month, or unknown), smoking (never, former, or within the past month), adiposity, time spent outside.
Model 3: Adjusted for age, sex, season, SES, smoking, adiposity, time spent outside, 25(OH)D.

Table 2.3. Difference in 25(OH)D (ng/mL) levels by quartile of BPb and urine As and metal levels.

	Q1		Q2		Q3		Q4	
Pb		β	95 CI	β	95 CI	β	95 CI	P trend
Model 1	ref	1.6	(-0.4, 3.6)	0.3	(-1.7, 2.4)	-0.1	(-2.1, 2.0)	0.70
Model 2	ref	0.6	(-1.3, 2.4)	0.3	(-1.6, 2.2)	-0.6	(-2.6, 1.3)	0.48
As								
Model 1	ref	1.1	(-0.9, 3.1)	0.9	(-1.1, 3.0)	2.1	(0, 4.2)	0.07
Model 2	ref	0.2	(-1.6, 2.1)	-0.2	(-2.1, 1.7)	1.3	(-0.7, 3.2)	0.30
Cd								
Model 1	ref	0.5	(-1.5, 2.5)	1.3	(-0.7, 3.3)	0.7	(-1.3, 2.7)	0.37
Model 2	ref	0.7	(-1.2, 2.5)	1.6	(-0.3, 3.4)	0.6	(-1.2, 2.5)	0.37
Mo								
Model 1	ref	1.5	(-0.5, 3.4)	1.8	(-0.2, 3.8)	3.8	(1.7, 5.8)	<0.01
Model 2	ref	0.7	(-1.2, 2.5)	0.5	(-1.3, 2.3)	2.5	(0.6, 4.4)	0.02
Tl								
Model 1	ref	1.2	(-0.8, 3.2)	1.1	(-0.9, 3.1)	1.9	(-0.2, 3.9)	0.10
Model 2	ref	1.1	(-0.7, 2.9)	0.6	(-1.2, 2.5)	1.9	(0.0, 3.8)	0.10
U								
Model 1	ref	0.1	(-1.9, 2.2)	2.1	(0.1, 4.1)	2.7	(0.6, 4.7)	<0.01
Model 2	ref	0.1	(-1.7, 2.0)	1.3	(-0.6, 3.1)	1.0	(-0.9, 2.9)	0.18

Model 1: Adjusted for age and sex.
Model 2: Model 2: Adjusted for age, sex, season, SES (family income <3000 pesos/month, >= 3000 pesos/month, or unknown), smoking (never, former, or within the past month), adiposity, time spent outside.
P-trend was calculated by entering the exposure variable as a continuous variable.
The cutpoints for quartiles were as follows: Pb 2.9, 4.0, 5.6 μg/dL; As: 26.5, 36.6, 47.7 μg/g creatinine; Cd: 0.1, 0.2, 0.3 μg/g creatinine; Mo: 48.7, 63.0, 83.3 μg/g creatinine; Tl: 02, 0.3, 0.4 μg/ g creatinine; U: 0.03, 0.04, 0.06 μg/g creatinine.

Table 2.4 Difference in $1,25(OH)_2D$ (pg/mL) levels by quartile of BPb and urine As and metal levels.

	Q1	Q2		Q3		Q4		
Pb		β	95 CI	β	95 CI	β	95 CI	P trend
Model 1	ref	-0.2	(-4.6, 4.3)	-2.4	(-7.0, 2.1)	0.8	(-3.8, 5.4)	0.96
Model 2	ref	-0.6	(-5.1, 3.9)	-1.6	(-6.2, 3.0)	1.1	(-3.7, 5.9)	0.75
Model 3	ref	-0.9	(-5.3, 3.5)	-1.7	(-6.3, 2.8)	1.4	(-3.3, 6.1)	0.64
As								
Model 1	ref	2.5	(-1.9, 7.0)	6.0	(1.5, 10.4)	6.4	(1.8, 11.0)	<0.01
Model 2	ref	2.4	(-2.1, 6.9)	6.2	(1.7, 10.8)	5.8	(1.1, 10.5)	<0.01
Model 3	ref	2.3	(-2.2, 6.7)	6.3	(1.9, 10.8)	5.2	(0.6, 9.9)	0.01
Cd								
Model 1	ref	-0.8	(-5.3, 3.6)	0.7	(-3.8, 5.1)	-0.2	(-4.6, 4.3)	0.65
Model 2	ref	-1.2	(-5.7, 3.3)	1.0	(-3.5, 5.5)	0.4	(-4.2, 4.9)	0.77
Model 3	ref	-1.5	(-5.9, 2.9)	0.3	(-4.1, 4.7)	0.1	(-4.4, 4.5)	0.78
Mo								
Model 1	ref	-0.7	(-5.1, 3.8)	3.7	(-0.7, 8.2)	3.7	(-0.7, 8.3)	0.03
Model 2	ref	-1.3	(-5.8, 3.2)	3.3	(-1.2, 7.8)	3.2	(-1.4, 7.9)	0.05
Model 3	ref	-1.6	(-6.0, 2.8)	3.1	(-1.3, 7.5)	2.0	(-2.6, 6.6)	0.13
Tl								
Model 1	ref	-1.6	(-6.1, 2.8)	-3.0	(-7.5, 1.5)	-3.4	(-8.0, 1.2)	0.12
Model 2	ref	-1.3	(-5.7, 3.2)	-2.6	(-7.1, 1.9)	-2.0	(-6.7, 2.6)	0.31
Model 3	ref	-1.8	(-6.2, 2.5)	-2.9	(-7.4, 1.5)	-3.0	(-7.6, 1.6)	0.17
U								
Model 1	ref	3.9	(-0.6, 8.3)	4.9	(0.4, 9.3)	8.1	(3.6, 12.6)	<0.01
Model 2	ref	4.7	(0.2, 9.1)	5.0	(0.5, 9.4)	8.2	(3.6, 12.9)	<0.01
Model 3	ref	4.6	(0.3, 9.0)	4.4	(0.0, 8.8)	7.8	(3.2, 12.3)	<0.01

Model 1: Adjusted for age and sex.
Model 2: Model 2: Adjusted for age, sex, season, SES (family income <3000 pesos/month, >= 3000 pesos/month, or unknown), smoking (never, former, or within the past month), adiposity, time spent outside.
Model 3: Adjusted for age, sex, season, SES, smoking, adiposity, time spent outside, 25(OH)D.
P-trend was calculated by entering the exposure variable as a continuous variable.
The cutpoints for quartiles were as follows: Pb 2.9, 4.0, 5.6 μg/dL; As: 26.5, 36.6, 47.7 μg/g creatinine; Cd: 0.1, 0.2, 0.3 μg/g creatinine; Mo: 48.7, 63.0, 83.3 μg/g creatinine; Tl: 02, 0.3, 0.4 μg/ g creatinine; U: 0.03, 0.04, 0.06 μg/g creatinine.

Table 2.5 Difference in vitamin D status per doubling of uranium and arsenic levels in a co-exposure model*.

	25(OH)D (ng/mL)			$1,25(OH)_2D$ (pg/mL)		
	β	95%CI	p-value	β	95%CI	p-value
Model 1						
As	0.3	(-1.0, 1.5)	0.68	2.7	(0.1, 5.4)	0.04
U	0.7	(-0.1, 1.4)	0.08	1.3	(-0.3, 2.9)	0.12
Model 2						
As	-0.1	(-1.2, 1.1)	0.91	2.3	(-0.4, 5.1)	0.09
U	0.2	(-0.4, 0.9)	0.51	1.6	(0.0, 3.3)	0.05
Model 3						
As	-			2.4	(-0.3, 5.0)	0.08
U	-			1.5	(-0.1, 3.1)	0.06

* Both uranium and arsenic were included in the same model.
Model 1: Adjusted for age and sex.
Model 2: Model 2: Adjusted for age, sex, season, SES (family income <3000 pesos/month, >= 3000 pesos/month, or unknown), smoking (never, former, or within the past month), adiposity, time spent outside.
Model 3: Adjusted for age, sex, season, SES, smoking, adiposity, time spent outside, 25(OH)D.

CHAPTER 3: Association of inflammatory biomarkers with 25-hydroxyvitamin D and 1,25-dihydroxyvitamin D

Rachel D. Zamoiski, MPH,[1] Eliseo Guallar, MD, DrPH,[2,3] Gonzalo G. Garcia-Vargas, MD, DrSc,[4,5] Stephen J. Rothenberg, PhD,[6] Carol Resnick,[1] Marisela Rubio-Andrade,[4] Amy J. Steuerwald,[7,8] Patrick J. Parsons, PhD,[7,8] Virginia Weaver, MD, MPH,[1,3,9] Ana Navas-Acien, MD, PhD,[1,2] Ellen K. Silbergeld, PhD.[1]

1. Department of Environmental Health Sciences, Johns Hopkins Bloomberg School of Public Health, Johns Hopkins University, Baltimore, MD, USA
2. Department of Epidemiology, Johns Hopkins Bloomberg School of Public Health, Johns Hopkins University, Baltimore, MD, USA
3. Welch Center for Prevention, Epidemiology, and Clinical Research, Johns Hopkins Bloomberg School of Public Health, Johns Hopkins University, Baltimore, MD, USA.
4. Facultad de Medicina, Universidad Juárez del Estado de Durango, Gómez Palacio Durango, México.
5. Secretaría de Salud del Estado de Coahuila, Coahuila, Mexico.
6. Instituto Nacional de Salud Pública, Centro de Investigación en Salud Poblacional, Cuernavaca, Morelos, Mexico.
7. Laboratory of Inorganic and Nuclear Chemistry, Wadsworth Center, New York State Department of Health, Albany, NY, USA.
8. Department of Environmental Health Sciences, School of Public Health, University at Albany, Albany, NY, USA.

9. Johns Hopkins University School of Medicine, Baltimore, MD.

Corresponding author: Ellen K. Silbergeld, Johns Hopkins Bloomberg School of Public Health, 615 N. Wolfe St, E6644, Baltimore MD 21205, 410.955.8678, esilberg@jhsph.edu

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3.1 Abstract

Background: Epidemiological studies of 25(OH)D suggest that it is associated with lower levels of inflammation. Laboratory-based studies of $1,25(OH)_2D$ suggest that it may downregulate inflammation, however these associations have not been studied in a population-based study.

Objectives: To evaluate the associations between both 25(OH)D and $1,25(OH)_2D$ with markers of inflammation, including C-reactive protein (CRP), tumor necrosis

factor alpha (TNF-α), interleukin-1β (IL-1β), interleukin-6 (IL-6), intracellular adhesion molecule 1 (ICAM-1), vascular cell adhesion molecule 1 (VCAM-1), P-selectin, and E-selectin.

Methods: Cross-sectional study of 512 adolescents from Mexico. Serum 25(OH)D and $1,25(OH)_2D$ were measured using a chemiluminescent immunoassay and a radioimmunoassay, respectively. Inflammation was measured in serum. Multivariable linear models and a Tobit model were used to estimate the association between each vitamin D metabolite and each inflammatory cytokine, controlling for age, sex, adiposity, smoking, and income.

Results: 25(OH)D was inversely associated with ICAM-1 and VCAM-1, and was positively associated with P-selectin and IL-1β. $1,25(OH)_2D$ was positively associated with ICAM-1, VCAM-1, P-selectin, and TNF-α.

Conclusions: We found non-uniform associations between 25(OH)D and inflammation. Vitamin D may play a role in regulating some, but not all, inflammatory pathways. Further research is needed to confirm these findings. The associations seen with $1,25(OH)_2D$ and inflammation are positive, which is contradictory to prior studies suggesting anti-inflammatory effects of $1,25(OH)_2D$. Further research is needed to evaluate the health correlates of serum 1,25(OH)2D.

3.2 Background

Vitamin D is a hormone produced in the skin upon exposure to UVB radiation from sunlight. The main circulating form of vitamin D, 25(OH)D, is produced in the liver after production of vitamin D in the skin. 25(OH)D is then hydroxylated in the kidney to $1,25(OH)_2D$, which is the active form of vitamin D that binds to the vitamin D receptor (DeLuca, 2004; Holick, 2007). While vitamin D deficiency has long been recognized as a cause of rickets, more recent data suggest that lower levels of 25(OH)D may also increase susceptibility to many other chronic diseases, such as atherosclerosis, hypertension, asthma, and certain types of cancer (Brøndum-Jacobsen et al., 2012; Giovannucci et al., 2008; Holick, 2004; Institute of Medicine (US) Committee to Review Dietary Reference Intakes for Vitamin D and Calcium et al., 2011).

Lower levels of vitamin D have also been linked to increased risks of infection and autoimmune disease(Adams & Hewison, 2008; Etten & Mathieu, 2005; Holick, 2007). Several studies have found an association between lower levels of 25(OH)D and higher levels of inflammatory markers, including IL-6 and CRP (Chandler et al., 2014; Laird et al., 2014; Shepherd et al., 2014). However, several randomized controlled trials have failed to show an effect of vitamin D supplementation on decreasing inflammation, suggesting that the association between 25(OH)D and inflammation may not be causal(Asemi et al., 2013).

Previous studies on vitamin D and inflammation have measured 25(OH)D in epidemiological studies, and used $1,25(OH)_2D$ in laboratory studies. In the present study, we examined associations between markers of inflammation and both forms of vitamin D. We sought to replicate previous findings of an inverse association between markers of inflammation and 25(OH)D, and to see if this relationship is observed for $1,25(OH)_2D$ as well.

3.3 Methods

This analysis was drawn from a study of adolescents from Torreon designed to assess the relationships between metal exposures and markers of cardiovascular health, including inflammation. Subjects were recruited from Torreon, a city in northern Mexico that is home to the Met-Mex Penoles smelter complex, which is a large lead-zinc smelter located amid residential areas (García-Vargas et al., 2014) (Albalak et al., 2003; Benin et al., 1999). The study population consisted of boys and girls, ages 12-15 years during the recruitment period of October 2009 to June 2010. All participants had participated in an earlier census of blood lead levels and were divided into five strata of earlier blood lead levels, and then recruited randomly from each stratum as part of the main study design to ensure an even distribution of blood lead values across the study population.

The study was approved by the Institutional Review Boards of Juarez University of Durango State, the Johns Hopkins Bloomberg School of Public Health, and the New

York State Department of Health. All study participants, parents, and legal guardians gave signed, informed consent.

Data were collected in the study in two home visits and one clinic visit. The home visit included interviews with the participants and their families and included information on family structure and income and household smoking. We used percent fat mass as a marker of adiposity, and measured impedance with a Tanita scale (Model BC-418, Tokyo, Japan). Blood samples were collected from each participant during the clinic visit in EDTA purple top tubes (Becton Dickinson, Franklin Lakes, NJ). Specimens were refrigerated immediately and then taken the same day to the Laboratory of Toxicology at the Juarez University of Durango State where they were aliquoted and frozen at -80˚C. The details of these methods have been published elsewhere (García-Vargas et al., 2014).

Vitamin D was measured in serum at Heartland Assays, a vitamin D laboratory in Ames, Iowa. 25(OH)D was measured using a chemiluminescent immunoassay on a Diasorin Liaison analyzer. $1,25(OH)_2D$ was measured using radioimmunoassay. The details of these assays have been published elsewhere (Hollis & Horst, 2007). In addition to the samples sent for the entire study population, we randomly selected a sub-set of 40 samples to be sent as blind duplicates, 20 for 25(OH)D and 20 for $1,25(OH)_2D$. The coefficient of variation was 8.0% for 25(OH)D and 16.3% for $1,25(OH)_2D$.

Several inflammatory cytokines and signaling molecules were measured in this study. The biomarkers of inflammation measured in this study were C-reactive protein (CRP), intercellular adhesion molecule-1 (ICAM-1), vascular cell adhesion molecule-1 (VCAM-1), E-selectin, P-selectin, interleukin-6 (IL-6), interleukin-1β (IL-1β), and tumor necrosis factor α (TNF-α). These biomarkers were measured in serum using ELISA at the Biochemistry Laboratory at the Johns Hopkins Bayview Medical Center, General Clinical Research Center.

Statistical analyses were conducted using Stata 12 (Stata Corp, College Station, TX). All biomarkers were log-transformed to correct for skewed distributions, except P-selectin, which was found to be normally distributed. For all of the biomarkers except IL-1β, multiple linear regression models were performed to assess associations between them and both 25(OH)D and 1,25(OH)2D. Since IL-1β levels were below the limit of detection in a significant number of the sample (133/512), a Tobit model was used to account for the large number of censored observations. 25(OH)D and $1,25(OH)_2D$ were each used as predictor variables in separate models. For each biomarker, we ran three models with progressive adjustment. First, we ran a crude model including only vitamin D and the biomarkers related to inflammation. Next, we adjusted for age (continuous) and sex. Third, we adjusted for age (continuous), sex, family income (≥ 3000 pesos/month, < 3000 pesos/month, and unknown), smoking (never smokers, former smokers, and current smokers), and percent fat mass (continuous).

We used added-variable plots to identify potential influential outliers and then reanalyzed the data excluding these outliers (data not shown). The results of all these sensitivity analyses were similar to the original analyses.

3.4 Results

The mean age of study participants was 13.9 years, and 262 (51.2%) were male (Table 1). The geometric mean of 25(OH)D concentration was 23.6 ng/mL, and 55.9 pg/mL for $1,25(OH)_2D$. The Spearman correlation coefficient between 25(OH)D and $1,25(OH)_2D$ was 0.21 ($p < 0.001$).

Higher 25(OH)D was associated with lower levels of ICAM-1, VCAM-1, and E-selectin, and with higher levels of P-selectin. There was no association between 25(OH)D and CRP, IL-6, or TNF-α. After adjusting for age, sex, socio-economic status, smoking, and adiposity, each doubling of 25(OH)D was associated with an decrease of 0.1 log ICAM-1 ($p<0.01$), a decrease of 0.01 log VCAM-1 ($p=0.02$), and an increase of 7.0 ng/dL P-selectin ($p=0.01$) (Table 2). After adjusting for age and sex, each doubling of 25(OH)D was associated with a decrease of 0.2 log E-selectin ($p=0.01$), however this association lost significance with additional adjustment for socioeconomic status, smoking, and adiposity (Table 2). Higher 25(OH)D was also associated with increased IL-1β after controlling for age, sex, socio-economic status, smoking, and adiposity. Each doubling of 25(OH)D was associated with an increase in log IL-1β of 1.5 (Table 4).

Higher $1,25(OH)_2D$ was associated with higher ICAM-1, VCAM-1, P-selectin, and TNF-α. There was no association between $1,25(OH)_2D$ and CRP, E-selectin, or IL-6. After adjusting for age, sex, socio-economic status, smoking, and adiposity, each doubling of $1,25(OH)_2D$ was associated with an increase of 0.1 log ICAM-1 ($p<0.01$), an increase of 0.1 log VCAM-1 ($p<0.01$), and an increase of 0.1 log TNF-α ($p=0.01$) (Table 3). The association of $1,25(OH)_2D$ and P-selectin was borderline significant, with each doubling of $1,25(OH)_2D$ associated with an increase in 5.0 ng/dL P-selectin ($p=0.06$) (Table 3). $1,25(OH)_2D$ was not significantly associated with IL-1β in any of the models.

3.5 Discussion

In this cross-sectional study of 512 adolescents, we found that 25(OH)D was associated with lower levels of ICAM-1 and VCAM-1, and higher levels of P-selectin and IL-1β, and that $1,25(OH)_2D$ was associated with higher levels of ICAM-1, VCAM-1, P-selectin, and TNF-α. We were not able to replicate findings from previous studies of an inverse association between 25(OH)D and CRP.

To our knowledge, the positive association of $1,25(OH)_2D$ with serum ICAM-1, VCAM-1, P-selectin, and TNF-α is a novel finding, as $1,25(OH)_2D$ is not typically measured in clinical or epidemiological studies. Prior *in vitro* studies of $1,25(OH)_2D$ and inflammation have suggested that it may be associated with anti-inflammatory

responses (Etten & Mathieu, 2005). Specifically, $1,25(OH)_2D$ has been shown to decrease IL-1, IL-6, and TNF-α (Guillot et al., 2010). In the present study, those cytokines had either no association (IL-1 and IL-6) or a positive association (TNF-α) with $1,25(OH)_2D$. While we cannot determine causality given that this is a cross-sectional study, these findings highlight the need for additional epidemiological studies on $1,25(OH)_2D$.

Prior studies had suggested that 25(OH)D is inversely associated with increased inflammation. Low 25(OH)D is associated with increased risk for autoimmune diseases including multiple sclerosis, type 1 diabetes, and rheumatoid arthritis (Ponsonby, Lucas, & Mei, 2005). Studies that have looked at 25(OH)D and various markers of inflammation have found associations between 25(OH)D and selected markers of inflammation, although not with all markers of inflammation measured or combined markers of inflammation. One study on levels of 25(OH)D and several markers of inflammation found a significant inverse relationship between 25(OH)D and IL-6, but not with other inflammatory markers measured (Shea et al., 2007). Another study looked at associations of 25(OH)D with various markers of inflammation, and found an inverse association with TNF-α but no association with IL-6 or CRP (Peterson & Heffernan, 2008). These findings are inconsistent with our study, since we found no association between 25(OH)D either IL-6 or TNF-α. Our findings were consistent with earlier studies in finding no association between levels of 25(OH)D and TNF-α.

The inverse association of 25(OH)D with ICAM-1 and VCAM-1 is consistent with prior studies suggesting anti-inflammatory properties of 25(OH)D, however, to our knowledge, there are no epidemiological studies of 25(OH)D with either ICAM-1 or VCAM-1. The positive association of 25(OH)D with P-selectin and IL-1β is inconsistent with prior studies suggesting 25(OH)D may be anti-inflammatory. These inconsistencies could could be due to many factors, including sample size, a limited age range of participants, and environmental exposures.

Biomarkers of inflammation, such as those measured in this study, are important clinically because of their associations with cardiovascular disease. C-reactive protein is a stronger predictor of cardiovascular events, such as heart attack or stroke, than LDL (Ridker et al., 2002), and patients with inflammatory diseases such as Crohn's disease, psoriasis, and rheumatoid arthritis are at increased risk for cardiovascular events (Jussila, Virta, Pukkala, & Färkkilä, 2014; Mankad & Gabriel, 2014; Shaharyar et al., 2014). Reducing inflammation is therefore a mechanistic target for prevention of cardiovascular morbidity and mortality (Libby, 2002).

Although prior studies suggest an inverse association between 25(OH)D and markers of inflammation, trials of vitamin D supplementation have generally failed to demonstrate measurable decreases in inflammatory markers in healthy participants (Barnes et al., 2011; Pittas, Harris, Stark, & Dawson-Hughes, 2007). One trial of vitamin D supplementation and weight loss did show a significant decrease

in TNF-α in participants who received vitamin D compared to control participants, however there were no differences in IL-6, the other biomarker of inflammation measured in that study (Zittermann et al., 2009). These findings suggest that the inverse association between 25(OH)D and inflammation may not be causal. These findings also do not address the issue of 1,25(OH)2D, since while 25(OH)D will increase significantly with supplementation, $1,25(OH)_2D$ remains relatively constant(Himmelstein, Clemens, Rubin, & Lindsay, 1990).

The strengths of this study include a large sample size, measurements of several biomarkers of inflammation, and two forms of vitamin D status, including 1,25(OH)2D, which is not commonly measured in epidemiological studies. This study does have several limitations, however. As this is a cross-sectional study, causality cannot be inferred for any of the associations seen. Smoking, a known risk factor for increased inflammation, was assessed using self-report, which may be biased. We cannot exclude the possibility of unmeasured confounders, such as genetics or nutrition, affecting our results. Finally, the study population consisted of adolescents from a smelter town in Mexico, and it is uncertain if these results can be generalized to other age groups or populations living elsewhere.

Finally, in this study, we found differences in direction of associations between two forms of vitamin D and our biomarkers of inflammatory response. These results suggest that there may be value in measuring both 25(OH)D and 1,25(OH)2D, as they may relate to biomarkers and health status differentially.

Table 3.1 Descriptive characteristics of study participants (N = 512).

Characteristic	Count or Geometric Mean	% or SE
Age (years)	13.9	0.1
Male	262	51.2%
Monthly family income below 3000 pesos (230)	311	60.7%
Never smoker	308	60.2%
Past smoker	149	29.1%
Current smoker	55	10.7%
Fat mass (%)	24.9	0.4
25(OH)D (ng/mL)	23.6	0.3
$1,25(OH)_2D$ (pg/mL)	55.9	0.8
CRP	0.58	0.04
ICAM-1	333.1	4.1
VCAM-1	528.7	5.5
E-selectin	43.9	0.8
P-selectin	82.9	1.2
IL-6	0.75	0.02
TNF-α	5.4	0.1
IL-1β	379 positive	74% positive

Table 3.2 Difference in inflammatory biomarker levels associated with a doubling of 25(OH)D levels

	Model 1			Model 2			Model 3		
	β	95% CI	p	β	95% CI	p	β	95% CI	p
CRP	-0.1	-0.4, 0.2	0.57	-0.1	-0.4, 0.2	0.54	0.2	-0.1, 0.4	0.20
ICAM-1	-0.2	-0.2, -0.1	<0.01	-0.1	-0.2, -0.1	<0.01	-0.1	-0.1, 0.0	<0.01
VCAM-1	-0.1	-0.1, 0.0	<0.01	-0.1	-0.1, 0.0	0.01	-0.1	-0.1, 0.0	0.02
E-selectin	-0.1	-0.2, 0.0	<0.01	-0.1	-0.2, 0.0	0.01	0.0	-0.1, 0.0	0.18
P-selectin	5.3	0.5, 10.1	0.03	5.7	0.8, 10.5	0.02	7.0	1.9, 12.0	0.01
IL-6	-0.1	-0.2, 0.1	0.35	0.0	-0.2, 0.1	0.58	0.0	-0.1, 0.2	0.56
TNF-α	0.0	0.0, 0.1	0.58	0.0	0.0, 0.1	0.48	0.0	0.0, 0.1	0.23

Model 1: Unadjusted
Model 2: Adjusted for age and sex
Model 3: Adjusted for age, sex, SES, smoking, adiposity

Table 3.3 Difference in inflammatory biomarker levels associated with a doubling of $1,25(OH)_2D$ levels

	Model 1			Model 2			Model 3		
	β	95% CI	p	β	95% CI	p	β	95% CI	p
CRP	-0.1	-0.4, 0.2	0.53	-0.1	-0.4, 0.2	0.45	0.1	-0.1, 0.4	0.32
ICAM-1	0.0	0.0, 0.1	0.10	0.0	0.0, 0.1	0.29	0.1	0.0, 0.1	<0.01
VCAM-1	0.1	0.1, 0.2	<0.01	0.1	0.1, 0.1	<0.01	0.1	0.1, 0.2	<0.01
E-selectin	0.0	0.0, 0.1	0.24	0.0	-0.1, 0.1	0.94	0.0	0.0, 0.1	0.23
P-selectin	4.4	-0.7, 9.5	0.09	3.6	-1.6, 8.7	0.17	5.0	-0.2, 10.2	0.06
IL-6	0.0	-0.1, 0.2	0.67	0.0	-0.1, 0.2	0.72	0.1	0.0, 0.2	0.16
TNF-α	0.1	0.0, 0.1	<0.01	0.1	0.0, 0.1	0.06	0.1	0.0, 0.1	0.01

Model 1: Unadjusted
Model 2: Adjusted for age and sex
Model 3: Adjusted for age, sex, SES, smoking, adiposity

Table 3.4 Tobit model for change in log IL-1β per doubling of vitamin D levels

	Model 1			Model 2			Model 3		
	β	95% CI	p	β	95% CI	p	β	95% CI	p
25(OH)D	1.3	0.6, 2.0	<0.01	1.4	0.7, 2.1	<0.01	1.5	0.8, 2.2	<0.01
$1,25(OH)_2D$	-0.5	-1.2, 0.1	0.12	-0.5	-1.2, 0.2	0.17	-0.4	-1.1, 0.2	0.20

Model 1: Unadjusted
Model 2: Adjusted for age and sex
Model 3: Adjusted for age, sex, SES, smoking, adiposity

CHAPTER 4: Arsenic and metals are not associated with antinuclear antibodies in adolescents in Torreon, Mexico

Rachel D. Zamoiski, MPH,[1] Jennifer F. Nyland, PhD[2], Noel R. Rose MD PhD[3], Eliseo Guallar, MD, DrPH,[4,5] Gonzalo G. Garcia-Vargas, MD, DrSc,[6,7] Stephen J. Rothenberg, PhD,[8] Carol Resnick,[1] Marisela Rubio-Andrade,[6] Amy J. Steuerwald,[9,10] Patrick J. Parsons, PhD,[9,10] Virginia Weaver, MD, MPH,[1,5,11] Ana Navas-Acien, MD, PhD,[1,4] Ellen K. Silbergeld, PhD.[1]

1. Department of Environmental Health Sciences, Johns Hopkins Bloomberg School of Public Health, Johns Hopkins University, Baltimore, MD, USA
2. Department of Pathology, Microbiology, and Immunology, University of South Carolina School of Medicine, Columbia, SC, USA
3. Departments of Pathology and of Molecular Microbiology and Immunology, Johns Hopkins University, Baltimore, MD, USA
4. Department of Epidemiology, Johns Hopkins Bloomberg School of Public Health, Johns Hopkins University, Baltimore, MD, USA
5. Welch Center for Prevention, Epidemiology, and Clinical Research, Johns Hopkins Bloomberg School of Public Health, Johns Hopkins University, Baltimore, MD, USA
6. Facultad de Medicina, Universidad Juárez del Estado de Durango, Gómez Palacio Durango, México
7. Secretaría de Salud del Estado de Coahuila, Coahuila, Mexico
8. Instituto Nacional de Salud Pública, Centro de Investigación en Salud Poblacional, Cuernavaca, Morelos, Mexico
9. Laboratory of Inorganic and Nuclear Chemistry, Wadsworth Center, New York State Department of Health, Albany, NY, USA
10. Department of Environmental Health Sciences, School of Public Health, University at Albany, Albany, NY, USA
11. Department of Medicine, Johns Hopkins School of Medicine, Johns Hopkins University, Baltimore, MD, USA

Corresponding author: Ellen K. Silbergeld, Johns Hopkins Bloomberg School of Public Health, 615 N. Wolfe St, E6644, Baltimore MD 21205, 410.955.8678, esilberg@jhsph.edu

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3.1 Abstract

Background: Prior data indicate that mercury (Hg) may increase risks of inflammation, autoimmunity and autoimmune disease. No epidemiological studies, to our knowledge, have looked to other metals to see if the relationship with autoimmunity can be generalized to other exposures.

Objectives: To evaluate the association between blood lead (BPb) and urine arsenic (As), cadmium (Cd), uranium (U), thallium (Tl), tungsten (W), molybdenum (Mo), and antimony (Sb) with antinuclear antibodies (ANA).

Methods: Cross-sectional study of 511 adolescents in Torreon, a smelter town in northern Mexico. BPb was measured using atomic absorption spectrometry. Urine As, Cd, U, Mo, Tl, W, and Sb were measured using inductively coupled plasma mass spectrometry. ANA was measured using indirect immunofluorescence microscopy. Logistic regression was used to evaluate associations between trace elements and positive ANA or high ANA (positive at ≥1:40 dilution)

Results: No significant associations seen with any of the trace elements measured and either positive or high ANA.

Conclusions: No evidence suggests that these trace elements are affecting biomarkers of autoimmunity or enhancing the risk for autoimmune disease. The effect seen on autoimmunity from Hg may be unique to Hg and not generalizable to other metals known to influence inflammation.

Keywords: Antinuclear antibody, metals, arsenic

3.2 Introduction

Hg, an immunotoxic metal, has been reported to increase levels of autoimmunity and inflammation, and to enhance the risk of autoimmune disease in susceptible individuals as well as animal models of autoimmune disease. Populations with higher exposures to mercury exhibit higher rates of positive ANA and ANoA (Silva et al., 2004) (Gardner et al., 2010b) as well as higher levels of pro-inflammatory cytokines (Gardner et al., 2010b). In addition, one study (Cooper et al., 2004) reported that patients with systemic lupus erythematosus were more likely than controls to report occupational exposure to Hg. Animal studies where Hg was administered to mice also show an increase in autoimmunity in exposed mice compared to control mice (Havarinasab & Hultman, 2006; Pollard et al., 2001) as well as exacerbation of disease in animal models of lupus and autoimmune cardiomyopathy (Nyland, Fillion, et al., 2011a; Via et al., 2003).

While prior studies support an association between Hg and autoimmunity, few studies have explored a relationship between other metals with autoimmunity. Like Hg, some metals, namely Pb, and Cd, as well as As, may increase markers of inflammation, which are often associated with autoimmunity. One study showed that individuals occupationally exposed to Pb had higher levels of IFN-γ (Mishra et al., 2003). Other *in vitro* studies suggest that exposing cells to Pb or Cd results in an increase in the levels of pro-inflammatory cytokines released by the cells (Flohé et al., 2002; Krocova et al., 2000). As has also been linked to increased release of pro-inflammatory cytokines, including IL-6 and TNF-α, both in mice and in humans (Das et al., 2005) (Wu et al., 2008).

Our goal in the present study was to see if the association between Hg and autoimmunity could be generalized to other metals or As. We evaluated the association of positive ANA and high ANA (positive at a titer of 40) with biomarkers of exposure to As and metals in a population of girls and boys, ages 12-15, from a smelter town in northern Mexico. We hypothesized that odds of positive and high ANA would increase with higher exposures.

3.3 Methods

The study population was composed of adolescents who resided in the town of Torreon, located in northern Mexico. This location was selected due to the presence of the Met-Mex Penoles smelter, which is a large primary lead-zinc smelter

surrounded by residential areas (Benin et al., 1999) (Albalak et al., 2003). All subjects were participants of an ongoing survey of BPb conducted by the State of Coahuila Department of Health since 1999, and all subjects had at least one BPb measurement prior to 2004. The prior BPb levels were divided into five strata, and subjects were chosen randomly from each stratum to ensure a range of exposures. Subjects were recruited from October 2009 until June 2010, and all subjects were aged 12-15 years at the time of recruitment.

Study personnel visited each subject's home twice, and each subject had one visit to the study clinic. Questionnaires were used to obtain demographic information, dietary information, and information on family structure. In the study clinic, we collected a fasting blood specimen and a spot urine specimen. The blood specimen was obtained in EDTA purple-top tubes (Becton-Dickinson, Franklin Lakes, NJ). The urine specimen was obtained in plastic containers washed with 10% HNO_3 and deionized water. All specimens were immediately refrigerated and taken on the same day of collection to the Laboratory of Toxicology at the Juarez University of Durango State. The specimens were then aliquoted and frozen at -80˚ C. The details of these procedures have been published elsewhere (García-Vargas et al., 2014).

BPb was measured in whole blood at the Environmental Toxicology Laboratory at Juarez University of Durango State with a graphite furnace atomic absorption spectrometer with Zeeman background correction. Any sample with a coefficient of

variation above 5% was re-analyzed, and the average coefficient of variation was 3.9%. The limit of detection was 0.7 μg/dL.

Urine Cd, As, U, Mo, Tl, W, and Sb were all measured at the Trace Elements Section at the Laboratory of Inorganic and Nuclear Chemistry, Wadsworth Center, New York State Department of Health, Albany, New York. The measurements were conducted using a Perkin Elmer ELAN DRC II inductively coupled plasma-mass spectrometer (ICP-MS) equipped with Dynamic Reaction Cell (DRC-) technology (PerkinElmer Life and Analytical Sciences, Shelton, Connecticut, USA). Cd was corrected for interference from Mo using a previously validated procedure based on the individual's Mo level. The details of these methods have been described elsewhere (Pollack et al., 2013). Arsenic was not speciated and is reported as total As. Concentrations were corrected for urine creatinine, to adjust for urine dilution. Urine creatinine was measured with a Dimension clinical chemistry system using a Flex reagent cartridge in an enzymatic assay (Siemens Dimension Vista 1500; Siemens Medical Solutions USA, Inc., Malvern, PA, United States).

We chose to measure these metals and As for several reasons. First, we wanted to expand upon previous findings of Pb, Cd, and As with respect to increasing inflammation and to see if those exposures would also be related to biomarkers of autoimmunity. Second, we suspected that levels of Pb, Cd, As, and U would be high in this population, due to the presence of the smelter, as well as groundwater

contaminated with As and U. Finally, the method of analysis generates the data for the other metals, and they are included as an exploratory analysis.

ANA was measured in serum at the Department of Pathology, Microbiology, and Immunology at the University of South Carolina School of Medicine in Columbia, South Carolina using indirect immunofluorescence microscopy using slides prepared with human epithelial cells (INOVA Diagnostics, San Diego, CA). The details of this assay have been described elsewhere (Gardner et al., 2010b; Nyland, Fillion, et al., 2011a; Nyland, Wang, et al., 2011b).

Serum samples were diluted 2-fold, starting at 1:10, and the inverse of the most dilute sample where ANA was observed was reported as the titer. If no ANA was observed at the lowest dilution, this was reported as a negative result.

Statistical analyses were carried out using Stata 12 (Stata Corp, College Station, TX). Metal and As levels were log base 2 transformed to correct for skewed distributions and were used as continuous variables. We ran models for two different outcomes: positive ANA and high ANA, defined as positive ANA at 1:40 dilution. Logistic regression was used for each outcome, and we used two models. First, we ran an unadjusted model that showed only the change in odds of positive or high ANA per doubling of metal or As. Then, we ran a model adjusting for age (continuous) and sex.

As a sensitivity analysis, we excluded participants who reported eating fish in the previous week to lessen the influence of higher levels of arsenobetaine and other organic arsenical compounds found in fish (Navas-Acien et al., 2011).

3.4 Results

The study population consisted of 250 girls and 261 boys, for a total of 511 participants. The mean (standard deviation) age of study participants was 14.0 (1.1) years. 78 (15.3%) of participants had positive ANA. 31/261 (11.9%) boys and 47/250 (18.8%) girls had positive ANA.

The median, 25th percentile, and 75th percentile of the trace elements measured are presented in Table 1.

None of the trace elements measured were associated with either positive or high (≥40 titer) ANA in the unadjusted model, or after adjusting for age and sex. The results of the models run are presented in Table 2. As a sensitivity analysis, the model with As was run again after excluding the 63 participants who reported eating fish in the preceding week, with no change in results (data not shown).

3.5 Discussion

In this cross-sectional study of 511 adolescents from a smelter town in Mexico, we found no association between metals or arsenic with ANA. We looked at the odds of both positive and high ANA, and we looked at unadjusted models as well as models adjusted for age and sex.

These results suggest that mercury compounds may be uniquely associated with increased titers of ANA. Many prior studies have reported on increased serum ANA and ANoA in animal models. Our earlier epidemiological studies of persons with exposures to metallic Hg and/or methyl mercury reported increases in odds of high ANA and ANoA associated with increasing exposure to Hg (Nyland, Fillion, et al., 2011a) (Gardner et al., 2010b). In this study, relatively few (15.3%) participants had detectable ANA, which is considerably lower than the prevalence of detectable ANA reported in populations exposed to relatively high levels of Hg. One of our prior studies on artisanal gold miners (who utilize elemental Hg in amalgamation) reported that 54.1% of participants had detectable ANA titers (Silva et al., 2004)

The strengths of this study include high-quality measurements of eight different trace elements and the availability of ANA in a population exposed to non-mercurial trace elements. Some limitations that need to be considered, however. Autoimmunity and autoimmune diseases are thought to be influenced by many different factors, including age and family history. This study population consisted

of adolescents, so we cannot exclude the possibility that they were too young to be susceptible to autoimmunity induced by exposure to metals or As. One prior study did find increased ANA titers in a Hg-exposed population that included adolescents, after adjusting for age, although these adolescents were older than the individuals in this analysis (Nyland, Fillion, et al., 2011a). We have no information on family history of autoimmune disease, so we could not stratify our analysis to examine the role of positive family history on risk of autoimmunity.

In this study, we found no association between metals and As, and risk for autoimmunity. These results suggest that the association between Hg and ANA may be unique to Hg and not generalizable to other trace elements.

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Table 4.1: Median, 25th percentile, and 75th percentile of trace elements

	Median	25th %ile	75th %ile
Pb	4.0	2.9	5.6
As	36.6	26.5	47.8
Cd	0.22	0.15	0.33
U	0.04	0.03	0.06
W	0.13	0.07	0.22
Sb	0.11	0.08	0.15
Tl	0.27	0.19	0.38
Mo	63.2	48.7	83.4

Units for Pb are μg/dL. Units for all others are μg/g creatinine.

Table 4.2: Odds ratio of ANA associated with doubling of metals and arsenic

	Model 1			Model 2		
	Odds ratio	95% CI	p-value	Odds ratio	95% CI	p-value
Pb						
ANA ≥10	0.8	0.6, 1.1	0.21	0.9	0.6, 1.2	0.48
ANA ≥40	0.8	0.4, 1.5	0.47	0.9	0.5, 1.8	0.82
Cd						
ANA ≥10	0.9	0.7, 1.1	0.19	0.9	0.7, 1.1	0.21
ANA ≥40	0.8	0.5, 1.3	0.40	0.8	0.5, 1.3	0.43
As						
ANA ≥10	0.8	0.5, 1.2	0.23	0.8	0.5, 1.2	0.29
ANA ≥40	0.5	0.2, 1.1	0.08	0.5	0.2, 1.2	0.10
U						
ANA ≥10	1.0	0.8, 1.2	0.74	1.0	0.8, 1.2	0.96
ANA ≥40	0.8	0.5, 1.3	0.33	0.8	0.5, 1.3	0.44
Sb						
ANA ≥10	0.9	0.7, 1.3	0.70	0.9	0.7, 1.3	0.74
ANA ≥40	0.8	0.5, 1.4	0.50	0.8	0.5, 1.4	0.43
W						
ANA ≥10	1.1	0.9, 1.2	0.46	1.1	0.9, 1.2	0.33
ANA ≥40	0.9	0.7, 1.2	0.43	0.9	0.7, 1.2	0.61
Tl						
ANA ≥10	0.8	0.6, 1.1	0.26	0.9	0.6, 1.2	0.44
ANA ≥40	0.7	0.4, 1.3	0.25	0.7	0.4, 1.4	0.34
Mo						
ANA ≥10	0.8	0.5, 1.2	0.26	0.8	0.5, 1.2	0.33
ANA ≥40	0.7	0.3, 1.5	0.32	0.6	0.3, 1.5	0.29

Model 1: Unadjusted

Model 2: Adjusted for age and sex

CHAPTER 5: No interaction between 25-hydroxyvitamin D and blood lead on increasing blood pressure in NHANES IV 2001-2006

Rachel D. Zamoiski MPH[1], Eliseo Guallar MD DrPH[2,3] , Virginia Weaver MD MPH[1,2,4], Ana Navas-Acien MD PhD[1,2], Ellen K. Silbergeld PhD[1]

1. Department of Environmental Health Sciences, Johns Hopkins Bloomberg School of Public Health, Johns Hopkins University, Baltimore, MD, USA.
2. Welch Center for Prevention, Epidemiology, and Clinical Research, Johns Hopkins Bloomberg School of Public Health, Johns Hopkins University, Baltimore, MD, USA.
3. Department of Epidemiology, Johns Hopkins Bloomberg School of Public Health, Johns Hopkins University, Baltimore, MD, USA
4. Johns Hopkins University School of Medicine, Baltimore, MD, USA.

5.1 Abstract

Background: Hypertension is a significant public health problem characterized by high systolic or diastolic blood pressure. Exposure to lead is well-known risk factor for increasing blood pressure, and exposure to ultraviolet B radiation and higher levels of vitamin D are associated with decreased blood pressure.

Objectives: To describe the associations between vitamin D and lead with systolic and diastolic blood pressure, and to determine if higher vitamin D may lessen the effect of lead on increasing blood pressure.

Methods: Data was used from NHANES IV. Subjects were ages 18 and older, not pregnant, and not taking anti-hypertensive medication. Multivariable linear models with blood pressure as the outcome variable was used to assess the effect of lead on blood pressure, with a lead/vitamin D interaction term. The sample was also stratified into four quartiles depending on vitamin D level, and the effect of lead on blood pressure was compared in the lowest quartile to the highest quartile.

Results: The full sample consisted of 10,627 participants. A lead/vitamin D interaction term was significant for systolic blood pressure after adjusting for age, sex, race, and BMI (interaction=-1,1, p=0.03), but not after additional adjustment for smoking and education (interaction=-1.2, p=0.18). There were no significant differences between low and high vitamin D with effect of lead on systolic blood pressure (0.5 mmHg per doubling of lead, p=0.34 and 0.0 mmHg per doubling of lead, p=0.92 respectively) or on diastolic blood pressure (0.9 mmHg per doubling of lead, p=0.01 and 1.2 mmHg per doubling of lead, p<0.01 respectively).

Conclusions: No evidence that higher 25(OH)D attenuates the increase in blood pressure seen with exposure to lead. Future research should focus on longitudinal studies of vitamin D to better understand its relationship with blood pressure.

5.2 Background

Hypertension, defined as systolic blood pressure (SBP) or diastolic blood pressure (DBP) of 140 mmHg or 90mmHg respectively, is an important public health problem.(Fields et al., 2004) The prevalence of hypertension in the United States is estimated at around 30%,(Fields et al., 2004; Wong, Lopez, Tang, & Williams, 2006) and it is a major risk factor for both all-cause mortality and mortality due to vascular disease.(Lewington et al., 2002) Hypertension is responsible for 13% of all deaths worldwide, and is also the leading risk factor for mortality in the world.(World Health Organization, 2009) As such, there is interest in identifying exposures that may increase blood pressure, as well as treatments that may decrease blood pressure and in turn, lower the risk of cardiovascular disease and mortality.

Lead (Pb) has been associated with hypertension, as well as other cardiovascular diseases, including coronary heart disease, peripheral arterial disease, and stroke. (Bhatnagar, 2006) Higher levels of lead have been linked to greater risk for all-cause mortality and cardiovascular mortality.(Lustberg & Silbergeld, 2002) Prior studies have led to the conclusion that the relationship between Pb and blood pressure is causal, and have implicated exposure to Pb as an important risk factor for increasing blood pressure.(Navas-Acien, Guallar, Silbergeld, & Rothenberg, 2006)

Prior studies have noted that higher vitamin D (25(OH)D) is associated with lower blood pressure. (Almirall, Vaqueiro, Bare, & Anton, 2010; Tamez & Thadhani, 2012; Zhao et al., 2010) One randomized trial of ultraviolet A (UVA) and ultraviolet B (UVB) irradiation showed that exposure to UVB increased 25(OH)D and lowered both SBP and DBP, while no changes were associated with UVA exposure.(Krause et al., 1998) These data suggest that increasing 25(OH)D may have a direct effect on lowering blood pressure. 1,25(OH)2D, which is the active metabolite of 25(OH)D that binds to the vitamin D receptor (VDR), inhibits the production of renin, which in turn may lead to decreased blood pressure.(Li et al., 2004)

Increasing age is another important risk factor for increasing blood pressure.(Rodriguez, Labarthe, Huang, & Lopez-Gomez, 1994) As people age, their risk of hypertension increases. One prior study showed that individuals with higher 25(OH)D had less of an age-associated increase in blood pressure.(Judd et al., 2008) In the present study, we sought to determine if higher levels of 25(OH)D would attenuate the effect of Pb on blood pressure, just as it does with the effect of age on blood pressure. We hypothesized that the effect of BPb on SBP and DBP would be lower in individuals who had higher levels of 25(OH)D as compared to individuals with lower 25(OH)D.

5.3 Methods

We used data from the National Health and Nutrition Examination Survey (NHANES) 2001-2006. NHANES is a complex, multistage sample, representative of the non-institutionalized US population, conducted by the National Center for Health Statistics. All participants gave informed consent, and participation consisted of an interview at the participant's home, and a physical exam conducted in a mobile exam center (MEC). (National Center for Health Statistics, 2011) Our sample included participants age 18 years and older, who had BPb, 25(OH)D, and blood pressure measurements. We excluded pregnant women and anyone who reported taking medication for hypertension.

Demographic information was collected during the interview at the participant's home. Anthropometric body measurements were collected during the visit to the MEC. Body mass index was calculated using weight and height (kg/m^2). Weight was measured on a Toledo digital scale in pounds, and then converted to kilograms. For participants weighing 440 pounds or more, weight was measured using 2 Seca digital scales, with one foot on each scale, and the results from each scale were added together. The details of the anthropometric methods are available elsewhere.(National Center for Health Statistics, 2002)

SBP and DBP were measured in each participant using a mercury sphygmomanometer in the MEC. SBP and DBP were determined by calculating the

mean of all measurements for each participant.(National Center for Health Statistics, 2004) Most participants (n=4725, 87.3%) had 3 BP measurements available, while 442 (8.2%) had 2 measurements, and 243 (4.5%) had 1.

Blood samples were drawn in the MEC and used for the analyses of 25(OH)D and BPb. After collection, samples were processed and frozen at -20° C before being sent to the National Center for Environmental Health for analysis.

25(OH)D was measured in serum using a Diasorin radioimmunoassay with a ^{125}I tracer. This method is capable of detecting 25(OH)D levels between 5-100 ng/mL, which encompasses the expected range of 25(OH)D values in a population. Each sample was run in duplicate. Any samples below 5 ng/mL or above 70 ng/mL were reanalyzed, as well as any sample with a coefficient of variation above 10%. The details of this assay are available elsewhere.(National Center for Environmental Health, 2001a)

BPb was measured in blood using atomic absorption with a PerkinElmer SIMAA 6600 multielement atomic absorption spectrometer with Zeeman background correction. The samples were each run in duplicate, and were repeated when the two measured differed by more than 1.0 μg/dL. This method is capable of detecting BPb levels between 0.6 and 50 μg/dL. For BPb values above 40 μg/dL, the sample was diluted and re-analyzed. The details of this assay are available elsewhere.(National Center for Environmental Health, 2001b)

Statistical analyses were conducted using Stata 12 (Stata Corp, College Station, TX). The svy command was used along with sampling weights provided in the dataset to account for the non-random sampling design of NHANES. We analyzed BPb as a log-transformed continuous variable, and we first looked at 25(OH)D as a continuous variable over the entire study population. Multiple linear regression was used with BPb and 25(OH)D as continuous variables, plus an interaction term with both continuous variables. An unadjusted model was run first, followed by a model adjusting for age, sex, ethnicity, and BMI, and then a model additionally adjusting for smoking and education. SBP and DBP were each used as outcome variables.

We then divided the group by 25(OH)D status into four quartiles. The lowest quartile was used for the low 25(OH)D group, and the highest quartile was used for the high 25(OH)D group. The middle two quartiles were excluded in this analysis. Multiple linear regression models were performed, stratified by 25(OH)D group, with either SBP or DBP as the outcome variable. BPb was log-transformed to account for a skewed distribution.

Logistical regression models were also used to determine the odds of high blood pressure in either SBP or DBP, defined as SBP greater than or equal to 140 mmHg, or DBP greater than or equal to 90 mmHg. An unadjusted model of BPb was run first, then a model adjusting for age, sex, ethnicity, and BMI, and finally a model adjusting for the previous covariates plus education and smoking.

5.4 Results

The sample consisted of 10627 participants. The mean age was 41.9 years, and 5599 participants (52.7%) were male. The mean BPb was 1.4 μg/dL, and the mean 25(OH)D level was 21.9 ng/mL. The mean SBP was 119.0 mmHg, and 70.2 mmHg for DBP (Table 1).

For the stratified analyses, there were 2917 people in the low 25(OH)D group and 2493 people in the high 25(OH)D group, yielding a total sample size of 5410. As compared to the low 25(OH)D group, the high 25(OH)D group was older ($p=0.08$), more likely to be male ($p<0.01$), more likely to be white and less likely to be black or Mexican-American ($p<0.01$), had more education ($p<0.01$), had lower BMI ($p<0.01$), and had lower SBP and DBP ($p<0.01$). BPb did not differ between the two groups ($p=0.15$) (Table 2).

In the unadjusted model of the effect of BPb on SBP, each doubling of BPb was associated with an increase in SBP of 3.9 mmHg ($p<0.01$) in the combined sample (Table 3). After adjusting for age, sex, ethnicity, and BMI, each doubling of BPb was associated with an increase of 0.3 mmHg ($p=0.34$), and after additional adjustment for education and smoking, each doubling of BPb was associated with an increase of 0.2 mmHg ($p=0.47$). When looking at odds of having SBP greater than or equal to 140 mmHg, each doubling of BPb was associated with a log odds increase of 0.5 ($p<0.01$) (Table 4). After adjusting for age, sex, ethnicity, and BMI, each doubling

was associated with an increase in log odds of 0.2 (p=0.04) and an increase of 0.1 (p=0.09) after additional adjustment for education and smoking.

After adding an interaction term of BPb and 25(OH)D to these models, the effect of BPb on SBP was similar (Table 5). The interaction terms were statistically significant in the unadjusted model and the model adjusting for age, sex, race, and BMI, but after adding smoking and education to the model, the interaction term was no longer significant (p=0.18) (Table 5).

When stratifying by 25(OH)D, the effect of BPb on SBP was marginally higher in the low 25(OH)D group than in the high 25(OH)D group (Figure 1; Table 6). In the low 25(OH)D group, each doubling of BPb was associated with an increase in SBP of 4.1 mmHg (p<0.01), while in the high 25(OH)D, each doubling was associated with an increase of 3.6 mmHg (p<0.01). After controlling for age, sex, race, and BMI, the effect of BPb on SBP remained higher in the low 25(OH)D group but was no longer statistically significant. Each doubling of BPb was associated with an increase of 0.5 mmHg (p=0.33) in the low 25(OH)D group and 0.1 mmHg (p=0.80) in the high 25(OH)D group. Results were similar when adjusting for smoking and education in addition, with each doubling of BPb associated with an increase in 0.5 mmHg (p=0.34) and 0.0 mmHg (p=0.92) in the low and high group respectively. Similar patterns were seen when BPb was analyzed in quartiles instead of as a continuous variable (Table 8). An interaction term of 25(OH)D and BPb was not statistically significant (data not shown).

For DBP, each doubling of BPb was associated with an increase of 1.4 mmHg ($p<0.01$). After adjusting for age, sex, ethnicity, and BMI, each doubling of BPb was associated with an increase of 0.9 mmHg ($p<0.01$), and 1.0 mmHg ($p<0.01$) after adding education and smoking (Table 3). The log odds of DBP greater than or equal to 90 mmHg per doubling of BPb was 0.4 ($p<0.01$) in the unadjusted model (Table 4). After adjusting for age, sex, ethnicity, and BMI, the log odds per doubling of BPb was 0.2 ($p=0.03$). After additional adjustment for education and smoking, the log odds per doubling of BPb was 0.2 ($p=0.02$). The interaction between BPb and 25(OH)D was not statistically significant for any of the models run (Table 5).

When stratifying by 25(OH)D, the effect of BPb on DBP was marginally greater in the low 25(OH)D group, but only in the participants with the highest levels of BPb (Figure 2). Without adjusting for anything, each doubling of BPb was associated with an increase in DBP of 1.4 mmHg ($p<0.01$) and 1.4 mmHg ($p<0.01$) in the low 25(OH)D group and the high 25(OH)D group respectively (Table 7). After adjusting for age, sex, ethnicity, and BMI, each doubling of BPb was associated with an increase in DBP of 0.8 mmHg ($p=0.02$) and 0.9 mmHg ($p<0.01$) in the low and high groups, and 0.9 mmHg ($p=0.01$) and 1.2 mmHg ($p<0.01$) after additional adjustment for education and smoking. While those analyses of BPb as a continuous variable do not show any apparent differences, the results are different when quartiles of BPb are used instead. In the unadjusted model, quartile 4 of BPb is associated with an increase in DBP of 4.0 mmHg in the low 25(OH)D group and 3.3 mmHg in the high

25(OH)D group (Table 9). In the model adjusting for age, sex, ethnicity, and BMI, quartile 4 is associated with an increase in 2.7 mmHg and 2.0 mmHg in the low and high groups respectively, and 2.9 mmHg and 2.7 mmHg in the low and high groups after additional adjustment for education and smoking. Additional models using an interaction term of BPb and 25(OH)D were not significant (data not shown).

5.5 Discussion

In this cross-sectional study of the general US adult population, we found that higher BPb was associated with higher SBP and DBP, and that higher 25(OH)D does not significantly attenuate the association between BPb and both SBP and DBP.

Although we were able to replicate previous findings of increasing BP with increasing BPb, these associations were no longer significant after adjusting for important confounders, including age, sex, ethnicity, and BMI in the models using BP as a continuous outcome variable. When hypertension, defined as SBP of greater than or equal to 140 mmHg or DBP of greater than or equal to 90 mmHg, was used as a dichotomous outcome variable, BPb was associated with an increased odds of hypertension, even after controlling for age, sex, ethnicity, BMI, education, and smoking. This is consistent with prior studies that use a dichotomous BP outcome and not a continuous one. (W. R. Harlan, Landis, Schmouder, Goldstein, & Harlan, 1985; Rothenberg et al., 2002)

Although some of these relationships were not statistically significant, we observed slightly higher effects of BPb on BP in participants with low 25(OH)D as compared to high 25(OH)D. When looking at the effect of BPb on SBP, this difference was seen when analyzing BPb as a continuous variable, and when BPb was divided into quartiles, the difference was only seen in the highest quartile of BPb. With DBP, this difference was only seen in the highest quartile of BPb.

The strengths of this study include a large sample size that is representative of the general non-institutionalized United States population, high-quality measurements of BPb, SBP, and DBP, and good information on potential confounders. This study is limited by its cross-sectional design. Although previous studies have suggested that the relationship between Pb exposure and increased BP is causal, we cannot conclude that any differences between the high and low 25(OH)D groups are due to actual differences in 25(OH)D. Many important determinants of 25(OH)D levels are also characteristics of overall physical health, including age and BMI. Just as older and heavier people are more at risk for cardiovascular disease, they are also more likely to have lower 25(OH)D. Although we adjusted for these factors in our analyses, we cannot dismiss the possibility that participants with higher 25(OH)D are healthier overall, which could translate to being less susceptible to effects of BPb on BP. In addition, although many prior studies show an association between higher 25(OH)D and lower risk of cardiovascular disease, to our knowledge there have not yet been large-scale randomized controlled trials of vitamin D supplementation, or UVB exposure, to determine causality.

Our goal in this study was to determine if 25(OH)D attenuates the relationship between BPb and BP, just as a prior study showed that 25(OH)D attenuates the relationship between increasing age and BP.(Judd et al., 2008) Although we did replicate the lower effect of the BPb on BP in participants with high 25(OH)D, the difference between high and low 25(OH)D was not as pronounced in this study as in the prior one. In the prior study, there was a statistically significant interaction between age and 25(OH)D, and we found no significant interaction between BPb and 25(OH)D.

In this study, we found that higher 25(OH)D is not associated with a significantly reduced effect of BPb on either SBP or DBP. We conclude that 25(OH)D may not lessen the effect of BPb on increasing blood pressure. Future studies should use longitudinal designs to better understand the effect of 25(OH)D on blood pressure.

Figure 5.1 Association between SBP and BPb stratified by 25(OH)D

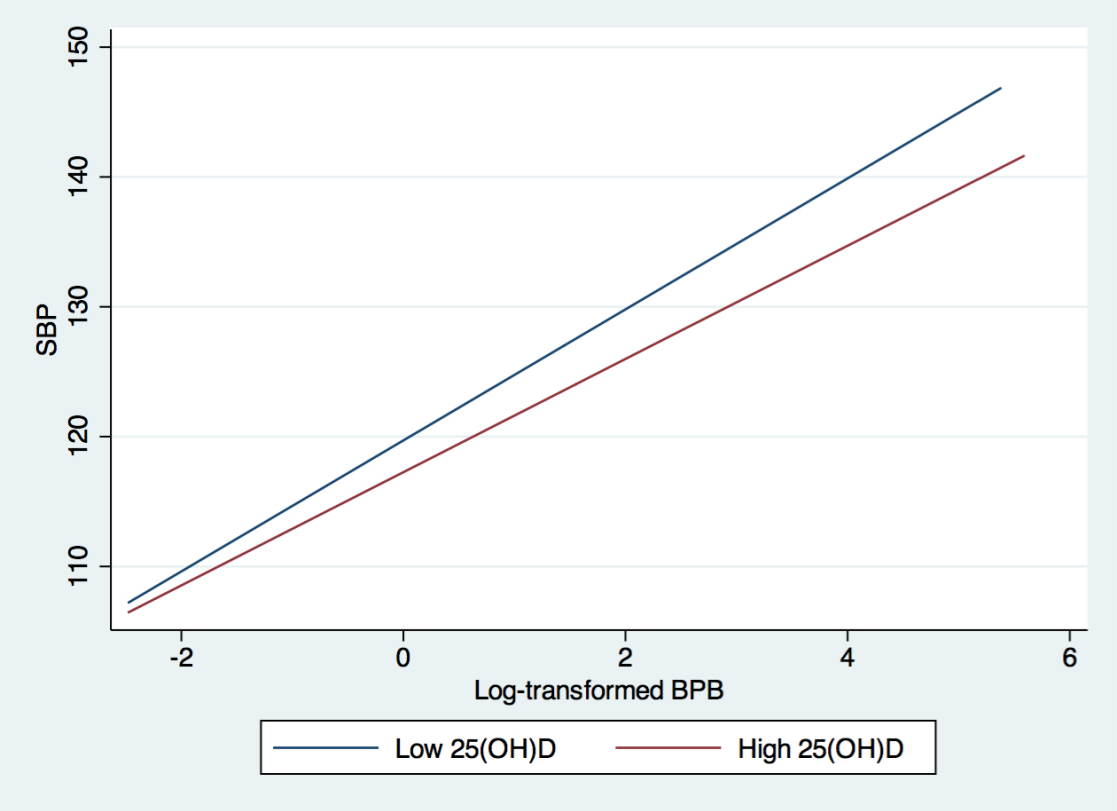

Figure 5.2 Association between DBP and BPb stratified by 25(OH)D

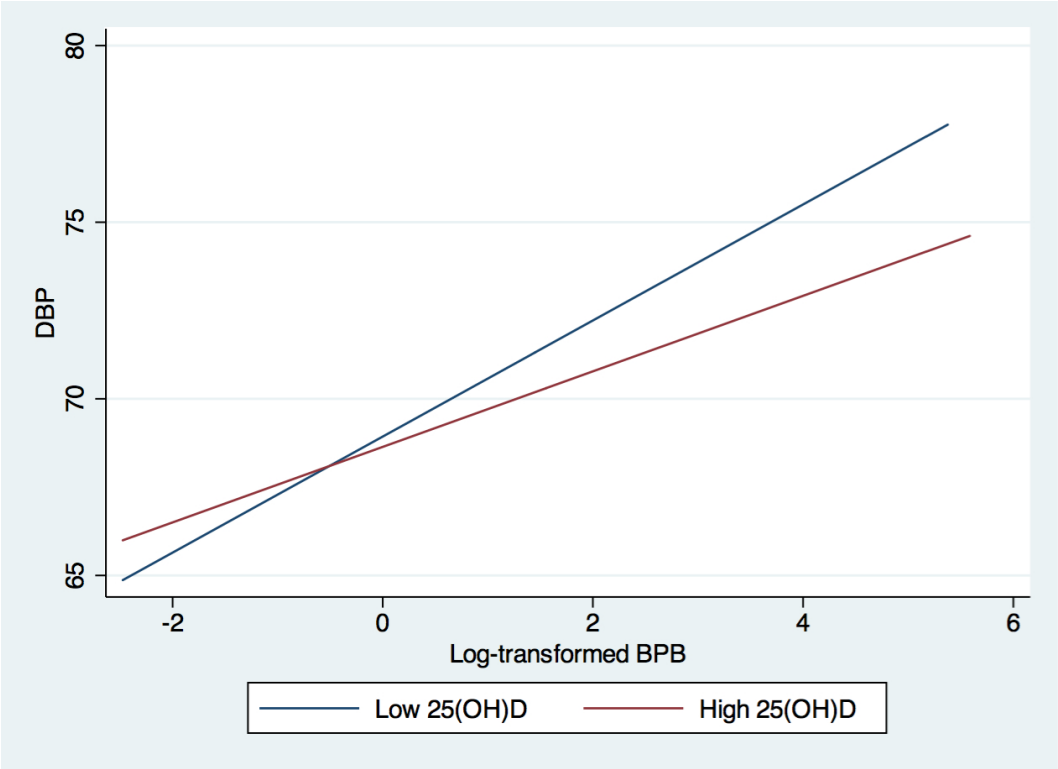

Table 5.1 Descriptive characteristics of study participants (n=10627)

Characteristic	Mean (SE) or n(%)
Age (years)	41.9 (0.4)
Male	5599 (52.7)
Female	5028 (47.3)
BPb (μg/dL)	1.4 (1.0)
25(OH)D (ng/mL)	21.9 (1.0)
SBP (mmHg)	119.0 (1.0)
DBP (mmHg)	70.2 (1.0)

Table 5.2 Descriptive characteristics of study participants by 25(OH)D status (n=5410)

Characteristic	Low 25(OH)D	High 25(OH)D	P
N	2917	2493	
Age (years)	40.8 (0.5)	41.8 (0.4)	0.08
Male	1351 (16.1)	1305 (31.3)	
Female	1566 (21.1)	1188 (31.3)	<0.01
White non-Hispanic	602 (15.0)	1972 (56.6)	
Black non-Hispanic	1320 (11.9)	79 (0.7)	
Mexican American	745 (4.9)	319 (2.2)	<0.01
Currently smoke every day	574 (10.0)	489 (13.9)	
Currently smoke but not every day	101 (1.4)	98 (2.7)	
Currently non-smoker	419 (5.9)	587 (14.7)	<0.01
Less than high school	1070 (9.8)	494 (7.8)	
High school diploma	716 (9.5)	684 (17.1)	
More than high school	1127 (18.0)	1313 (37.7)	<0.01
Body mass index	29.1 (0.2)	25.8 (0.2)	<0.01
BPb (μg/dL)	1.5 (1.0)	1.4 (1.0)	0.15
25(OH)D (ng/mL)	11.4 (0.1)	34.4 (0.2)	<0.01
SBP (mmHg)	122.2 (0.4)	118.3 (0.5)	<0.01
DBP (mmHg)	71.7 (0.4)	70.4 (0.3)	0.01

Categorical values are counts (weighted %), continuous variables are means (SE). BPb is the geometric mean.

Table 5.3 Change in BP with each doubling of BPb

	SBP		DBP	
	β	p	β	p
Model 1	3.9 (3.2, 4.5)	<0.01	1.4 (1.0, 1.9)	<0.01
Model 2	0.3 (-0.3, 1.0)	0.34	0.9 (0.4, 1.4)	<0.01
Model 3	0.2 (-0.4, 0.9)	0.47	1.0 (0.5, 1.6)	<0.01

Model 1: Unadjusted
Model 2: Adjusted for age, sex, race (white, black, Mexican American, other Hispanic, other, unknown), BMI
Model 3: Adjusted for age, sex, race (white, black, Mexican American, other Hispanic, other, unknown), BMI, education (< high school, high school, > high school), smoking (every day, currently but not every day, non-smoker)

Table 5.4 Change in log odds of SBP >= 140 and DBP >=90 with each doubling of BPb

	SBP		DBP	
	β	p	β	p
Model 1	0.5 (0.4, 0.6)	<0.01	0.4 (0.2, 0.5)	<0.01
Model 2	0.2 (0.0, 0.3)	0.04	0.2 (0.0, 0.4)	0.03
Model 3	0.1 (0.0, 0.3)	0.09	0.2 (0.0, 0.4)	0.02

Model 1: Unadjusted
Model 2: Adjusted for age, sex, race (white, black, Mexican American, other Hispanic, other, unknown), BMI
Model 3: Adjusted for age, sex, race (white, black, Mexican American, other Hispanic, other, unknown), BMI, education (< high school, high school, > high school), smoking (every day, currently but not every day, non-smoker)

Table 5.5 Change in BP with each doubling of BPb with a BPb/25(OH)D interaction term

	Pb	p	Interaction	p
	SBP			
Model 1	7.8 (3.6, 12.1)	<0.01	-0.8 (-1.8, 0.1)	0.08
Model 2	5.2 (1.0, 9.4)	0.02	-1.1 (-1.2, -0.1)	0.03
Model 3	5.5 (-2.4, 12.5)	0.17	-1.2 (-2.9, 0.6)	0.18
	DBP			
Model 1	1.0 (-1.6, 4.1)	0.49	0.0 (-0.6, 0.7)	0.90
Model 2	1.9 (-1.4, 5.2)	0.25	-0.2 (-0.9, 0.5)	0.53
Model 3	0.6 (-5.6, 6.8)	0.85	0.1 (-1.3, 1.5)	0.87

Model 1: Unadjusted
Model 2: Adjusted for age, sex, race (white, black, Mexican American, other Hispanic, other, unknown), BMI
Model 3: Adjusted for age, sex, race (white, black, Mexican American, other Hispanic, other, unknown), BMI, education (< high school, high school, > high school), smoking (every day, currently but not every day, non-smoker)

Table 5.6 Change in SBP with each doubling of BPb, stratified by 25(OH)D

	Low 25(OH)D		High 25(OH)D	
	β	p	β	p
Model 1	4.1 (3.2, 5.0)	<0.01	3.6 (2.9, 4.3)	<0.01
Model 2	0.5 (-0.5, 1.4)	0.33	0.1 (-0.6, 0.8)	0.80
Model 3	0.5 (-0.5, 1.6)	0.34	0.0 (-0.6, 0.7)	0.92

Model 1: Unadjusted
Model 2: Adjusted for age, sex, race (white, black, Mexican American, other Hispanic, other, unknown), BMI
Model 3: Adjusted for age, sex, race (white, black, Mexican American, other Hispanic, other, unknown), BMI, education (< high school, high school, > high school), smoking (every day, currently but not every day, non-smoker)

Table 5.7 Change in DBP with each doubling of BPb, stratified by 25(OH)D

	Low 25(OH)D		High 25(OH)D	
	β	p	β	p
Model 1	1.4 (0.7, 2.0)	<0.01	1.4 (0.9, 1.9)	<0.01
Model 2	0.8 (0.1, 1.6)	0.02	0.9 (0.4, 1.5)	<0.01
Model 3	0.9 (0.2, 1.6)	0.01	1.2 (0.6, 1.8)	<0.01

Model 1: Unadjusted
Model 2: Adjusted for age, sex, race (white, black, Mexican American, other Hispanic, other, unknown), BMI
Model 3: Adjusted for age, sex, race (white, black, Mexican American, other Hispanic, other, unknown), BMI, education (< high school, high school, > high school), smoking (every day, currently but not every day, non-smoker)

Table 5.8 Change in SBP by quartile of BPb, stratified by 25(OH)D

		Low 25(OH)D β	High 25(OH)D β
Model 1			
	Q1	ref	ref
	Q2	3.2 (1.3, 5.2)	4.6 (2.8, 6.4)
	Q3	6.4 (4.1, 8.8)	7.9 (6.7, 9.1)
	Q4	10.6 (8.3, 12.9)	9.5 (7.8, 11.2)
	p trend	<0.01	<0.01
Model 2			
	Q1	ref	ref
	Q2	-1.0 (-3., 1.1)	0.4 (-1.3, 2.2)
	Q3	-0.3 (-2.7, 2.0)	0.3 (-1.1, 1.7)
	Q4	1.2 (-1.4, 3.7)	0.5 (-1.2, 2.1)
	p trend	0.32	0.66
Model 3			
	Q1	ref	ref
	Q2	-0.9 (-3.1, 1.2)	0.4 (-1.4, 2.3)
	Q3	-0.3 (-2.7, 2.2)	0.2 (-1.3, 1.7)
	Q4	1.2 (-1.5, 3.9)	0.4 (-1.3, 2.1)
	p trend	0.32	0.75

Model 1: Unadjusted
Model 2: Adjusted for age, sex, race (white, black, Mexican American, other Hispanic, other, unknown), BMI
Model 3: Adjusted for age, sex, race (white, black, Mexican American, other Hispanic, other, unknown), BMI, education (< high school, high school, > high school), smoking (every day, currently but not every day, non-smoker)

Table 5.9 Change in DBP by quartile of BPb, stratified by 25(OH)D

		Low 25(OH)D	High 25(OH)D
		β	β
Model 1			
	Q1	ref	ref
	Q2	1.5 (0.1, 3.0)	2.1 (0.6, 3.5)
	Q3	3.8 (1.9, 5.7)	4.0 (2.7, 5.3)
	Q4	4.0 (2.5, 5.4)	3.3 (2.0, 4.6)
	p trend	<0.01	<0.01
Model 2			
	Q1	ref	ref
	Q2	0.5 (-1.1, 2.1)	1.6 (0.1, 3.1)
	Q3	2.6 (0.5, 4.6)	2.8 (1.4, 4.2)
	Q4	2.7 (0.9, 4.4)	2.0 (0.6, 3.4)
	p trend	<0.01	<0.01
Model 3			
	Q1	ref	ref
	Q2	0.6 (-1.0, 2.2)	1.9 (0.4, 3.4)
	Q3	2.7 (0.7, 4.6)	3.2 (1.7, 4.7)
	Q4	2.9 (1.2, 4.6)	2.7 (1.2, 4.2)
	p trend	<0.01	<0.01

Model 1: Unadjusted
Model 2: Adjusted for age, sex, race (white, black, Mexican American, other Hispanic, other, unknown), BMI
Model 3: Adjusted for age, sex, race (white, black, Mexican American, other Hispanic, other, unknown), BMI, education (< high school, high school, > high school), smoking (every day, currently but not every day, non-smoker)

CHAPTER 6: Conclusion

6.1 Summary of findings

The first goal of this research was to test the associations between metals and vitamin D in light of earlier reports on the inhibition of $1,25(OH)_2D$ synthesis by exposure to Pb and Cd. My findings were inconsistent with these reports, but there are important differences between my study and these earlier reports. First, while the biomarkers of Pb and Cd exposure in adolescents in in Torreon were elevated compared to national survey data in the US, they were substantially lower than those reported in the earlier studies. Thus, my results suggest that at lower levels of exposure to Pb and Cd, there is no detectable inhibition of 1α-hydroxylase and therefore no reduction in circulating levels of $1,25(OH)_2D$.

Unexpectedly I found a positive association between $1,25(OH)_2D$ and both As and U, even after controlling for 25(OH)D. This is a novel finding that has not been reported before. This finding questions the importance of nephrotoxicity in inhibiting $1,25(OH)_2D$ synthesis, since As and U, as well as Pb and Cd, are nephrotoxic (Chiu & Yang, 2005; Hong, Jin, & Zhang, 2004; Magdo et al., 2007; Vicente-Vicente et al., 2010),. However, one explanation for the positive association seen also supports a nephrotoxic mechanism: that damage to the renal proximal tubule results in a partial Fanconi syndrome, causing mineral wasting and in response, the body upregulates $1,25(OH)_2D$. In addition, I observed for the first time

that both Mo and Tl were positively associated with 25(OH)D. The mechanisms of this association are unclear and this finding highlights the need for further research on the interactions of metals with vitamin D.

In the study on vitamin D and inflammation, I found that 25(OH)D was positively associated with certain inflammatory biomarkers, and inversely associated with others, while $1,25(OH)_2D$ was positively associated with inflammatory biomarkers. The positive association between $1,25(OH)_2D$ and inflammation is a novel finding and provides new information on the link between vitamin D and inflammation. Since this is a cross-sectional study, I cannot determine causality from these observed associations. Randomized controlled trials of vitamin D supplementation have failed to show a significant decrease in inflammation associated with supplementation. In addition, *in vitro* studies of $1,25(OH)_2D$ suggest that it decreases the release of inflammatory cytokines on a cellular level. Therefore, the positive association between $1,25(OH)_2D$ and inflammation may represent reverse causality or an unmeasured confounder. Reverse causality in this case would mean that higher levels of inflammation cause an increase in $1,25(OH)_2D$. A confounder would be something that increases both $1,25(OH)_2D$ and inflammation, and is not in any causal pathway between $1,25(OH)_2D$ and inflammation.

Using data from NHANES, I found that similar to Torreon, Pb and 25(OH)D were unrelated. The effect of Pb on blood pressure was similar across all levels of

25(OH)D, suggesting that 25(OH)D does not protect against the harmful effects of Pb on blood pressure.

Although there are numerous studies linking Hg with autoimmunity, there are limited data on other metals in human populations. For that reason, I examined associations between biomarkers of metals and ANA, a biomarker of autoimmunity, in this study. I found no evidence that any of the eight metals measured, Pb, Cd, As, U, W, Tl, Mo, and Sb, increase risk for autoimmunity. Metal dose was not significantly associated with odds of positive ANA at any titer, or odds of positive ANA at a high titer (defined as ≥40). This suggests that Hg may be unique in its effect of increasing biomarkers of autoimmunity, and other metals will not increase autoimmunity as Hg does. In addition, one proposed mechanism for Hg-induced autoimmunity is through tissue damage, however these results suggest that tissue damage alone is not enough to induce autoimmunity since many of these metals, particularly Pb, are highly nephrotoxic and cause tissue damage without autoimmunity.

6.2 Public health implications and future research

The work presented in this dissertation provides insight into the health correlates of vitamin D, the value of measuring $1,25(OH)_2D$, and the viability of vitamin D as a public health tool.

The association between $1,25(OH)_2D$ and inflammation has significant public health implications and highlights the need for more research on vitamin D. The higher levels of $1,25(OH)_2D$ seen with exposure to higher levels of U and As, which may be related to kidney toxicity, and the higher levels of $1,25(OH)_2D$ seen with higher levels of inflammation suggest that $1,25(OH)_2D$ has potential value as a predictor of negative health status. Future research should incorporate $1,25(OH)_2D$ into epidemiological studies to identify its health correlates in cross-sectional studies, and also utilize longitudinal study designs to ascertain its value in predicting future health events.

In this dissertation, I chose to measure both $25(OH)D$ and $1,25(OH)_2D$ even though $1,25(OH)_2D$ is not typically measured in epidemiological studies. Current thinking is that $1,25(OH)_2D$ is not a good biomarker of vitamin D status because it remains relatively constant, even with sun exposure and supplementation (Himmelstein et al., 1990). However, the results of these studies suggest that it may prove to be a reliable marker for other areas of health and these results should challenge current thinking on the value of measuring $1,25(OH)_2D$. Future research should focus on identifying the clinical and prognostic values of measuring $1,25(OH)_2D$. The finding that $1,25(OH)_2D$ is positively associated with inflammation, and is therefore a potential predictor of adverse health effects, is a novel finding that could have significant public health implications.

My results suggest that vitamin D may not be an effective tool in treating increases in blood pressure caused by exposure to Pb. The public health significance of this finding is that efforts to treat increased blood pressure caused by Pb exposure should focus on limiting exposure to Pb, and it highlights the importance of continuing to reduce population-wide exposures to Pb. Although Pb levels in blood have decreased dramatically since the 1970s, elevated Pb continues to be a problem in both children and adults (Centers for Disease Control and Prevention (CDC), 2013; 2014).

Apart from any potential value of vitamin D in metal toxicity, this dissertation opens questions about the role of vitamin D in preventing or predicting disease and dysfunction and its potential role as a public health tool. If future longitudinal studies and randomized controlled trials provide evidence that increasing vitamin D improves morbidity and mortality, there will be huge public health implications. Vitamin D is unique as a public health intervention because it comes from the sun.

There are several potential advantages of sunlight as the primary source of vitamin D as opposed to supplementation, when considering a public health campaign to increase vitamin D. First, cost, as sunlight is free. Another advantage is lack of toxicity, as hypervitaminosis D is only a concern with oral supplementation, and not sunlight exposure. Finally, the half-life of vitamin D is longer when vitamin D is produced from sun exposure as compared to ingested orally from supplementation (Haddad, Matsuoka, Hollis, Hu, & Wortsman, 1993). The clinical implications of a

longer half-life from sun exposure have not been studied, and are an important area for future research. The mechanism for this longer half-life is that a higher proportion of vitamin D binds to the vitamin D binding protein when it is produced endogenously as compared to being ingested orally. The potential differential effects of vitamin D produced endogenously as compared to vitamin D taken orally, and the role of vitamin D binding protein, should be a focus of future research on vitamin D. While studies on UVB radiation are scarce, likely due to fears of skin cancer, one study of UVA and UVB radiation found significant effects of UVB radiation on reducing blood pressure in a short time (6 weeks) and a small sample size (17 total, 9 individuals receiving UVB) (Krause et al., 1998). Future studies could compare the efficacy of increasing vitamin D by UVB exposure as compared to oral supplementation to see if one method is more effective than the other.

It is important to continue work in environmental risk factors for autoimmune diseases. Autoimmune disease affect approximately 7% of the US population, and they are lifelong, chronic conditions that are very expensive to treat(F. W. Miller et al., 2012). Environmental risk factors in combination with genetic risk factors likely play an important role in the etiology of autoimmune disease, yet there remains much to understand about the role of environmental exposures as a risk factor for autoimmune disease, and the mechanisms for an association (F. W. Miller, 2011). Future research should continue to attempt to identify environmental risk factors for autoimmune disease, and negative findings should be reported as well. My finding that the effects of Hg on autoimmunity may be unique to Hg has important

public health implications, because it underlies the importance of further research on Hg and autoimmunity, and identifying the unique mechanisms that lead to autoimmunity and autoimmune disease from exposure to Hg.

CHAPTER 7: References

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APPENDIX

Spatial Clustering of Toxic Trace Elements in Adolescents around the Torreón, Mexico Lead-Zinc Smelter

Gonzalo G. Garcia-Vargas, ScD1,2, Stephen J. Rothenberg, PhD3, Ellen K. Silbergeld, PhD4,5, Virginia Weaver, MD4,6,7, Rachel Zamoiski, MPH4, Carol Resnick, BA4, Marisela Rubio-Andrade, MSc1, Patrick J. Parsons, PhD8,9, Amy J. Steuerwald, MS8,9, Ana Navas-Acién, PhD4-6, Eliseo Guallar, DrPH4-6

1 Facultad de Medicina, Universidad Juárez del Estado de Durango, Gómez Palacio Durango, México.
2 Secretaría de Salud del Estado de Coahuila, Coahuila, México.
3 Instituto Nacional de Salud Pública, Centro de Investigación en Salud Poblacional, Cuernavaca, Morelos, México.
4 Department of Environmental Health Sciences, Johns Hopkins Bloomberg School of Public Health, Baltimore, MD, USA.
5 Department of Epidemiology, Johns Hopkins Bloomberg School of Public Health, Baltimore, MD, USA.
6 Welch Center for Prevention, Epidemiology, and Clinical Research, Johns Hopkins Bloomberg School of Public Health, Baltimore, MD, USA.
7 Department of Medicine, Johns Hopkins University School of Medicine, Baltimore, MD, USA

8 Laboratory of Inorganic and Nuclear Chemistry, Wadsworth Center, New York State Department of Health, Albany, NY, USA.

9 Department of Environmental Health Sciences, School of Public Health, University at Albany, Albany, NY, USA.

Corresponding Author: S.J. Rothenberg, PhD, Centro de Salud Poblacional, Instituto Nacional de Salud Pública, Avenida Universidad No. 655, Col. Sta. Ma. Ahuacatitlán, CP 62508, Cuernavaca, Morelos, México. Telephone: +52-739-395-0662. E-mail: drlead@prodigy.net.mx

Abstract

Background

High blood lead levels in children and elevated soil and dust arsenic, cadmium, and lead were previously found in Torreón, northern Mexico, host to the world's fourth largest lead-zinc metal smelter.

Objectives

To determine spatial distributions of adolescents with higher blood lead and creatinine-corrected urine total arsenic, cadmium, molybdenum, thallium, and uranium around the smelter.

Methods

Cross-sectional study of 512 male and female subjects 12-15 years of age. We measured blood lead by graphite furnace atomic absorption spectrometry and urine trace elements by inductively coupled plasma-mass spectrometry, with dynamic reaction cell mode for arsenic. We constructed multiple regression models

including sociodemographic variables and adjusted for subject residence spatial correlation with spatial lag or error terms. We applied local indicators of spatial association statistics to model residuals to identify hot-spots of significant spatial clusters of subjects with higher trace elements.

Results

We found spatial clusters of subjects with elevated blood lead (range 3.6-14.7 μg/dL) and urine cadmium (0.18-1.14 μg/g creatinine) adjacent to and downwind of the smelter and elevated urine thallium (0.28-0.93 μg/g creatinine) and uranium (0.07-0.13 μg/g creatinine) near ore transport routes, former waste, and industrial discharge sites.

Conclusions

Spatial clustering of adolescents with high blood lead and urine cadmium adjacent to and downwind of the smelter and residual waste pile, areas identified over a decade ago with high lead and cadmium in soil and dust, suggests that past and/or present plant operations continue to present health risks to children in those neighborhoods.

Introduction

Children and adolescents in Torreón, an industrial city in the State of Coahuila in northern Mexico, have shown elevated blood lead levels (BPb) for at least three decades (1-3). Documented sources of exposure to toxic metals and metalloids in Torreón include high levels of lead, cadmium, and arsenic in roadside dust in

residential areas downwind and adjacent to the Met-Mex Peñoles plant, the largest lead-zinc-silver smelter complex in Latin America and the fourth largest in the world (4), and moderately high arsenic concentrations in drinking water throughout the city (8 μg/L – 75 μg/L) (5). While BPb levels in children have decreased substantially from 1999 (mean 17.0 μg/dL) to 2010 (mean 5.5 μg/dL) (6) following a program of emission control, environmental remediation and child lead surveillance at the turn of the century, lead exposure is still a major public health problem in Torreón.

As in other smelter towns (7-8), the location of the smelter and local area characteristics such as the direction of prevailing winds might be important determinants of the geospatial distribution of elevated BPb among Torreón residents. The goal of this paper was to determine the spatial distribution of concentrations of toxic trace elements based on subject residence in a sample of adolescents living in Torreón and to determine if higher concentrations of any of the trace elements clustered around known or suspected industrial sources.

Methods

Study Sample

Between August 2009 and June 2010, we performed the "Cuida tu Corazon" ("Take care of your heart"; C2C) study to evaluate the association of BPb and urine antimony (Sb), arsenic (As), cadmium (Cd), molybdenum (Mo), thallium (Tl),

tungsten (W), and uranium (U) with cardiovascular and renal function measures (to be reported separately) among 512 males and females 12 to 15 years of age living in Torreón. Residential neighborhoods border the Peñoles smelter complex in the south of the city (Figure 1) (3). The dominant wind pattern is from the north and east (9). In 1999-2000 the Health Department of the State of Coahuila conducted a census of all children and adolescents residing within 1.5 km of the smelter complex. The original census continued annually from 2002. We selected children and adolescents from the census who had at least one BPb determination performed prior to 2004 and who would be 12 to 15 years of age at the time of the study (N = 6,254). We divided the study population into 5 strata based on the initial BPb determination for each census subject (<10.0, 10.0 – 14.9, 15.0 – 19.9, 20.0 – 44.9, and ≥45.0 μg/dL), randomly selecting subjects within each stratum to achieve a target sample of 512 participants. The study was approved by the Institutional Review Boards of Juarez University of Durango State, the Johns Hopkins Bloomberg School of Public Health and the New York State Department of Health's Wadsworth Center. All subjects and their parents or legal guardians provided signed informed consent.

Data Collection

Study questionnaires were administered by trained study interviewers in home interviews and clinic visits. We used clinic visits to collect additional data, including detailed physical measurements and blood and urine specimens for trace element analysis.

Blood specimens were collected in EDTA purple top tubes (Becton Dickinson, Franklin Lakes, NJ) and refrigerated immediately. Spot urine specimens were collected in plastic containers that had been washed with 10% HNO3 overnight and rinsed with 18.2 MΩcm deionized water. Refrigerated blood and urine specimens were transported daily to the Laboratory of Toxicology of Juárez University, Durango State, where specimens were aliquoted and frozen at -80 ºC pending analysis.

Laboratory Analyses

BPb concentrations were measured in duplicate at the Laboratory of Toxicology of Juárez University using a graphite furnace atomic absorption spectrometer equipped with longitudinal Zeeman-effect background correction (Analyst 800, Perkin Elmer Norwalk, CT), according to the method described by Miller et al. (10). Specimens with a coefficient of variation (CV) >5% (N = 11) were reanalyzed in duplicate until CV <5%. The limit of detection (LOD) was 0.7 µg/dL and 2 subjects were <LOD. National Institute of Standards and Technology Standard Reference Material 955b, Lead in Bovine Blood (NIST, Gaithersburg, MD) was used to ensure the accuracy and traceability of BPb measurements to international standards. The laboratory successfully participates in the BPb Inter Laboratory Program of Quality Control from the Facultad de Medicina, Universidad de Zaragoza, Zaragoza, Spain and in the Wisconsin State Laboratory of Hygiene's Proficiency Testing Program for BPb.

Urine concentrations of Sb, total (unspeciated) As, Cd corrected for molybdenum oxide interference, Mo, Tl, W, and U were measured in the Trace Elements section of the Laboratory of Inorganic and Nuclear Chemistry at the New York State Department of Health's Wadsworth Center (Albany, New York, USA). The analyses were performed using an ELAN DRC II inductively coupled plasma-mass spectrometer (PerkinElmer Life and Analytical Sciences, Shelton, Connecticut, USA) equipped with Dynamic Reaction Cell technology (11). Multielement calibration standards were prepared from a NIST-traceable stock solution (High Purity Standards, Charleston, SC) and a six-point, calibration curve used for each element. Method accuracy was assessed via analysis of NIST Standard Reference Material (SRM) 2670a, Toxic Elements in Urine (Freeze-Dried), and SRM 2668, Toxic Elements in Frozen Human Urine. The LODs for Sb, As, Cd, Mo, Tl, W, and U were 0.2, 1.1, 0.02, 1.0, 0.02, 0.06, and 0.001 μg/L, respectively. The corresponding percentages of participants with urine element levels <LOD were 76.0, 0.0, 0.8, 0.0, 0.4, 22.9, and 0.2%, respectively. Because of the high proportion of participants with urine antimony and urine tungsten concentrations <LOD, we do not present further statistical analyses of these elements.

Urine creatinine concentrations were measured by an enzymatic assay (Siemens Dimension Vista 1500; Siemens Medical Solutions USA, Inc., Malvern, PA, United States). Urine trace element concentrations were normalized to urine creatinine concentrations to account for variations in urine dilution in spot urine specimens and results were expressed as mg/g creatinine.

Water samples from 12 separate well-heads of the municipal water system serving the subject area were collected by the municipal water system authorities of Torreón County in 2010 (12). During the study data collection period, the Torreón city water system had no centralized water treatment system for distributing potable water. Thus water from each well was distributed to specific neighborhoods and each well-head water arsenic concentration served to represent arsenic in water delivered to those neighborhoods. Measurements of total arsenic in water were made by the Torreón municipal water system (12) using a standard method based on hydride generation atomic absorption spectrometry, following the procedure recommended by Perkin Elmer (13).

We identified study participant residences on a geo-referenced Torreón street map as well as the metal smelter, the main pile of smelter waste, other known unofficial plant dumping areas (Garcia-Vargas, personal communication), a covered canal unused since 1957 that formerly transported liquid industrial waste from the metal smelter and urban waste, and rail and truck routes transporting ore to the plant, to identify possible geographic determinants of element concentrations in sample adolescents (Figure 2). We used a wind rose for 2011 (9), the natural year closest to the measurement of elements in our sample, to determine prevailing winds. Predominant and strongest winds are from the arc north-northwest through the southeast. There was almost a complete absence of winds from the south and southwest.

Statistical Analysis

Trace element concentrations were markedly right skewed and were natural log-transformed for statistical analysis. We calculated descriptive statistics for the elements and selected control variables stratified by sex. Sex differences in continuous variables were determined by t-test and in categorical variables by the Fisher test.

We constructed ordinary least squares (OLS) multiple regression models for log-transformed BPb and for log-transformed creatinine-corrected urine trace element concentrations using available demographic variables as predictors (apart from residence location). These variables included mean-centered age, sex, family income, subject former and current smoking, private or public school attendance, living at same address for at least 10 years, use of municipal water, commercial bottled water or municipal water with a filter for domestic water consumption, consumption of milk or cola beverage at least once per week, seafood consumption during the previous week, and municipal water arsenic concentrations. We included milk and cola intake because milk produced in the Torreón region was reported to contain arsenic (14) and many soft drinks are bottled in Torreón, presumably using local well water, except for Coca-Cola™, the largest selling brand in the city, which is bottled elsewhere. Since some types of seafood are reported to contribute organoarsenic species, such as arsenbetaine, to the diet (15), we used a dichotomous variable to code seafood eating within the week prior to blood and

urine sampling for trace element analysis. Although municipal water arsenic concentrations were used primarily to adjust spatial models of urine arsenic concentrations for known regional differences in water arsenic, we also used water arsenic as a proxy for other potential water-borne metal sources. We calculated Spearman correlations between water arsenic concentrations by well and measured trace element means in subjects grouped by well areas. We used Stata 11 and 12 (StataCorp, College Station, TX) for all descriptive analyses and preliminary multiple regression modeling.

Spatial Analyses

We used GeoDa 1.4 (16) to construct spatial models. We calculated a distance-based spatial weight matrix with a cutoff value of 0.9 km using decimal degree geographic coordinates of subject homes provided by the Mexican Institute for Geographical and Statistical Information (17). We diagnosed OLS BPb and urine trace element models for the presence of two types of spatial dependence, spatial error and spatial lag, using robust Lagrange Multiplier tests. Both types of dependence indicate a violation of the OLS assumption of residual independence; spatial lag dependence additionally indicates a violation of the assumption of independent observations of the dependent variable.

If model diagnostics indicated statistically significant spatial error or lag dependence, we reanalyzed the models adjusted for spatial error or spatial lag dependence to reduce global spatial clustering to statistical insignificance and

eliminate OLS assumption violations, providing coefficients and standard errors of model variables uninfluenced by the above OLS violations.

We calculated model residuals, adjusted for spatial error or spatial lag where necessary, as estimates of element concentrations not explained by model variables and not subject to spatial clustering of model explanatory variables. These residuals were used to calculate Moran's I (18), a statistic representing global spatial associations based on inverse spatial weighting derived from distances between subject residences.

We used local indicators of spatial association (LISA) statistics (19) to measure the inverse distance-weighted association of trace element model residuals with the trace element residuals of neighboring subjects to identify local spatial clustering. As detailed in (19), these statistics classified study participants into 4 groups for each element distribution: hot-spots (subjects with high element levels relative to the sample living in close proximity to other subjects with high element levels); cold-spots (subjects with low element levels living in close proximity to other subjects with low element levels); "cluster outliers" (high element level subjects living in close proximity to other subjects with low element levels); and other subjects, not otherwise identified in the LISA analysis. Hot-spots had model residuals indicating model underestimation of element concentrations and cold-spots had model residuals indicating model overestimation of element concentrations. To simplify the interpretation, we mapped only significant hot- and

cold-spots (frequency-based probabilities ≤0.05, determined by 10,000 random permutations of the data set), with all other subjects grouped together. We also used LISA analysis on unadjusted trace element concentrations for comparison to the model residual analysis. We excluded no data points among the elements examined with spatial analysis.

We plotted contour maps of untransformed BPb and creatinine-adjusted urine trace element concentrations using the natural neighbor technique (20) to show spatial correspondence of trace element levels with LISA evidence of local spatial clustering. A digitized map of Torreón was overlaid on the combined LISA and contour map to locate hot- and cold-spots. Data and map manipulation were performed in Surfer 8.09 (Golden Software, Inc., Golden, CO).

To ensure that individual subject addresses could not be recovered from the maps, all plotted subject residence locations were degraded to 10 m accuracy in addition to the best-case 7.9 m accuracy specified for single frequency satellite-based GPS determination (21). In addition, symbols used to indicate subject residence on our maps were 40 m wide. All spatial statistical models, however, used the full accuracy of the GPS-determined coordinates in calculating spatial clustering.

Results

Quality control data for urine trace element levels are shown in Supplemental Table1. Distribution of urine trace element concentrations among study participants uncorrected by urine creatinine are shown in Supplemental Table 2. Spearman correlations among all trace elements are shown in Supplemental Table 3. Geometric mean (95% CI of mean) of blood lead was 4.0 (3.8, 4.2) μg/dL. Geometric means (95% CI of mean) of creatinine-correct urine trace elements (arsenic, cadmium, molybdenum, thalium, and uranium, respectively) were 36.5 (35.1, 38.0), 0.23 (0.22, 0.24), 63.7 (61.4, 66.2), 0.27 (0.26, 0.28), and 0.04(0.039, 0.045) μg/g. Urine trace element levels were significantly higher for males compared to females except for cadmium and molybdenum (Table 1). Other control variables showed no sex differences.

Spearman correlations between well water arsenic and trace elements in subjects grouped by well areas were positive and significant ($p \leq 0.05$) for creatinine-corrected urine As, Mo, Tl, and U (Table 2). Figure 3 shows the spatial distribution of subjects classified by well water As. Summaries of trace element models are found in Table 3. Only variables significant at $p \leq 0.05$ are shown along with the type of spatial dependency adjustment used, if any. Full models are shown in Supplemental Table 4 A-F. Model R2 varied from 0.107 (Mo) to 0.239 (As). In multiple regression models, male sex was significantly associated with higher

concentrations of trace elements except Cd and Mo, and increasing age was significantly associated with lower concentrations of trace elements except Cd.

Spatial Results

Figure 4A-F shows hot- and cold-spot grouping (LISA maps) of all metals based solely on measured concentration. There are strong tendencies for hot-spots to cluster in either the northwest or southeast sectors with cold-spots occupying the opposite pole. These metal concentration gradients are responsible for the significant Moran's I statistic indicating spatial dependence of element concentrations. Clustering seen using measured element concentration can be due to many factors, including point or area sources, prevailing winds, anisotropic spatial sample distribution and sample characteristic distribution. Thus, for elements showing significant age, sex, and/or sociodemographic associations, uneven distribution of such sample characteristics can influence the apparent spatial clustering of higher and lower measured element concentrations.

Figure 5A-F contains combined contour-post maps showing measured element concentration contours with LISA hot and cold-spots based on model residuals. Global spatial correlations have been statistically removed from the model residual maps. The hot and cold-spots remaining were not influenced by possible uneven distribution of other determined subject characteristics but could still be influenced by variables not explicitly in the model, including point and area element sources as

well as prevailing wind patterns. Table 4 shows measured subject trace element concentrations of subjects identified with model residual hot and cold-spots.

The highest levels of water As from municipal wells were found in the southeast (Figure 3), where the major grouping of measured arsenic hot-spots was also found (Figure 4A). In adjusted models, no hot-spots for As in subjects were found in the southeast, since water As was explicitly accounted for in the urine As model, along with lagged spatial dependence (Figure 5A).

Hot and cold-spot geographic locations for urine Cd (Figure 5B) and BPb (Figure 5D) substantially overlapped. Those hot-spots were principally located downwind and immediately adjacent to the metal refinery waste pile in the north of the Peñoles property and the smelter operations area south of the property.

Tl and U hot-spots occupied similar but non-overlapping locations in the southeast of the study area. Higher Tl levels (Figure 5E) were clustered downwind of the former sewage and industrial waste canal and ore transport rail lines. Higher U levels (Figure 5F) were clustered around the former plant open waste dump, now covered, and downwind of the rail and truck ore transport routes. Mo (Figure 5C) showed no model LISA hot-spots.

Discussion

In this cross-sectional study of BPb and urine trace elements in male and female adolescents residing in Torreón, Mexico, we found significant spatial clustering of higher BPb and urine Cd levels adjacent to and downwind of the Peñoles smelter complex. We also found significant spatial clustering of higher urine Tl and U adjacent to and downwind of the truck and rail route staging areas for delivery of raw materials to the smelter complex. The only spatial component considered in previous studies of trace element contamination in Torreón was crude distance to the smelter complex, indicating that the smelter was a possible point source for some elements (4,22). Our study adds detailed location and distance spatial components and the evaluation of multiple trace elements to the study of toxic exposures in this geographic area.

Since the turn of this century, Peñoles has embarked on a program of limiting stack emissions from their refinery and enlarging critical bagging enclosures. They have also performed environmental remediation by street cleaning operations around the plant since May 1999, buying properties of residents from 1999-2001 in the areas where the highest BPb were found, relocating these residents to other areas, and planting the evacuated areas with trees. They continue to maintain a childhood lead surveillance program in cooperation with state health authorities. Nevertheless, over 86% of adolescents in BPb hot-spots in the present study exceeded the CDC reference level of 5 μg/dL (23).

Before the Peñoles remediation program, two groups made independent measurements of soil Pb, Cd, and As in residential areas as a function of distance to the smelter (4,22). Soil concentrations had combined ranges of 1,640-17,320 μg/g for Pb, 80-1,497 μg/g for Cd, and 50-570 μg/g for As. Soil element levels were higher in sites closest to the smelter. The CDC (24) performed a survey sample-design study of BPb in children aged 1-6 years and dust lead levels around the smelter complex in March 2001, after the start of cleanup efforts. In addition to finding significantly higher BPb in children closest to the plant, they also found lead dust in the children's indoor play areas up to 216 μg/g and in their outdoor play areas up to 464 μg/g. Soil lead in the outdoor play areas within 1.5 km of the plant ranged from 25-2179 μg/g and soil lead beyond that distance from 24-589 μg/g.

Sparse rain (yearly average 23 cm) and frequent high winds (average maximum daily wind over 18 km/hour) combine to produce dusty conditions in Torreón with dust storms typically preceding desert thunderstorms (25). Total suspended particulates measured from the air quality monitoring network within Torreón throughout the year frequently exceed the Official Mexican Norm limiting the 24-hour average to <210 μg/m3 (26). 24-hour air Pb concentration measured during April-December, 2007 at 6-day intervals was highest at the station about 1 km from the western border (downwind) of the Peñoles property, ranging between 0.2 to 1.1 μg/m3 (26). 24-hour air Cd and As measured at the same location varied between 0.01-0.14 μg/m3 and 0.01-0.05 μg/m3, respectively. The US EPA set the National

Ambient Air Quality Standard for Lead to 0.15 μg/m3 (rolling 3-month average) in 2008 (27).

After adjusting our model of As for sociodemographic variables and the spatial pattern of As concentration in water, we found only a few subjects clustered for higher urine As. The small cluster location did not suggest wind-transported As from the smelter operation, even though previous studies (4,22) and the known association of As with some precious metal ores indicated the possibility of some plant contribution to As burden. Most of the total urine As in our study appeared to come from As in the water supply and diet. Nine water district wells providing water to the study area exceeded the EPA limit of 10 μg/L for As in drinking water and six exceeded the 25 μg/L Mexican norm. Since we did not speciate urine As, some fraction of urinary As in our subjects may have come from dietary organoarsenic compounds. Despite including model adjustments for fish and milk consumption, such adjustments will only partially adjust total As for organoarsenic species. Measurement of speciated urine As would have provided better assessment of health risk from this metalloid.

Other limitations need to be considered in the interpretation of our data. All urine element determinations were made with spot urine specimens, which require correction for urine dilution and may be affected by diurnal variation in excretion. This was a cross-sectional study; longitudinal associations with seasonal wind and rainfall patterns were not possible.

In addition, the sample was chosen with the goal of providing roughly equal numbers of male and female children in selected earlier BPb ranges who previously participated in the long-term surveillance program started in 1999. The children in the surveillance program and this study were self-selected by their parents and did not necessarily represent the population of children living in Torreón.
Our study also had several strengths, including use of standardized field data collection techniques, rigorous quality control of trace element measurements, and adequate power of the study to detect statistically significant results.

The published literature documented elevated levels of Pb, Cd, and As in soil, dust, and air close to the Peñoles plant before and during the clean-up efforts of the plant operators. Our data showed clusters of children with elevated BPb adjacent to and downwind of the Peñoles smelter complex over a decade after the start of the company's efforts to reduce exposure to the surrounding residential population. Despite the remedial actions of the company and heightened population awareness of the problem, clusters of subjects with BPb in these areas >5 μg/dL likely indicate ongoing exposure. Furthermore, while Pb exposure in this population has received substantial attention, our data also indicate that the smelter complex directly or indirectly contributes to Cd, Tl, and U exposures.

Our findings are based on spatial statistical models of subject trace element concentrations and typical wind patterns in the area. The most current soil and dust

samples are from 1999-2002 and these are not precisely located in reference to the plant. To determine the role of past and present plant operations in measured BPb and urine element hot-spots, renewed soil sampling for trace element analysis in and around hot-spot areas should be undertaken. Air particulate sampling for Pb and Cd on the plant perimeter would help determine if current controls on stack emissions and refining residues are sufficient to prevent continued accumulation of elevated soil and dust trace element levels in affected neighborhoods.

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Figure 1. Aerial view of the Peñoles Met-Mex metal smelter and surrounding communities, circa 1999. The view is to the south. The smelter waste pile occupies the center foreground, the smelter and offices are in the center background, behind the waste pile. Photo from: Programa para mejorar la calidad del aire en la región de la Comarca Lagunera 2010-2015. SEMARNAT (Secretaría de Medio Ambiente y Recursos Naturales), no date, no copyright. Accessed: 3 December 2012 from http://www.semarnat.gob.mx/temas/gestionambiental/calidaddelaire/Documents/Calidad%20del%20aire/Proaires/ProAires_Vigentes/9_ProAire%20Comarca%20Lagunera%202010-2015.pdf . Color contrast values of photo have been adjusted to enhance detail.

Figure 2

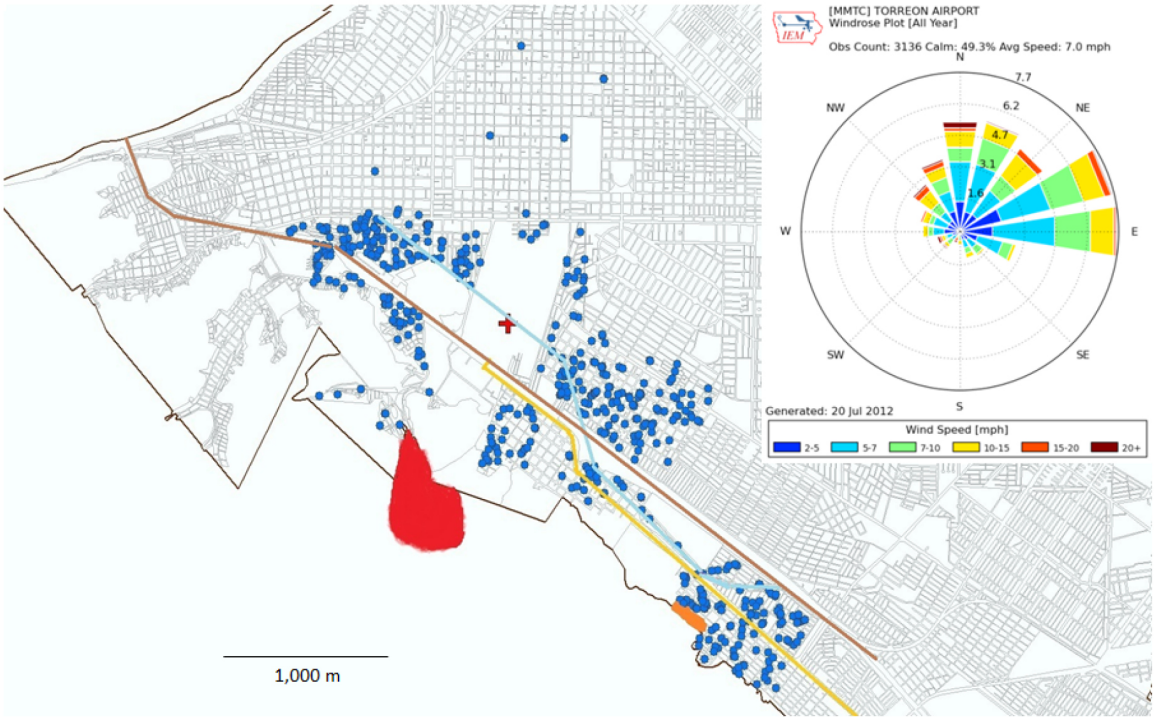

Figure 2. Geographically referenced map of Torreón, Coahuila.
Blue dots represent the residence sites of study participants. The red cross represents the center of Peñoles Met-Mex smelter complex. North is up as shown in the wind-rose inset. Other possible sources for toxicological element exposure are: ( ) Confinement of residual materials from Zn-Cd plant (Jarosite); ( ) Former open dump of smelter debris, now closed and covered; ( ) Ore truck transport route; ( ) A former irrigation channel converted to conduct urban sewage and smelter industrial waste, now abandoned; ( ) Railway transport route for ores. The wind rose shows that winds are predominantly from the arc north-northwest to east-southeast.

Figure 3 A-F

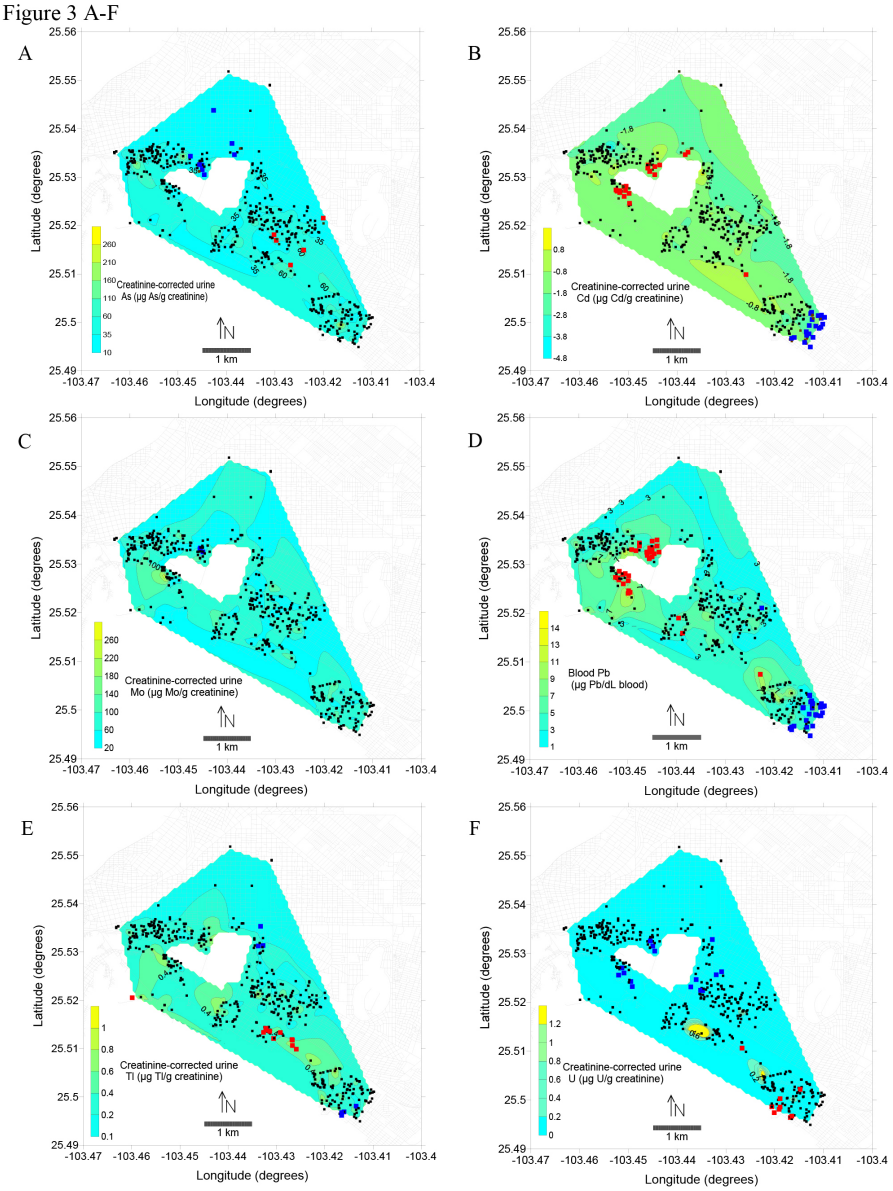

Figure 3. Post map of subject residence location coded by water As concentration measured at well heads servicing the subject area. Open circles are well locations, with same color code as the residence locations. Several wells had identical (nearest µg/L) As concentrations.
Two wells with the lowest and two wells with the highest water As are combined on the map. See Figure 2 for more details.

Figure 4 A-F

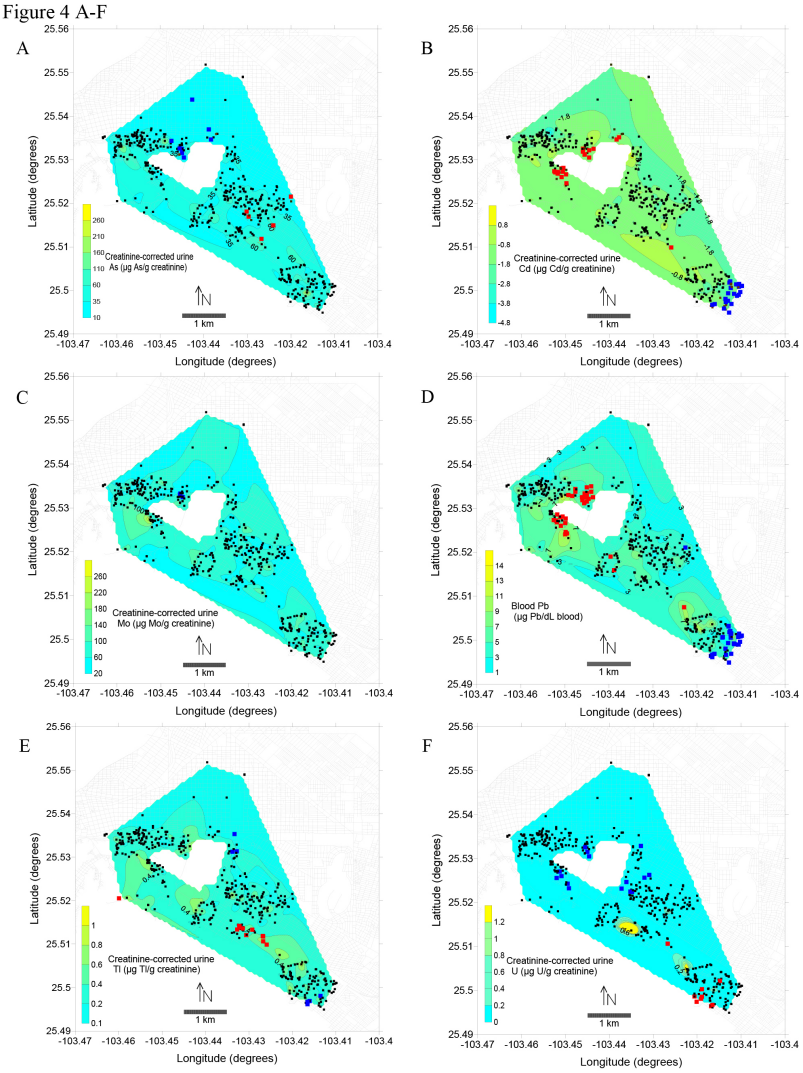

Figure 4A-F. Post maps of significant (frequency-based probability < 0.05) hot (■) and cold-spot (■) subject locations determined from measured (unadjusted by models) trace element concentrations. Black symbols were subjects that did not significantly cluster into hot or cold-spots. The unfilled irregular polygon in the center-upper left sector represents the area occupied by the Peñoles smelter complex (southern part of the polygon) and the major waste pile from plant operations (northern part of the polygon). Other industrial plants occupy the arm of the polygon extending west-northwest of the Peñoles property.

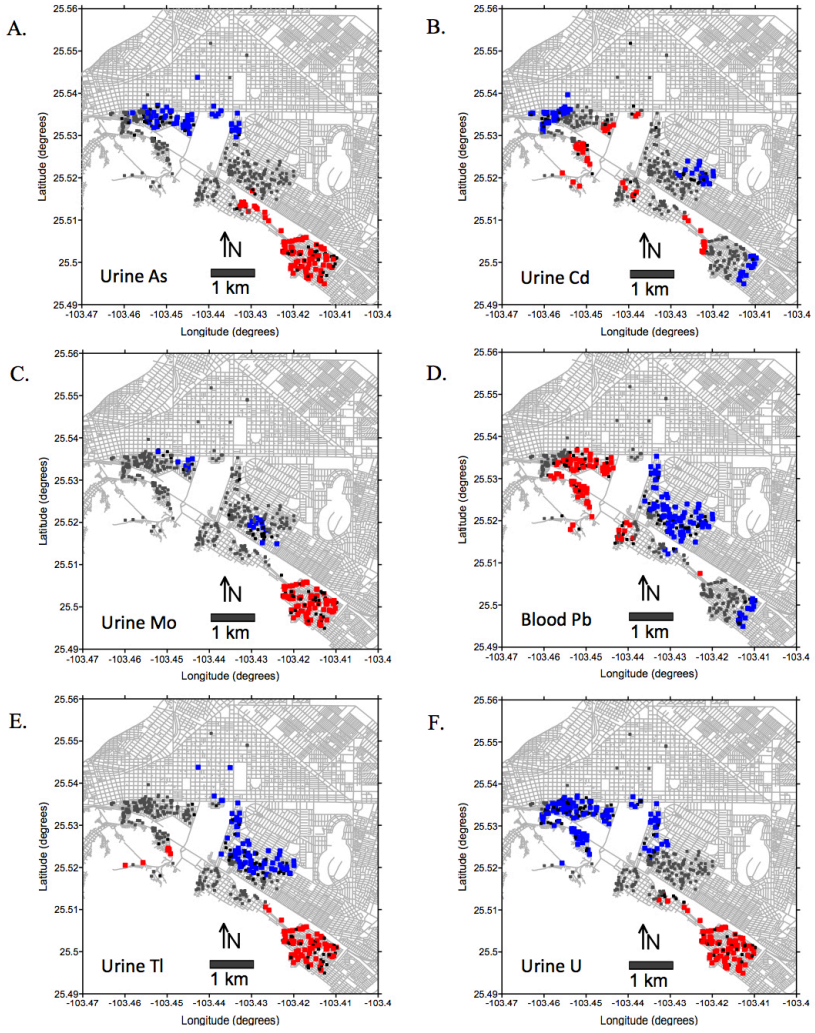

Figure 5A-F. Contour-post maps of (A) Arsenic, (B) Cadmium, (C) Molybdenum, (D) Lead, (E) Thallium, and (F) Uranium in study subjects.
Contour maps of measured trace element concentrations were formed by the natural neighbor technique. Some subject locations on the borders of the contour space fall outside the contour area but elemental concentrations contribute to the contours within the contour area. All trace elements except blood lead were creatinine-corrected urine concentrations. Post overlay of significant (frequency-based probability < 0.05) element hot (■) and cold-spot (■) locations were determined from residuals of demographic models, corrected for global spatial dependence where needed (see Methods). See Figure 4 legend for more details.

Table 1. Descriptive statistics of sample by sex

	Females (N=250)			Males (N=262)			
Variable[1]	Mean[2]	Count	SD[3]	Mean[2]	Count	SD[3]	P-value[4]
Urine Arsenic (As)	33.6		1.6	39.5		1.6	0.0001
Urine Cadmium (Cd)	0.22		2.0	0.23		2.0	0.58
Urine Molybdenum (Mo)	61.7		1.5	65.7		1.6	0.11
Blood Lead (Pb)	3.5		1.7	4.6		1.7	<0.00005
Urine Thallium (Tl)	0.25		1.7	0.29		1.7	0.0004
Urine Uranium (U)	0.04		2.3	0.04		2.0	0.01
Age (years)	13.9		1.2	14.0		1.1	0.26
School grade	6.9		1.3	6.9		1.3	0.94
Income (> 3000/month)		78			66		0.09
Smoke (within last month)		92			102		0.12
Occupational risk		133			132		0.54
Eat fish (≥1/week)		24			39		0.08
Drink milk (≥1/week)		134			130		0.38
Drink cola (≥1/week)		121			128		0.93

[1]Urine trace element units μg element/g of creatinine (creatinine-corrected); blood Pb units μg of lead/dL of blood.
[2]Element means are geometric means; other means are arithmetic means.
[3]Element standard deviations are geometric SD; other standard deviations are arithmetic SD.
[4]t-test p-values for variables with means; Fisher's exact p-values for variables with counts.
p-values for categorical variables with count summaries are calculated by comparison with the omitted dummy variable: Income: < 3000/m; Smoke: never smoked; Parental Occupational Risk for any trace element exposure: no occupational risk; Eat fish, drink milk/cola: <1/week

Table 2. Spearman correlations of water As measured at 12 well heads serving the sample area and subject trace element concentration means grouped by well service area.

Subject trace element[1]	rho	P-values
Urine As	0.860	<0.001
Urine Cd	0.435	0.158
Urine Mo	0.677	0.016
Blood Pb	0.544	0.068
Urine Tl	0.947	<0.001
Urine U	0.632	0.028

[1]All urine elements creatinine-corrected

Table 3. Summary of final demographic models showing only variables significant at p≤0.05.

Element[1]	Final Model	Significant Variables[2]	Model R^2
Urine Arsenic ln(As)	Spatial Lag	+Sex (male)# -Age† +Occupational Risk* -Private School* -Use Bottled Water# +Fish† +Milk#	0.239
Urine Cadmium ln(Cd)	Spatial Lag	+Occupational Risk* -Use Bottled Water** -Drink Cola*	0.127
Urine Molybdenum ln(Mo)	OLS	-Age† -Family Income (> 3000 Mexican Pesos/Month)* +Arsenic in Tap Water#	0.107
Blood Lead ln(Pb)	Spatial Error	+Sex (male)† -Age# -Income> 3000* -Private School* +Arsenic in Tap Water# -Drink Cola#	0.224
Urine Thallium ln(Tl)	Spatial Error	+Sex (male)† -Age† -Use Bottled Water** +Arsenic in Tap Water†	0.215
Urine Uranium ln(U)	Spatial Lag	+Sex (male)** -Age† -Not Current Smoker* -Private School# -Use Bottled Water#	0.160

[1]All urine elements creatinine-corrected.

[2] Statistically significant non-informative categorical responses ("don't know" "won't say" "missing") not shown. p-values of categorical variables are from comparison with omitted categories: Sex (female), Parental Occupational Risk for all trace elements (no occupational or hobby risk), Water use (tap water from municipal lines), School (public school), Family Income (≤$3000 Mexican Pesos/month), Lived at same address <10 years; Smoker (never smoked), fish (<1 portion/week), milk and cola (<1 glass/week). All demographic models used the same set of variables, except for corrections of spatial dependencies where needed. See Supplemental Table S1A-F for complete models including all variables, coefficients and 95% confidence intervals.

+,- Sign of coefficient
*p<0.05; **p<0.01; #p<0.005; †p<0.0001

Table 4. Measured element concentrations in subjects associated with local indicators of spatial association (LISA) hot and cold-spot spatial clusters determined from residuals of demographic models.

Trace Elements	N	Measured Geometric Mean (μg element/ g creatinine)[1]	Geometric Standard Deviation	Low [2]	High[2]
Urine Arsenic					
Hot Spots	24	48.0	1.3	25.8	75.9
Cold Spots	8	21.5	1.4	12.1	31.2
Urine Cadmium					
Hot Spots	21	0.38	1.55	0.18	1.14
Cold Spots	21	0.12	1.49	0.06	0.25
Urine Molybdenum					
Hot Spots	0	-	-	-	-
Cold Spots	6	44.5	1.4	26.0	57.4
Blood Lead[1]					
Hot Spots	35	6.9	1.4	3.6	14.7
Cold Spots	32	2.7	1.4	1.4	4.4
Urine Thallium					
Hot Spots	13	0.42	1.35	0.28	0.93
Cold Spots	6	0.18	1.28	0.13	0.25

Urine Uranium					
Hot Spots	6	0.08	1.30	0.07	0.13
Cold Spots	10	0.03	1.28	0.02	0.04

[1]Blood lead units are µg/dL

[2]The range of hot spots and cold spots for some elements overlaps because the clusters were based on model residuals and the descriptive statistics were based on measured element concentrations of those same subjects.

Supplemental material for Chapter 2

Appendix Table 5. Spearman correlation matrix of metals and arsenic.

	Pb	As	Cd	Mo	Tl	U
Pb	1.00					
As	0.24	1.00				
Cd	0.43	0.26	1.00			
Mo	0.09	0.37	0.17	1.00		
Tl	0.26	0.35	0.32	0.24	1.00	
U	0.18	0.56	0.24	0.18	0.26	1.00

Appendix Table 6. Difference in 25(OH)D (ng/mL) per doubling of metal dose by sex.

	Girls			Boys		
	β	95%CI	p-value	β	95%CI	p-value
Model 1						
As	0.4	(-1.1, 2.0)	0.59	0.9	(-0.7, 2.5)	0.24
U	0.9	(0.1, 1.8)	0.03	0.4	(-0.6, 1.5)	0.41
Tl	0.6	(-0.7, 2.0)	0.36	1.8	(0.4, 3.2)	0.01
Model 2						
As	-0.1	(-1.5, 1.4)	0.93	0.5	(-1.0, 1.9)	0.55
U	0.4	(-0.4, 1.2)	0.30	-0.1	(-1.1, 0.9)	0.86
Tl	0.4	(-0.9, 1.7)	0.54	1.9	(0.6, 3.2)	<0.01

Model 1: Adjusted for age
Model 2: Model 2: Adjusted for age, sex, season, SES (family income <3000 pesos/month, >= 3000 pesos/month, or unknown), smoking (never, former, or within the past month), adiposity, time spent outside.

Appendix Table 7. Difference in $1,25(OH)_2D$ (pg/mL) per doubling of metal dose by sex.

	Girls			Boys		
	β	95%CI	p-value	β	95%CI	p-value
Model 1						
As	4.7	(1.3, 8.1)	0.01	2.5	(-1.0, 6.0)	0.15
U	2.6	(0.7, 4.5)	0.01	1.1	(-1.2, 3.5)	0.35
Model 2						
As	4.4	(0.7, 8.0)	0.02	2.8	(-0.8, 6.4)	0.13
U	3.0	(0.9, 5.0)	<0.01	1.6	(-0.8, 3.9)	0.20
Model 3						
As	4.4	(0.8, 8.0)	0.02	2.6	(-0.9, 6.1)	0.15
U	2.8	(0.8, 4.8)	0.01	1.6	(-0.7, 3.9)	0.18

Model 1: Adjusted for age
Model 2: Model 2: Adjusted for age, season, SES (family income <3000 pesos/month, >= 3000 pesos/month, or unknown), smoking (never, former, or within the past month), adiposity, time spent outside.
Model 3: Adjusted for age, season, SES, smoking, adiposity, time spent outside, 25(OH)D

Appendix Table 8. Difference in 25(OH)D and 1,25$(OH)_2$D per doubling of As in non-fish eaters

	25(OH)D (ng/mL)			1,25$(OH)_2$D (pg/mL)		
	β	95%CI	p-value	β	95%CI	p-value
Model 1						
As	1.1	(-0.2, 2.3)	0.10	4.1	(1.4, 6.8)	<0.01
Model 2						
As	0.2	(-1.0, 1.4)	0.70	4.1	(1.3, 6.9)	0.01
Model 3						
As	-			4.0	(1.2, 6.7)	0.01

Model 1: Adjusted for age and sex.
Model 2: Model 2: Adjusted for age, sex, season, SES (family income <3000 pesos/month, >= 3000 pesos/month, or unknown), smoking (never, former, or within the past month), adiposity, time spent outside.
Model 3: Adjusted for age, sex, season, SES, smoking, adiposity, time spent outside, 25(OH)D.

Rachel D. Zamoiski, MPH

Home Address:
707 President St. Apt. 1406
Baltimore, MD 21202
443.545.4988
rachel.zamoiski@gmail.com

Office Address:
Johns Hopkins School of Public Health
615 N. Wolfe St, W6110
Baltimore, MD 21205
rdz@jhmi.edu

Born: December 12, 1981
Baltimore, MD

Education

Expected August 2014	**Doctor of Philosophy,** Environmental Health Sciences, Johns Hopkins Bloomberg School of Public Health, Baltimore, MD **Dissertation:** The associations of vitamin D and metal exposures with inflammation, autoimmunity, and blood pressure **Certificates:** Vaccine Science and Policy; Risk Sciences and Public Policy
May 2007	**Master of Public Health,** Johns Hopkins Bloomberg School of Public Health, Baltimore, MD **Capstone:** Clinical comparisons, familial aggregation, and genetic association of hallucinations and delusions in major mood disorders
May 2004	**Bachelor of Arts,** Psychology, University of Pennsylvania, Philadelphia, PA. Minor: Biological Basis of Behavior. **Senior Thesis:** Procrastination, depressive symptoms, stress, and health in undergraduates

Honors and Awards

2011-2014	Sommer Scholarship, Johns Hopkins Bloomberg School of Public Health
2011-2014	Procter & Gamble Dissertation Research Fellowship, Johns Hopkins Bloomberg School of Public Health
2003-2004	Dean's List, University of Pennsylvania

Experience

Sep 2008-Aug 2009

Executive Assistant to the Chief Financial Officer, American Jewish Committee, New York, NY.

- Assisted in running the finance office of a large (200+ employees) non-profit organization during the 2008 financial crisis
- Wrote and implemented new travel and expense policies
- Prepared financial information for presentation to board members

Oct 2007-Aug 2008

Epidemiologist, Ackerman Academy of Dermatopathology, New York, NY.

- Conducted literature searches and wrote critiques of research on sun exposure and risk of melanoma

Jan 2008-May 2008

Intern, The Leukemia and Lymphoma Society, New York, NY.

- Communicated with patients and prepared patient education programs

Jun 2004-May 2006

Research Assistant, Department of Psychiatry and Behavioral Sciences, Johns Hopkins School of Medicine, Baltimore, MD.

- Screened and enrolled research participants for three nationwide studies of the genetics of bipolar disorder and major depression
- Interviewed psychiatric patients to determine eligibility for participation
- Produced communication materials updating past research participants on progress and new findings
- Assisted in grant writing and grant submission
- Supervised volunteers
- Attended weekly rounds and lectures

May 2001-Aug 2001

Intern, Harvey Institute for Human Genetics, Greater Baltimore Medical Center, Baltimore, MD.

- Analyzed data on chromosomal causes of miscarriages

Teaching Experience

2010-2013

Teaching Assistant, Johns Hopkins Bloomberg School of Public Health, Baltimore, MD.

Methods in Quantitative Risk Assessment

- Served as a teaching assistant for this course twice

Environmental and Occupational Health Law and Policy

- Served as a teaching assistant for this course three times

Environmental Epidemiology

Advanced Topics on Control and Prevention of HIV and AIDS

Epidemiology and Public Health Impact of HIV and AIDS

Epidemiology and Natural History of Human Viral Infections

Academic Service

2012-2013

Student Representative, Environmental Health Sciences Education Committee, Johns Hopkins Bloomberg School of Public Health

2010-2012

President and President-Elect, Environmental Health Sciences Student Organization, Johns Hopkins Bloomberg School of Public Health

Publications

Zamoiski RD, Guallar E, Garcia-Vargas GG, Rothenberg SJ, Resnick C, Rubio-Andrade M, Steuerwald AJ, Parsons PJ, Weaver V, Navas-Acien A, Silbergeld EK. Association of arsenic and metals with 25-hydroxyvitamin D and 1,25- dihydroxyvitamin D levels among adolescents in Torreon, Mexico. Submitted.

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Payne JL, Klein SR, **Zamoiski R**, Zandi PP, Bienvenu OJ, Mackinnon DF, Mondimore FM, Schweizer B, Swartz KL; NIMH Genetics Initiative Bipolar Disorder Consortium, Crowe RP, Scheftner WA, Weissman MM, Levinson DF, DePaulo JR Jr, Potash JB. Premenstrual mood symptoms: study of familiarity and personality correlates in mood disorder pedigrees. Arch Womens Ment Health 2009 Feb;12(1):27-34.

Payne JL, Mackinnon DF, Mondimore FM, McInnis MG, Schweizer B, **Zamoiski R**, McMahon FJ, Nurnberger JI Jr, Rice JP, Scheftner W, Coryell W, Berrettini WH, Kelsoe JR, Byerley W, Gershon ES, Raymond Depaulo J Jr, Potash JB. Familial aggregation of postpartum mood symptoms in bipolar disorder pedigrees. Bipolar Disord 2008, 10:38-44.

Goes FS, Sadler B, Toolan J, **Zamoiski RD**, Mondimore FM, Mackinnon DF, Schweizer B; The Bipolar Disorder Phenome Group, Raymond Depaulo J Jr, Potash JB. Psychotic features in bipolar and unipolar depression. Bipolar Disord 2007, 9:901-906.

MacKinnon DF, **Zamoiski R**. Panic comorbidity with bipolar disorder: what is the manic-panic connection? Bipolar Disord 2006, 8:648-664.

Presentations

Zamoiski RD, Guallar E, Garcia-Vargas G, Rubio-Andrade M, Parsons PJ, Steuerwald AJ, Rothenberg SJ, Navas-Acien A, Weaver V, Resnick C, Silbergeld EK. Association of 25-hydroxyvitamin D and 1,25-dihydroxyvitamin D with metals in a cohort of adolescents in Torreon, Mexico. Poster presented at Society of Toxicology Annual Meeting, San Antonio, TX, March 2013.