Cadmium Burden and Blood Pressure

Megan Suter

A dissertation

submitted in partial fulfillment of the

requirements for the degree of

Doctor of Philosophy

University of Washington

2021

Reading Committee:

Daniel A. Enquobahrie, Chair

Kwun Chan

Howard Hu

Program Authorized to Offer Degree:

Department of Epidemiology

University of Washington

Abstract

Cadmium Burden and Blood Pressure

Megan Suter

Chair of the Supervisory Committee:

Daniel A. Enquobahrie

Department of Epidemiology

Background Hypertension, or high blood pressure (BP) affects 1 in 3 adults worldwide and is a leading cause of cardiovascular disease. A growing body of literature has implicated toxic heavy metals, such as cadmium (Cd), as contributors to higher BP and hypertensive disorders. Cd is extensively used in production of consumer products such as batteries, pigments, coatings, metal plating, and plastic stabilizers. Cd exposure in the general population is mainly through diet (leafy greens and root vegetables) and tobacco smoke. Importantly, the human body does not have an efficient means of eliminating Cd and thus it is accumulated in the kidneys for up to 40 years. Mechanisms of Cd's cardiovascular toxicity are not well-understood, however, the accumulation of Cd in kidney tissue increases oxidative stress through depletion of glutathione, resulting in damage to the kidney tissue and blood vessels. In addition, even though the placenta acts as a partial barrier to fetal Cd exposure, maternal Cd may damage the placenta, causing

growth impairment and/or fetal programming of adverse life course cardiovascular outcomes. Existing epidemiological studies on maternal Cd burden-offspring BP as well as adult Cd burden-BP associations have been limited by a lack of longitudinal data, inadequate control of important confounders, and limited assessment of potential modifiers (e.g., genetic susceptibility or exposure to other heavy metals). More specifically, the few studies that have examined impacts of prenatal Cd burden on childhood BP in the offspring have reported inconsistent findings. No study has examined how genetic variations impact fetal susceptibility to offspring Cd-related blood pressure changes. Furthermore, studies of Cd and BP in adults are mostly cross-sectional. This dissertation aims to address these gaps in the current literature.

Objectives The three aims of the dissertation project were 1) to determine the associations between prenatal Cd burden (as measured by maternal urinary Cd (UCd)), birthweight, and blood pressure in middle childhood, 2) to examine whether associations of maternal UCd burden with offspring BP differ by offspring genotypes 3) to investigate associations between Cd (measured in toenails) and longitudinal changes in BP, as well as risk of hypertension among adults and examine for potential effect modification by zinc (Zn).

Methods This dissertation utilized data from two large cohort studies – ELEMENT and CARDIA. ELEMENT (Early Life Exposure in Mexico to ENvironmental Toxicants) is a birth cohort study based in Mexico City, Mexico, designed to assess the effects of environmental toxicants on pregnancy outcomes and child development. CARDIA (Coronary Artery Risk Development in Young Adults) recruited young adults (18-25 years) at four centers in the United States to study determinants and development of clinical and subclinical cardiovascular disease. Analyses to address aim #1 used a subset of the ELEMENT cohort (N=202) for which

information on maternal urinary Cd and childhood BP among the offspring (age 7-15) was available. To address aim #2, we identified 113 mother-child dyads in ELEMENT with available offspring genetic data in addition to maternal urinary Cd (UCd) and offspring BP measurement in childhood. To address aim #3, we used data from 3745 participants in CARDIA, free of hypertension at baseline, with available measures of Cd in toenails, and longitudinal BP measures and the development of hypertension. To estimate associations of maternal UCd and child BP in ELEMENT, we used linear and logistic models, adjusted for covariates (child sex, age, height, postnatal UCd, maternal SES). To estimate associations of toenail Cd with BP and hypertension, we used estimating equations (GEE) and Cox proportional hazards models with interaction terms, adjusted for covariates (sex, race, education, study center, BMI, smoking status, alcohol use, physical activity). Effect modification was assessed with interaction terms and stratified analyses.

Results In ELEMENT, prenatal cadmium was not associated with birthweight (β=-41.47 grams, 95%CI: -128.03, 45.08), offspring SBP (β=-1.01 mmHg, 95%CI: -3.04, 1.00), DBP (β=-1.22 mmHg, 95%CI: -2.75, 0.29), or high BP (OR=0.65, 95%CI: 0.34, 1.23). In a subset of ELEMENT participants, we found evidence of maternal UCd and offspring genotype interaction on SBP and DBP for eight and two SNPs, respectively (interaction p-values<0.10). Maternal UCd was inversely associated with SBP among those with 0 copies of the *rs5909* (*HMGCR*) minor allele (β=-4.61 mmHg, 95%CI: -8.84, -0.38), at least one copy of the *rs10497900* (*PTH2R*) minor allele (β=-5.02 mmHg, 95%CI: -8.67, -1.38), and 0 copies of the *rs3771452* (*ADD2*) minor allele (β=-9.82 mmHg, 95%CI: -17.30, -2.35, p-value < 0.05) (interaction p-values 0.05, 0.05, and 0.02, respectively). There were suggestive (borderline) inverse associations of maternal UCd with lower DBP among those with at least one copy of the

rs208272 (*HMGCR*) minor allele (β=-5.57 mmHg, 95%CI: -11.17, 0.03, p-value=0.05) and 0 copies of the *rs4984* (*ADD2*) minor allele (β=-2.86 mmHg, 95%CI: -6.01, 0.29, p-value=0.07) (interaction p-values, 0.07 and 0.03, respectively). In CARDIA, toenail Cd concentration (log-μg/g) was not associated with SBP (β=0.09, 95%CI: -0.18, 0.35), DBP (β=-0.06, 95%CI: -0.26, 0.15), or risk of hypertension (HR=1.02, 95%CI: 0.98,1.07). We observed evidence of potential effect modification by sex in the association of Cd with hypertension (interaction p-value =0.05). The risk of hypertension was marginally positively associated with toenail Cd among females (HR=1.06, 95%CI: 0.99, 1.13), but not males (HR=0.98, 95%CI: 0.90, 1.05). There was no interaction of Zn and Cd in associations with hypertension, SBP, or DBP (p for interaction 0.72, 0.80, 0.53, respectively).

Conclusion We did not observe a statistically significant association between maternal UCd and childhood BP in all ELEMENT participants, however, contrary to our hypothesis, we observed an inverse association between maternal UCd and SBP and DBP in a subset of the populations defined by offspring genotypes of genes associated with oxidative stress and calcium (Ca) homeostasis. We did not find an association between toenail Cd concentration and longitudinal BP changes or risk of hypertension among adults, however, there was some indication of potential effect modification by sex. Future larger studies are needed to confirm and expand on our findings. If confirmed, our findings may lead to strategies for targeting interventions and BP screening efforts.

Dedication

In memory of my grandmother, Mary Anne Suter. She always believed in and supported my academic endeavors. I know that she would have loved to celebrate this milestone with me.

ACKNOWLEDGEMENTS

First and foremost, I wish to thank my dissertation chair, Dr. Daniel Enquobahrie, for his enduring support, kindness, and mentorship throughout the entirety of my doctoral degree process. Additionally, I would like to thank all the members of my dissertation committee for their thoughtful feedback and expert guidance: Drs. Howard Hu, Catherine Karr, Kwun (Gary) Chan, Sheela Sathyanarayana, and Joseph Flynn. I am grateful for the funding support, professional growth, and learning opportunities I received as a teaching assistant under Drs. Kristjana Asbjornsdottir, Noel Weiss, Wendy Barrington, Stephanie Farquhar, and Elizabeth Kirk. Thanks also to Dr. Lianne Sheppard for providing me with opportunities including funding support from the NIEHS BEBTEH T32 training grant, as well as the Department of Epidemiology for funding my work through a pilot grant. I also acknowledge my undergraduate professors at the University of Rochester, Drs. Edwin van Wijngaarden, Todd Jusko, Courtney Jones, and Nancy Chin, who helped foster my love of public health and epidemiology. In addition, I am grateful for the research participants of the ELEMENT and CARDIA cohorts, without whom this research would have, quite literally, been impossible.

Moving across the country to pursue my dreams at UW would not have been possible without the love and support of my parents, Monica and Frank Suter, and my brother, Patrick Suter.

Finally, I would like to thank the wonderful friends I made in Seattle during my 6 years of grad school. In particular, I would like to acknowledge Molly, Joey, and Maggie. I would not have made it here without you guys

Table of Contents

List of Tables

Chapter 1. Background and Overview

This purpose of this dissertation is to investigate associations of cadmium (Cd) burden (among mothers and young adults) with offspring and adult blood pressure (BP), respectively, and to examine modifiable (e.g., BMI, physical activity, zinc status) and non-modifiable (e.g., sex and genetic susceptibility) factors that might modify these associations.

Cardiovascular disease is the leading cause of morbidity and mortality in many parts of the world (Willey et al., 2014). Hypertension (high BP) is one of the most important risk factors for cardiovascular disease, and importantly, BP during childhood and adolescence is a significant predictor of blood pressure during adulthood and of future cardiovascular disease risk (Hao et al., 2017; Theodore et al., 2015). Despite progress made in hypertension control and treatment, roughly 1 in 3 (32%) adults and 4% of children have hypertension worldwide ((NCD-RisC), 2021; Song et al., 2019b).

Cd is a heavy metal that occurs naturally in earth's crust. It is resistant to corrosion, making it an attractive element in the production of consumer products such as batteries, pigments, coatings, metal plating, and plastic stabilizers.("Substance Priority List | ATSDR," 2017) However, it is also efficiently absorbed by plants; leafy green vegetables, potatoes, grains, soybeans, tobacco plants, and sunflower seeds accumulate particularly high levels of cadmium from the soil ("Substance Priority List | ATSDR," 2017). Thus, exposure to cadmium in the general population occurs primarily through diet or from tobacco smoke ("Substance Priority List | ATSDR," 2017). Importantly, the human body does not have an efficient means of eliminating Cd and thus it is accumulated in the kidneys for up to 40 years (Jarup, Berglund,

Elinder, Nordberg, & Vahter, 1998). Cd burden that reflects long-term exposure is characterized by accumulated Cd concentrations in tissue and urine (Prozialeck & Edwards, 2010).

A growing body of research has highlighted Cd as a contributor to increased BP and hypertensive disorders, though results have been inconsistent (Gallagher & Meliker, 2010). While not fully understood, animal and *in vitro* studies have suggested several mechanisms of Cd cardiovascular toxicity. Cd binds to albumin and is transported through the bloodstream to the liver (Genchi, Sinicropi, Lauria, Carocci, & Catalano, 2020). The liver and kidney synthesize metallothionein (MT), proteins that preferentially bind to heavy metals such as Cd, resulting in long-term accumulation of Cd in these organs (Genchi et al., 2020). Cd likely increases oxidative stress through depletion of glutathione (Almenara et al., 2013; Yiin, Chern, Sheu, Tseng, & Lin, 1999). The subsequent excessive generation of reactive oxygen species (ROS), contributes to vascular dysfunction and subsequent blood pressure changes (Rodrigo, González, & Paoletto, 2011). Animal studies suggest that Cd may inhibit nitric oxide (NO) synthase which functions to suppress acetylcholine-induced vascular relaxation, (Yoopan, Watcharasit, Wongsawatkul, Piyachaturawat, & Satayavivad, 2008).

Due to the tendency of Cd to bind to MTs in placental tissue, placental transfer of Cd is limited. However, Cd may have direct toxic effects on placental tissue, similar to those described above. Damage to vascular tissue of the placenta may result in detrimental changes in fetal growth, development, and programming (Z. Chen et al., 2014). Sub-optimal fetal growth can in turn program the fetus for adverse cardiovascular outcomes later in life, including hypertension (Crispi, Miranda, & Gratacós, 2018; Neggers et al., 1990; H. Wang et al., 2015).

Some studies have reported associations of Cd, even at low levels, with higher BP (An et al., 2017; Franceschini et al., 2017; Satarug, Nishijo, Ujjin, Vanavanitkun, & Moore, 2005;

Tellez-Plaza, Navas-Acien, Criniceanu, & Guallar, 2008), while others have found no associations (Mordukhovich et al., 2012; Schutte et al., 2008). Besides such inconsistencies, several gaps exist in previous literature. Few studies have been adequately powered to examine clinical hypertension. Existing studies have relied on cross-sectional data. Unlike other heavy metals, associations of maternal Cd with offspring BP are relatively under-investigated. The few studies that have examined prenatal Cd in relation to offspring BP have reported an absence of associations (Chatzi et al., 2019; Hawkesworth S, 2012). Importantly, these studies were limited to assessment of BP during early childhood and did not account for postnatal (concurrent) Cd exposure, a potentially important confounder. While previous research has uncovered differences in Cd accumulation and toxicity by genetic variations (Ehret et al., 2016), gene-Cd interactions in blood pressure have not been thoroughly studied. To date, no studies have examined prenatal Cd and offspring gene interactions on childhood BP of which we are aware.

This dissertation addresses the limitations of previous studies, providing an in-depth analysis of relationships between prenatal Cd and offspring BP, as well as relationships of Cd burden with longitudinal BP changes and incident hypertension among adults. We used data from two large cohort studies, ELEMENT (Early Life Exposure in Mexico to ENvironmental Toxicants) and CARDIA (Coronary Artery Risk Development in Young Adults). ELEMENT is birth cohort study based in Mexico City, Mexico, designed to assess the effects of environmental toxicants on pregnancy outcomes and child development. CARDIA recruited young adults at four centers in in the United States to study determinants and development of clinical and subclinical cardiovascular disease. The report of this project includes chapters 2-4 that describe work to address the three aims of the project and an overarching discussion (chapter 5). In aim #1 (Chapter 2), we sought to determine the association between prenatal Cd burden (as measured

by maternal urinary Cd) and blood pressure in middle childhood. This analysis used a subset of the ELEMENT cohort (N=202) for which maternal urinary Cd was available. In aim #2 (Chapter 3), we examined interactions between prenatal Cd burden and offspring genetic variations on blood pressure in middle childhood. This study used a subset of 113 mother-child dyads in ELEMENT. Finally, in aim #3 (Chapter 4), we used data from 3745 participants in CARDIA, free of hypertension at baseline, to determine associations between Cd in toenails and longitudinal changes in BP, as well as risk of hypertension. Additionally, we examined potential effect modifiers (sex, BMI, toenail Zn concentration, physical activity) of these associations. In Chapter 5, we summarized the main findings of the project, highlighted strengths and limitations of the project, and described potential areas of future research.

This research can help to enhance our understanding of mechanisms of Cd toxicity at different points in the life course and its effects on blood pressure. Findings can also be used to identify children and young adults at greatest risk of developing hypertensive disorders, as well as genetic and lifestyle factors that may impact these outcomes.

1.1 References

(NCD-RisC), N. R. F. C. (2021). Worldwide trends in hypertension prevalence and progress in treatment and control from 1990 to 2019: a pooled analysis of 1201 population-representative studies with 104 million participants. *Lancet*. doi:10.1016/S0140-6736(21)01330-1

Almenara, C. C., Broseghini-Filho, G. B., Vescovi, M. V., Angeli, J. K., Faria, T. e. O., Stefanon, I., . . . Padilha, A. S. (2013). Chronic cadmium treatment promotes oxidative stress and endothelial damage in isolated rat aorta. *PLoS One, 8*(7), e68418. doi:10.1371/journal.pone.0068418

An, H. C., Sung, J. H., Lee, J., Sim, C. S., Kim, S. H., & Kim, Y. (2017). The association between cadmium and lead exposure and blood pressure among workers of a smelting industry: a cross-sectional study. *Ann Occup Environ Med, 29*, 47. doi:10.1186/s40557-017-0202-z

Chatzi, L., Ierodiakonou, D., Margetaki, K., Vafeiadi, M., Chalkiadaki, G., Roumeliotaki, T., . . . Kippler, M. (2019). Associations of Prenatal Exposure to Cadmium With Child Growth, Obesity, and Cardiometabolic Traits. *Am J Epidemiol, 188*(1), 141-150. doi:10.1093/aje/kwy216

Chen, Z., Myers, R., Wei, T., Bind, E., Kassim, P., Wang, G., . . . Wang, X. (2014). Placental transfer and concentrations of cadmium, mercury, lead, and selenium in mothers, newborns, and young children. *J Expo Sci Environ Epidemiol, 24*(5), 537-544. doi:10.1038/jes.2014.26

Crispi, F., Miranda, J., & Gratacós, E. (2018). Long-term cardiovascular consequences of fetal growth restriction: biology, clinical implications, and opportunities for prevention of adult disease. *Am J Obstet Gynecol, 218*(2S), S869-S879. doi:10.1016/j.ajog.2017.12.012

Ehret, G. B., Ferreira, T., Chasman, D. I., Jackson, A. U., Schmidt, E. M., Johnson, T., . . . Consortium, W. T. C. C. (2016). The genetics of blood pressure regulation and its target organs from association studies in 342,415 individuals. *Nat Genet, 48*(10), 1171-1184. doi:10.1038/ng.3667

Franceschini, N., Fry, R. C., Balakrishnan, P., Navas-Acien, A., Oliver-Williams, C., Howard, A. G., . . . Tellez-Plaza, M. (2017). Cadmium body burden and increased blood pressure in middle-aged American Indians: the Strong Heart Study. *J Hum Hypertens, 31*(3), 225-230. doi:10.1038/jhh.2016.67

Gallagher, C. M., & Meliker, J. R. (2010). Blood and urine cadmium, blood pressure, and hypertension: a systematic review and meta-analysis. *Environ Health Perspect, 118*(12), 1676-1684. doi:10.1289/ehp.1002077

Genchi, G., Sinicropi, M. S., Lauria, G., Carocci, A., & Catalano, A. (2020). The Effects of Cadmium Toxicity *Int J Environ Res Public Health, 17*(11), 3782. doi:10.3390/ijerph17113782

Hao, G., Wang, X., Treiber, F. A., Harshfield, G., Kapuku, G., & Su, S. (2017). Blood Pressure Trajectories From Childhood to Young Adulthood Associated With Cardiovascular Risk: Results From the 23-Year Longitudinal Georgia Stress and Heart Study. *Hypertension, 69*(3), 435-442. doi:10.1161/HYPERTENSIONAHA.116.08312

Hawkesworth S, W. Y., Kippler M, Fulford AJC, Arifeen SE, Persson L, Moore SE, Vahter M. (2012). Early exposure to toxic metals has a limited effect on blood pressure or kidney function in later childhood, rural Bangladesh. *International Journal of Epidemiology*(42), 176-185.

Jarup, L., Berglund, M., Elinder, C. G., Nordberg, G., & Vahter, M. (1998). Health effects of cadmium exposure--a review of the literature and a risk estimate. *Scand J Work Environ Health, 24 Suppl 1*, 1-51.

Mordukhovich, I., Wright, R. O., Hu, H., Amarasiriwardena, C., Baccarelli, A., Litonjua, A., . . . Schwartz, J. (2012). Associations of toenail arsenic, cadmium, mercury, manganese, and lead with blood pressure in the normative aging study. *Environ Health Perspect, 120*(1), 98-104. doi:10.1289/ehp.1002805

Neggers, Y. H., Cutter, G. R., Acton, R. T., Alvarez, J. O., Bonner, J. L., Goldenberg, R. L., . . . Roseman, J. M. (1990). A Positive Association between Maternal Serum Zinc Concentration and Birth-Weight. *American Journal of Clinical Nutrition, 51*(4), 678-684.

Prozialeck, W. C., & Edwards, J. R. (2010). Early biomarkers of cadmium exposure and nephrotoxicity. *Biometals, 23*(5), 793-809. doi:10.1007/s10534-010-9288-2

Rodrigo, R., González, J., & Paoletto, F. (2011). The role of oxidative stress in the pathophysiology of hypertension. *Hypertens Res, 34*(4), 431-440. doi:10.1038/hr.2010.264

Satarug, S., Nishijo, M., Ujjin, P., Vanavanitkun, Y., & Moore, M. R. (2005). Cadmium-induced nephropathy in the development of high blood pressure. *Toxicol Lett, 157*(1), 57-68. doi:10.1016/j.toxlet.2005.01.004

Schutte, R., Nawrot, T., Richart, T., Thijs, L., Roels, H. A., Van Bortel, L. M., . . . Staessen, J. A. (2008). Arterial structure and function and environmental exposure to cadmium. *Occup Environ Med, 65*(6), 412-419. doi:10.1136/oem.2007.035576

Song, P., Zhang, Y., Yu, J., Zha, M., Zhu, Y., Rahimi, K., & Rudan, I. (2019). Global Prevalence of Hypertension in Children: A Systematic Review and Meta-analysis. *JAMA Pediatr, 173*(12), 1154-1163. doi:10.1001/jamapediatrics.2019.3310

Substance Priority List | ATSDR. (2017, 2017-10-03T03:11:38Z). Retrieved from https://www.atsdr.cdc.gov/spl/index.html

Tellez-Plaza, M., Navas-Acien, A., Criniceanu, C., & Guallar, E. (2008). Cadmium Exposure and Hypertension in the 1999–2004 National Health and Nutrition Examination Survey (NHANES). *Environmental Health Perspectives, 116*(1).

Theodore, R. F., Broadbent, J., Nagin, D., Ambler, A., Hogan, S., Ramrakha, S., . . . Poulton, R. (2015). Childhood to Early-Midlife Systolic Blood Pressure Trajectories: Early-Life Predictors, Effect Modifiers, and Adult Cardiovascular Outcomes. *Hypertension, 66*(6), 1108-1115. doi:10.1161/HYPERTENSIONAHA.115.05831

Wang, H., Hu, Y. F., Hao, J. H., Chen, Y. H., Su, P. Y., Wang, Y., . . . Xu, D. X. (2015). Maternal zinc deficiency during pregnancy elevates the risks of fetal growth restriction: a population-based birth cohort study. *Sci Rep, 5*, 11262. doi:10.1038/srep11262

Willey, J. Z., Moon, Y. P., Kahn, E., Rodriguez, C. J., Rundek, T., Cheung, K., . . . Elkind, M. S. (2014). Population attributable risks of hypertension and diabetes for cardiovascular disease and stroke in the northern Manhattan study. *J Am Heart Assoc, 3*(5), e001106. doi:10.1161/JAHA.114.001106

Yiin, S. J., Chern, C. L., Sheu, J. Y., Tseng, W. C., & Lin, T. H. (1999). Cadmium-induced renal lipid peroxidation in rats and protection by selenium. *J Toxicol Environ Health A, 57*(6), 403-413.

Yoopan, N., Watcharasit, P., Wongsawatkul, O., Piyachaturawat, P., & Satayavivad, J. (2008). Attenuation of eNOS expression in cadmium-induced hypertensive rats. *Toxicol Lett, 176*(2), 157-161. doi:10.1016/j.toxlet.2007.11.002

Chapter 2. Prenatal Cadmium Burden, Birth Weight, and Offspring Blood Pressure: the ELEMENT Study

2.1 Abstract

Background: Cadmium is a ubiquitous, toxic heavy metal associated with adverse health outcomes, including high blood pressure (BP), in adults. The impact of maternal cadmium burden on offspring birth weight and offspring BP is less clear.

Objectives: We investigated associations of prenatal cadmium burden with birth weight and offspring BP in middle childhood.

Methods: We analyzed data from 202 mother-child pairs recruited as part of the Early Life Exposure in Mexico to Environmental Toxicants (ELEMENT) study in Mexico City (1997-2000). Prenatal cadmium burden was characterized using maternal urine collected in the third trimester of pregnancy. Outcomes were offspring birth weight and BP (measured at a single time point age 7-15 years). BP status was defined based on the 2017 American Academy of Pediatrics Guidelines. Those with elevated or stage 1 hypertension were categorized as having high BP. Linear and logistic regression models were used to examine associations of specific-gravity adjusted cadmium (log-UCd, μg/L) with birth weight, BP, and hypertension, adjusted for potential confounders (sex, child age child height, offspring UCd, maternal ever-smoking, maternal SES). Potential effect modification by offspring sex was assessed using interaction terms and sex-stratified models.

Results: Study participants included 93 males and 109 females, with a mean age of 10.0 (SD=1.5) years. Median third trimester urinary cadmium concentration was 0.17 μg/L (IQR=0.12, 0.26). The prevalence of high BP was 19.3% (39/202). Prenatal cadmium was not associated with birthweight (β=-41.47 grams, 95%CI: -128.03, 45.08), offspring systolic BP (β=-

1.01 mmHg, 95%CI: -3.04, 1.00), diastolic BP (β=-1.22 mmHg, 95%CI: -2.75, 0.29), or high BP (OR=0.65, 95%CI: 0.34, 1.23). There was no evidence of effect modification by sex; associations were null among male and female children.

Conclusion: Cadmium burden in our study population was slightly lower than in other populations. Maternal third trimester cadmium was not associated with offspring birth weight or BP. Future larger studies conducted in diverse populations are needed to confirm these findings.

2.2 Introduction

Cadmium ranks 7th on the U.S. Agency for Toxic Substances and Disease Registry (ATSDR) substance priority list, due to its toxicity and potential for human exposure ("Substance Priority List | ATSDR," 2017). In the general population, exposure to cadmium is primarily from diet (Olsson et al., 2002) or tobacco smoke ("Substance Priority List | ATSDR," 2017). Research implicates cadmium burden as harmful to multiple organs and systems; it has been linked with numerous health outcomes in adults, including hypertension (Gallagher & Meliker, 2010; Tellez-Plaza et al., 2008). Cardiovascular diseases are leading causes of morbidity and mortality in many parts of the world, and hypertension is one of the most important risk factors for cardiovascular diseases (Willey et al., 2014). Importantly, blood pressure (BP) during childhood and adolescence is a significant predictor of BP during adulthood, hypertension, and of future cardiovascular disease risk (Hao et al., 2017; Theodore et al., 2015). Prevalence of hypertension among adolescents in Mexico City is estimated to be 10.6%, with an additional 10% of adolescents classified as pre-hypertensive (Juarez-Rojas et al., 2008). This is higher than the worldwide burden of hypertension in children and adolescents <19 years old, which is approximately 4.56%.(Song et al., 2019a) Thus, determining modifiable risk

factors for hypertension could have a substantial impact on health throughout the life course. Among modifiable risk factors are early life factors such as birth weight. Multiple studies have linked low birthweight with higher BP and risk of hypertension throughout the life course (Barker, Osmond, Golding, Kuh, & Wadsworth, 1989; Falkner, 2002).

Experimental studies suggest that placental cadmium accumulation, subsequent to increased maternal cadmium burden, may impair utero-placental blood flow and maternal-fetal nutrient transport, resulting in sub-optimal fetal growth which in turn may program the fetus for adverse cardiovascular outcomes later in life (Jones & Parer, 1983; Kippler, Hoque, Raqib, Ohrvik, Ekstrom, et al., 2010; Neggers et al., 1990; H. Wang et al., 2015) Mounting evidence supports associations between maternal cadmium burden and birth outcomes such as birth weight and infant growth (Johnston, Valentiner, Maxson, Miranda, & Fry, 2014; Lin, Doyle, Wang, Hwang, & Chen, 2011). However, inconsistencies in findings across populations persist. In addition, little is known about the impacts of prenatal cadmium exposure on offspring BP. To date, only two studies have examined associations between prenatal cadmium burden and BP in pediatric populations, and both have found no significant association (Chatzi et al., 2019; Hawkesworth S, 2012). However, evaluation of BP was limited to early childhood (age 4) and neither of these studies adjusted for postnatal cadmium exposure, a potentially important confounder in this association. Finally, several studies have shown infant-sex specific associations between maternal cadmium and birth size measures (Kippler et al., 2012; Romano, Enquobahrie, Simpson, Checkoway, & Williams, 2016) and sex-specific differences in associations between birthweight and BP (O'Sullivan, Wright, Pearce, & Parker, 2002). Additionally, our group found a significant association between maternal lead and offspring blood pressure in girls but not boys, suggesting sex-specific impacts of heavy metals on the

development of the cardiovascular system (A. Zhang et al., 2012). Thus, infant sex should be examined as a potential effect modifier for the association of prenatal Cd burden with offspring childhood blood pressure.

In the current study, we leveraged an existing birth cohort study in Mexico to investigate associations of maternal cadmium burden with offspring birth weight and middle childhood BP. We also examined whether potential associations are modified by offspring sex.

2.3 Materials and Methods

Study Setting and Study Population

The study setting was the three sequentially-recruited cohorts of the Early Life Exposure in Mexico to Environmental Toxicants (ELEMENT) study. ELEMENT was originally initiated to examine the impacts of prenatal and childhood lead exposure on neurodevelopment in Mexico City, but has expanded to broadly measure the impacts of environmental exposures on maternal and child health (Perng et al., 2019). A detailed description of recruitment and data collection methods have been published elsewhere (Perng et al., 2019). Briefly, from 1994-2003, healthy pregnant women were recruited from maternity hospitals serving low- to moderate-income population. At delivery, anthropometric data for mothers and children were collected, as well as information on baseline demographic and health status.

When children were 8-15 years of age (2008-2011), mother-child dyads were contacted and recalled for a follow-up assessment in middle childhood. Of the 1,272 mother-child dyads eligible for follow up, 49.6% completed the examination. The current study included a subset of mother-child dyads who were recruited from 1997-2003 and who participated in the follow-up. We included mother-child dyads for whom a maternal urine sample was provided in the third

trimester of pregnancy and data on maternal urinary cadmium and specific-gravity were non-missing. After these exclusions, the total analytic population is comprised of 202 mother-offspring dyads. The ethics and research committees of the Mexico National Institute of Public Health, the University of Michigan, and the University of Washington approved this research and all participants provided consent prior to enrollment.

Data Collection

Subjects were recruited at a regularly scheduled prenatal visit during their first trimester of pregnancy and followed up at each trimester of pregnancy. Baseline information on health status, and demographics was collected from all participants at delivery by a trained interviewer. Within twelve hours of delivery, anthropometric data from mother and newborn, venous, and cord blood samples were collected. Gestational age, based on date of last menstrual period, was abstracted from medical records. During the follow-up visit when children were 8-15 years old, a physical exam, BP measurements, urine collection, and venous blood sampling were performed on children.

Exposure

Urinary cadmium (UCd) was measured in 202 mothers in the third trimester of pregnancy (fasting spot) and 241 offspring (arbitrary spot) at the follow-up visit.(Chatzi et al., 2019) UCd is a commonly used biomarker for cadmium burden.(Caterina Vacchi-Suzzi & Meliker, 2016; Suwazono, Akesson, Alfven, Jarup, & Vahter, 2005) Due to cadmium's half-life of up to 38 years, it is felt to reflect long-term cadmium exposure from dietary and environmental sources.(Caterina Vacchi-Suzzi & Meliker, 2016) Urine was collected in sterile cups, transferred

to smaller containers for analysis within an hour, and then stored at -70 degrees Fahrenheit. Samples (0.5mL) were digested with equal volumes of nitric acid and left under a fume hood overnight. The digest was brought to 2% acid content with the addition of Milli-Q water. Urine cadmium concentration was analyzed using an Inductively Coupled Plasma Mass Spectrometer. Accuracy was determined using urinary reference material from Institut National de Santé Publique du Québec with a known concentration of cadmium (2.1 ug/L). Samples were run in 25 separate batches of 20 and each included a reference material. The measured mean (SD) of cadmium in the reference material was 1.7 (0.1) ug/L, which indicates an accuracy of 82%. UCd was specific gravity-adjusted for dilution, using the following formula: Urinary cadmium measurement for participant$_i$*(median specific gravity for entire cohort-1)/(specific gravity measurement for participant$_i$ -1) (Suwazono et al., 2005).

Outcomes

Birthweight (in grams) was measured at delivery. In analyses with birthweight as the outcome, we excluded infants born low birthweight (<2500g)(Organization, 2004). This decision was made to increase the sensitivity of our analysis, as low birthweight infants may have risk factors besides Cd exposure (e.g., genetic, maternal malnutrition).

A single BP reading was taken in the morning after subjects had rested quietly for 5 minutes and subjects were asked to abstain from caffeinated beverages for at least 12 hours before the follow-up visit. Systolic BP (SBP) and diastolic BP (DBP) were measured on participants' left arm using a standard mercury column sphygmomanometer and an appropriately sized cuff. Hypertension and elevated BP were defined by 2017 guidelines set by the American Academy of Pediatrics for children and adolescents (Flynn et al., 2017). In children 1-<13 years old, normal BP was defined as a SBP and/or DBP <90th percentile for age, sex, and height;

elevated BP was defined as either 1)SBP and/or DBP ≥90th-<95th percentile for age, sex, and height, or 2) 120 mmHg SBP and/or 80 mmHg DBP to <95th percentile (whichever is lower); hypertension was defined as either: 1) a SBP and/or DBP ≥95th percentile for age, sex, and height, or 2) ≥130mmHg SBP and/or ≥ 80 mmHg SBP (whichever is lower). In children and adolescents ≥13 years old, normal blood pressure was defined as <120 mmHg SBP and/or <80 mmHg DBP; elevated BP was defined as 120-129 mmHg SBP and DBP <80 mmHg; hypertension was defined as ≥130 mmHg and/or SBP ≥80 mmHg. In our analyses, we combined children with elevated BP and hypertension and considered them to have "high BP."

Covariates

Covariates were selected *a priori* based on previous literature and three sequential models were fitted to all models in which offspring blood pressure was the outcome: 1) A minimally adjusted model included sex, child age, and child height; 2) A partially adjusted model included the previous variables and the addition of offspring UCd; and 3) A fully adjusted model included the previous variables plus maternal ever smoking, and maternal socioeconomic status (SES). Models with birthweight as the outcome were limited to infants with normal birthweight (>2500 and <4500 grams) and were adjusted for child sex and maternal SES at study enrollment. We made this exclusion to increase the specificity to detect our association of interest; infants born low birthweight may have other risk factors for hypertension, independent of cadmium exposure. Maternal SES at study enrollment categorized into 7 levels, based on a standardized index from the Mexican Association of Marketing Research and Public Opinion Agencies (AMAI) that accounts for housing quality and household assets. ("Asociación Mexicana de Agencias de Inteligencia de Mercado y Opinión (AMAI) ")

Statistical analysis

We calculated univariate and bivariate summary statistics for maternal and child demographic and anthropometric characteristics. Statistically significant differences by quartile of maternal UCd were examined with one-way ANOVA tests for continuous variables and Pearson's Chi-square tests for categorical variables. Median and interquartile range (IQR) were calculated for maternal and offspring specific gravity adjusted UCd, stratified by maternal smoking history. A Wilcoxon Rank-sum test was used to compare distributions of maternal and postnatal UCd by maternal history of smoking. The distribution of UCd was examined graphically to visualize distribution and identify outliers. We retained 2 UCd values below the LOD (0.04 ug/L), however, a single value of maternal UCd (7.87ug/L) was deemed implausible and was removed from the analytic dataset.

Multiple linear regression models were used to quantify relationships between maternal UCd and continuous birth weight and offspring BP (systolic or diastolic). Maternal UCd was modeled as continuous and quartiles. In continuous analyses, UCd was $\log_{10}$ transformed to normalize the right skewed distribution. Logistic regression was used to determine associations of maternal UCd and risk of childhood high BP. To examine effect modification by child sex, we generated sex-stratified models. Additionally, all models were rerun with a child sex*exposure interaction term used to assess statistical significance of multiplicative interactions.

We conducted several sensitivity analyses to test associations under different scenarios. Analyses were repeated including the previously excluded upper outlier value to assess for meaningful differences in results. Since smoking history is associated with cadmium body burden (S. Adams & P. Newcomb, 2014; Olsson et al., 2002) and birthweight (Berntein et al., 2005), we conducted sensitivity analyses limited to never-smoking mothers. Additionally, we repeated analyses with unadjusted maternal UCd, and including specific-gravity as a covariate since specific gravity

could be independently related to other factors (e.g. maternal renal and cardiovascular system abnormalities) that are potentially related to offspring middle-childhood BP (Bulka, Mabila, Lash, Turyk, & Argos, 2017). We repeated analyses with birthweight as the outcome, including infants with birthweight <2500g. Finally, we added parity as a covariate in all models with birthweight as an outcome, since increasing parity is typically associated with higher birthweight (Shah & births, 2010). Statistical significance was determined using the p-value < 0.05 cutoff. All analyses were carried out using Stata 14.0 (Stata, 2015).

2.4 Results

Demographic and anthropometric characteristics of mother-child dyads are shown in **Table 2.1**. Our sample included 93 males and 109 females, with a mean age of 10.0 (SD=1.5) years at their follow-up visit. The prevalence of high BP was 19.3% (39/202) comprised of 7.4% with elevated BP and 11.9% with stage 1 hypertension. Maternal age (p=0.01) and child age (p<0.01) were significantly different across quartiles of maternal UCd (**Table 2.1)**. Mean birthweight in our sample was 3145.5 grams. While the difference was not statistically significant (p=0.16), mean birthweight tended to decrease in sequential quartiles of maternal UCd (**Table 2.1**). Mothers in the fourth quartile of UCd tended to be older compared to those in the other quartiles, as did their children at the time of the follow-up visit. Median third trimester maternal UCd concentration was 0.17 µg/L (IQR=0.12, 0.26) and 0.14 µg/L (IQR=0.11, 0.18) in offspring (**Table 2.2**). Maternal UCd was slightly higher in mothers with no history of smoking (N=99, Median=0.19, IQR=0.12, 0.27) compared to mothers who reported a history of smoking (N=102, Median=0.16, IQR=0.11, 0.24), although this difference was not statistically significant (p=0.13).

Results of models testing associations between maternal UCd, birthweight, and continuous BP are shown in (**Table 2.3)**. After adjustment for sex and maternal SES, maternal UCd was not significantly associated with birthweight (β=-41.47 grams, 95%CI: -128.03, 45.09) although the estimates indicated lower birth weight for offspring in the higher maternal UCd quartiles compared with offspring in the lowest maternal UCd quartile (**Table 2.3**). In fully adjusted models, a $\log_{10}$ µg/L increase in prenatal cadmium was not associated with child systolic BP (β=-1.01 mmHg, 95%CI: -3.04, 1.00), diastolic BP (β=-1.22 mmHg, 95%CI: -2.75, 0.29). Relative to those in the lowest quartile of maternal UCd concentration, those in the second quartile of maternal UCd concentration had significantly lower systolic BP (β=-5.20 mmHg, 95%CI=-9.03, -1.36). Additionally, a $\log_{10}$ µg/L increase in cadmium was not associated with odds of high BP (OR=0.65, 95%CI: 0.34, 1.23) (**Table 2.4**). There was no evidence of effect modification by sex for any of the outcomes and stratified models showed similar results for boys and girls.

When restricting the analysis to women with no history of smoking (N=99), we saw a slight increase in the magnitude of the association between third trimester UCd and odds of high BP (adjusted OR=1.22, 95%CI: 0.40, 3.72), as well as some suggestion of a dose response relationship with increasing UCd quartile and odds of high BP. (**Table 2.1-2.2**). Our results were similar when we repeated analyses with unadjusted maternal UCd and inclusion of specific gravity as a covariate (**Tables 2.3-2.4**). No meaningful differences were found when birthweight analyses included children whose birthweight was less than 2500 (n=9), although point estimates were slightly greater in magnitude (**Table 2.5**). In birthweight models, adjustment for parity resulted in a slight increase in point estimates, however, it should be noted that parity values

were missing for 52 individuals (**Table 2.6**). Finally, results did not change after inclusion of the participant with outlier UCd value (data not shown).

2.5 Discussion

We used data from a birth cohort study in Mexico City to examine associations between prenatal cadmium burden, measured by maternal UCd, birthweight, and middle-childhood BP. We found that prenatal cadmium exposure was not associated significantly with birthweight or middle-childhood BP. Further, we did not find evidence of sex-specific differences in associations.

Compared to other populations, UCd concentrations were lower among mothers and children in our cohort. Maternal UCd concentrations observed in our study (median=0.17 μg/L) was slightly lower than that observed among adults in the USA (0.25 μg/L) (S. V. Adams & P. A. Newcomb, 2014)and much lower than that in Bangladeshi women (0.81 μg/L) (Kippler et al., 2012) . The specific gravity-adjusted median UCd among children in our sample was 0.14 μg/L, which was similar to that in children aged 6-15 in the United States (0.11 μg/L) (Ciesielski et al., 2012) and slightly higher than among children in Uruguay (0.06 μg/L) (Burganowski et al., 2019) and Denmark (0.021 μg/L). However, the concentration was lower than among children in Bangladesh (0.30 μg/L) (Kippler, Nermell, et al., 2010), and Italy (0.38 μg/L) (Protano, Astolfi, Canepari, & Vitali, 2016).

This study contributes to the literature on investigations of maternal cadmium burden and offspring health. We did not find a significant association between higher maternal UCd and lower birthweight, which is inconsistent with some, but not all, previous studies. However, our point estimates suggested a trend of lower birthweight with increasing maternal (β=-41.47

grams, 95%CI: -128.03, 45.09). This is consistent with the Omega study (N=396), a prospective birth cohort study in Seattle, WA, which also reported a statistically insignificant lower birthweight associated with increasing maternal UCd (β=-29 grams, 95% CI:-70, 12) (Kippler et al., 2012; Romano et al., 2016). A larger study of 1616 infants in Bangladesh reported a statistically significant –31.0 gram (95%CI: –59, –2.8) decrease in birthweight associated with a 1-µg/L increase in maternal UCd (Kippler et al., 2012). However, the median maternal UCd in our study was much lower (0.17 µg/L) in our study, compared to Bangladeshi mothers (0.63 µg/L), which could, in part, account for the lack of significant findings in our study. Other studies found significant associations between prenatal cadmium exposure and standardized measures of birthweight (Johnston et al., 2014; Wai, Mar, Kosaka, Umemura, & Watanabe, 2017). It is possible that our study is underpowered to detect an association.

Our BP findings are consistent with the two previously published studies of prenatal cadmium exposure and offspring BP, although these studies were conducted in younger children. A study of Greek 4 year-olds reported that, relative to those in the first and second tertile of maternal UCd, those in the third tertile had no difference in their SBP (0.01 mmHg, 95%CI= -0.14, 0.16) or DBP (0.04, 95% CI=0.06, 0.13) (Chatzi et al., 2019). Similarly, in a study of1291 Bangladeshi 4.5-year-olds, log-UCd was not associated with SBP(-0.49 mmHg, 95%CI=-1.44, 0.45) or DBP(-0.21 mmHg, 95%CI=-1.02, 0.59).

In our study, after adjustment for confounders, compared to children of mothers in the lowest quartile of UCd, children of mothers in the second quartile of UCd had a significantly lower SBP (-5.33 mmHg, 95%CI=-9.12, -1.55) and DBP (-2.97 mmHg, 95%CI=-5.84, -0.10). This observation could be due to chance or uncontrolled (e.g., diet) or residual (e.g., socioeconomic status) confounding. Diet is a particularly important confounder since a main

source of Cd in this population is likely dietary (Moynihan et al., 2017). In a previous analysis of this cohort, maternal UCd was positively associated with intake of fruit and vegetables and offspring UCd was positively associated with intake of potatoes (Moynihan et al., 2017). This suggests that mothers and children with higher UCd likely have a different dietary profile than those with lower UCd levels, particularly one associated with lower blood pressure (Borgi et al., 2016). Some evidence suggests diets rich in fruit and vegetables may be associated with higher birthweight and lower risk of small for gestational age (SGA).(Murphy, Stettler, Smith, & Reiss, 2014). Thus, methods to adjust for dietary patterns—such as frequency of fruit and vegetable consumption—would improve this analysis. Another explanation for this findings is the complex relationships between different maternal UCd levels and offspring middle-childhood BP where lower level increases lead to potential regulatory feedback mechanisms (such as those that involve increased production of enzymes that counteract reactive oxygen species and reduce oxidative stress) that are protective from long term risk (Jan et al., 2015).

Interestingly, women who reported a history of smoking (50.8%) tended to have slightly lower urinary Cd concentrations (0.16 ug/L, IQR=0.11,0.24), compared to women who never smoked (0.19 ug/L, IQR=0.12, 0.27). The fact that tobacco is a major source of Cd, coupled with the long half-life of Cd would lead one to expect higher body burden among former smokers. In an analysis of NHANES participants from 1999-2010, smokers had a higher UCd concentration, even several years after smoking cessation.(S. Adams & P. Newcomb, 2014) Reasons for our observation may include the following: For most women, we did not have information about time since smoking cession or duration of smoking, so it is possible that smoking duration was brief and ceased several years before the pregnancy. Additionally, only 3 mothers reported smoking during pregnancy. Due to social desirability bias, particularly around smoking and

pregnancy, mothers could have under reported their smoking history minimizing the differences leading to a chance observation of lower cadmium among former smokers. Additionally, based on the high prevalence of ever-smoking among women, it is reasonable to believe that a large proportion of mothers and children were exposed to second-hand smoke. In sensitivity analyses limited to never-smoking mothers, (n=99), after adjustment for confounders, the association changed direction, but did not reach statistical significance. We also saw some indication of a dose response relationship with increasing quartile of UCd and odds of high blood pressure. While this finding needs to be confirmed, some studies have found stronger associations of Cd and BP among never smokers compared to ever smokers (Franceschini et al., 2017; Tellez-Plaza et al., 2008).

Strengths of our study include the study population that is representative of the intended source population. Our estimate of hypertension prevalence among children was 11.9%, similar to the 10.6% reported from a cross-sectional study of 1846 12–16-year-olds in Mexico City. Additionally, ELEMENT is comprised of a population that is relatively homogenous in regard to socioeconomic, and racial factors. Unlike previous studies, we provided a novel analysis of effects on BP in middle childhood and adolescence, rather than early childhood. Additionally, we were able to adjust for postnatal urinary Cd, likely an important confounder in our association of interest. Another strength is our use of maternal UCd as a biomarker, over alternatives such a cord blood Cd. Urinary cadmium is thought to be relatively temporally stable and minimally affected by short-term dietary exposures, making it an acceptable biomarker for long-term cadmium exposure.(S. Adams & P. Newcomb, 2014; Vacchi-Suzzi, Kruse, Harrington, Levin, & Meliker, 2016) Direct transfer of Cd to the fetus is limited and thus it is theorized that many of

the deleterious impacts maternal Cd on the fetus would be through its impact on the placenta (Kippler, Hoque, Raqib, Ohrvik, Ekstrom, et al., 2010).

Our study has several limitations. Importantly, our study size was small and our study may be underpowered to detect small (but potentially population level significant) associations, particularly in light of our relatively low population Cd exposure. Similarly, our study may not be powered to detect sex-specific differences in associations. Therefore, larger studies are needed. Second, despite a standardized protocol, a single measurement of BP is susceptible to measurement error. Categorization of hypertension status based on a single BP measurement could also lead to misclassification. We would expect this misclassification of the outcome to be non-differential with respect to UCd, and thus attenuate our estimates. This could potentially contribute to the null findings we observed in the current study. Children and adolescents may be particularly susceptible to whitecoat hypertension, the phenomenon in which BP readings increase only in an office setting. White coat hypertension prevalence is estimated at 13-52% in children and adolescents with elevated office BP (Miyashita, Flynn, & Hanevold, 2017). Therefore, studies with improved BP ascertainment through multiple measurements or ambulatory BP monitoring are needed. While we adjusted for several confounders, unmeasured and residual confounding is a potential limitation. Generalizability of our observations to non-Mexican populations may be limited. Future studies in diverse cohorts are needed.

We were unable to consider and explore critical windows of Cd exposure on fetal development or how Cd body burden changes during pregnancy. We relied on a single measurement of maternal UCd, taken in the 3rd trimester of pregnancy as a proxy for Cd body burden throughout pregnancy. Evidence suggests that blood and UCd levels increase during pregnancy, likely a result of depleted iron stores (Akesson et al., 2002). Also, some evidence

suggests that fetal exposure in early (L. Cheng et al., 2017) and middle pregnancy (H. Wang et al., 2016) may have the largest impacts on birthweight and fetal growth. Further studies should capture variations in Cd body burden throughout the entirety of pregnancy.

In conclusion, our study found no significant associations between prenatal cadmium exposure, birth weight, and middle-childhood BP similar to limited prior studies of this research on younger children. Future, larger longitudinal studies conducted among diverse well characterized populations, with more thorough BP ascertainment are needed to confirm these findings.

2.6 References

Adams, S., & Newcomb, P. (2014). Cadmium blood and urine concentrations as measures of exposure: NHANES 1999–2010. *Journal of Exposure Science and Environmental Epidemiology, 24*(2), 163-170.

Adams, S. V., & Newcomb, P. A. (2014). Cadmium blood and urine concentrations as measures of exposure: NHANES 1999-2010. *J Expo Sci Environ Epidemiol, 24*(2), 163-170. doi:10.1038/jes.2013.55

Asociación Mexicana de Agencias de Inteligencia de Mercado y Opinión (AMAI) Retrieved from https://nse.amai.org/niveles-socio-economicos-amai/

Barker, D. J., Osmond, C., Golding, J., Kuh, D., & Wadsworth, M. E. (1989). Growth in utero, blood pressure in childhood and adult life, and mortality from cardiovascular disease. *BMJ, 298*(6673), 564-567.

Berntein, I., Mongeon, J., Badger, G., Solomon, L., Heil, S., & Higgins, S. (2005). Maternal smoking and its association with birth weight. *Obstetrics and Gynecology, 106*(5), 986-991.

Bulka, C. M., Mabila, S. L., Lash, J. P., Turyk, M. E., & Argos, M. (2017). Arsenic and Obesity: A Comparison of Urine Dilution Adjustment Methods. *Environ Health Perspect, 125*(8), 087020. doi:10.1289/EHP1202

Burganowski, R., Vahter, M., Queirolo, E. I., Peregalli, F., Baccino, V., Barcia, E., . . . Kordas, K. (2019). A cross-sectional study of urinary cadmium concentrations in relation to dietary intakes in Uruguayan school children. *Sci Total Environ, 658*, 1239-1248. doi:10.1016/j.scitotenv.2018.12.220

Caterina Vacchi-Suzzi, D. K., James Harrington, Keith Levine, and Jaymie R., & Meliker. (2016). Is Urinary Cadmium a Biomarker of Long-term Exposure in Humans? A Review. *Curr Environ Health Rep, 3*(4), 450-458.

Chatzi, L., Ierodiakonou, D., Margetaki, K., Vafeiadi, M., Chalkiadaki, G., Roumeliotaki, T., . . . Kippler, M. (2019). Associations of Prenatal Exposure to Cadmium With Child Growth, Obesity, and Cardiometabolic Traits. *Am J Epidemiol, 188*(1), 141-150. doi:10.1093/aje/kwy216

Ciesielski, T., Weuve, J., Bellinger, D. C., Schwartz, J., Lanphear, B., & Wright, R. O. (2012). Cadmium exposure and neurodevelopmental outcomes in U.S. children. *Environ Health Perspect, 120*(5), 758-763. doi:10.1289/ehp.1104152

Falkner, B. (2002). Birth weight as a predictor of future hypertension. *American Journal of Hypertension, 15*(S2), 43S-45S.

Flynn, J. T., Kaelber, D. C., Baker-Smith, C. M., Blowey, D., Carroll, A. E., Daniels, S. R., . . . Management, S. S. (2017). Clinical Practice Guideline for Screening and Management of High Blood Pressure in Children and Adolescents. *Pediatrics, 140*(3). doi:ARTN e20171904

10.1542/peds.2017-1904

Gallagher, C. M., & Meliker, J. R. (2010). Blood and urine cadmium, blood pressure, and hypertension: a systematic review and meta-analysis. *Environ Health Perspect, 118*(12), 1676-1684. doi:10.1289/ehp.1002077

Hao, G., Wang, X., Treiber, F. A., Harshfield, G., Kapuku, G., & Su, S. (2017). Blood Pressure Trajectories From Childhood to Young Adulthood Associated With Cardiovascular Risk: Results From the 23-Year Longitudinal Georgia Stress and Heart Study. *Hypertension, 69*(3), 435-442. doi:10.1161/HYPERTENSIONAHA.116.08312

Hawkesworth S, W. Y., Kippler M, Fulford AJC, Arifeen SE, Persson L, Moore SE, Vahter M. (2012). Early exposure to toxic metals has a limited effect on blood pressure or kidney function in later childhood, rural Bangladesh. *International Journal of Epidemiology*(42), 176-185.

Jan, A., Azam, M., Siddiqui, K., Ali, A., Choi, I., & Haq, A. (2015). Heavy Metals and Human Health: Mechanistic Insight into Toxicity and Counter Defense System of Antioxidants. *Int J Mol Sci, 16*(12), 29592-29630.

Johnston, J. E., Valentiner, E., Maxson, P., Miranda, M. L., & Fry, R. C. (2014). Maternal cadmium levels during pregnancy associated with lower birth weight in infants in a North Carolina cohort. *PLoS One, 9*(10), e109661. doi:10.1371/journal.pone.0109661

Jones, C. T., & Parer, J. T. (1983). The effect of alterations in placental blood flow on the growth of and nutrient supply to the fetal guinea-pig. *J Physiol, 343*, 525-537.

Juarez-Rojas, J. G., Cardoso-Saldana, G. C., Posadas-Sanchez, R., Medina-Urrutia, A. X., Yamamoto-Kimura, L., & Posadas-Romero, C. (2008). Blood pressure and associated cardiovascular risk factors in adolescents of Mexico City. *Arch Cardiol Mex, 78*(4), 384-391.

Kippler, M., Hoque, A. M., Raqib, R., Ohrvik, H., Ekstrom, E. C., & Vahter, M. (2010). Accumulation of cadmium in human placenta interacts with the transport of micronutrients to the fetus. *Toxicol Lett, 192*(2), 162-168. doi:10.1016/j.toxlet.2009.10.018

Kippler, M., Nermell, B., Hamadani, J., Tofail, F., Moore, S., & Vahter, M. (2010). Burden of cadmium in early childhood: longitudinal assessment of urinary cadmium in rural Bangladesh. *Toxicol Lett, 198*(1), 20-25. doi:10.1016/j.toxlet.2010.04.029

Kippler, M., Tofail, F., Gardner, R., Rahman, A., Hamadani, J., Bottai, M., & Vahter, M. (2012). Maternal cadmium exposure during pregnancy and size at birth: a prospective cohort study. *Environ Health Perspect, 120*(2), 284-289.

Lin, C.-M., Doyle, P., Wang, D., Hwang, Y.-H., & Chen, P.-C. (2011). Does prenatal cadmium exposure affect fetal and child growth? *Occupational and Environmental Medicine, 68*, 641-646

Miyashita, Y., Flynn, J. T., & Hanevold, C. D. (2017). Diagnosis and management of white-coat hypertension in children and adolescents: A Midwest Pediatric Nephrology Consortium study. *J Clin Hypertens (Greenwich), 19*(9), 884-889. doi:10.1111/jch.13006

Neggers, Y. H., Cutter, G. R., Acton, R. T., Alvarez, J. O., Bonner, J. L., Goldenberg, R. L., . . . Roseman, J. M. (1990). A Positive Association between Maternal Serum Zinc Concentration and Birth-Weight. *American Journal of Clinical Nutrition, 51*(4), 678-684.

O'Sullivan, J., Wright, C., Pearce, M. S., & Parker, L. (2002). The influence of age and gender on the relationship between birth weight and blood pressure in childhood: a study using 24-hour and casual blood pressure. *European Journal of Pediatrics, 161*(8), 423-427. doi:10.1007/s00431-002-0985-x

Olsson, I., Bensryd, I., Lundh, T., Ottosson, H., Skerfvin, S., & Oskarsson, A. (2002). Cadmium in blood and urine--impact of sex, age, dietary intake, iron status, and former smoking--association of renal effects. *Environmental Health Perspectives, 110*, 1185-1190.

Organization, W. H. (2004). *World Health Organization, International Classification of Diseases, 10th Revision* (2 ed.). Geneva: World Health Organization.

Perng, W., Tamayo-Ortiz, M., Tang, L., Sanchez, B. N., Cantoral, A., Meeker, J. D., . . . Peterson, K. E. (2019). Early Life Exposure in Mexico to ENvironmental Toxicants (ELEMENT) Project. *BMJ Open, 9*(8), e030427. doi:10.1136/bmjopen-2019-030427

Protano, C., Astolfi, M. L., Canepari, S., & Vitali, M. (2016). Urinary levels of trace elements among primary school-aged children from Italy: The contribution of smoking habits of family members. *Sci Total Environ, 557-558*, 378-385. doi:10.1016/j.scitotenv.2016.03.073

Romano, M., Enquobahrie, D., Simpson, C., Checkoway, H., & Williams, M. (2016). Maternal body burden of cadmium and offspring size at birth. *Environ Res, 147*, 461-468.

Shah, P. S., & births, K. S. G. o. D. o. L. P. (2010). Parity and low birth weight and preterm birth: a systematic review and meta-analyses. *Acta Obstet Gynecol Scand, 89*(7), 862-875. doi:10.3109/00016349.2010.486827

Song, P., Zhang, Y., Yu, J., Zha, M., Zhu, Y., Rahimi, K., & Rudan, I. (2019). Global Prevalence of Hypertension in Children: A Systematic Review and Meta-analysis. *JAMA Pediatr*, 1-10. doi:10.1001/jamapediatrics.2019.3310

Stata, C. (2015). *Stata Statistical Software: Release 14*. College Station, TX: StataCorp LP.

Substance Priority List | ATSDR. (2017, October 3 2017). Retrieved from https://www.atsdr.cdc.gov/spl/index.html

Suwazono, Y., Akesson, A., Alfven, T., Jarup, L., & Vahter, M. (2005). Creatinine versus specific gravity-adjusted urinary cadmium concentrations. *Biomarkers, 10*(2-3), 117-126. doi:10.1080/13547500500159001

Tellez-Plaza, M., Navas-Acien, A., Criniceanu, C., & Guallar, E. (2008). Cadmium Exposure and Hypertension in the 1999–2004 National Health and Nutrition Examination Survey (NHANES). *Environmental Health Perspectives, 116*(1).

Theodore, R. F., Broadbent, J., Nagin, D., Ambler, A., Hogan, S., Ramrakha, S., . . . Poulton, R. (2015). Childhood to Early-Midlife Systolic Blood Pressure Trajectories: Early-Life Predictors, Effect Modifiers, and Adult Cardiovascular Outcomes. *Hypertension, 66*(6), 1108-1115. doi:10.1161/HYPERTENSIONAHA.115.05831

Vacchi-Suzzi, C., Kruse, D., Harrington, J., Levin, K., & Meliker, J. R. (2016). Is Urinary Cadmium a Biomarker of Long-term Exposure in Humans? A Review. *Curr Environ Health Rep, 3*(4), 450-458.

Wai, K. M., Mar, O., Kosaka, S., Umemura, M., & Watanabe, C. (2017). Prenatal Heavy Metal Exposure and Adverse Birth Outcomes in Myanmar: A Birth-Cohort Study. *Int J Environ Res Public Health, 14*(11). doi:10.3390/ijerph14111339

Wang, H., Hu, Y. F., Hao, J. H., Chen, Y. H., Su, P. Y., Wang, Y., . . . Xu, D. X. (2015). Maternal zinc deficiency during pregnancy elevates the risks of fetal growth restriction: a population-based birth cohort study. *Sci Rep, 5*, 11262. doi:10.1038/srep11262

Willey, J. Z., Moon, Y. P., Kahn, E., Rodriguez, C. J., Rundek, T., Cheung, K., . . . Elkind, M. S. (2014). Population attributable risks of hypertension and diabetes for cardiovascular disease and stroke in the northern Manhattan study. *J Am Heart Assoc, 3*(5), e001106. doi:10.1161/JAHA.114.001106

Zhang, A., Hu, H., Sánchez, B. N., Ettinger, A. S., Park, S. K., Cantonwine, D., . . . Tellez-Rojo, M. M. (2012). Association between prenatal lead exposure and blood pressure in children. *Environ Health Perspect, 120*(3), 445-450. doi:10.1289/ehp.1103736

2.7 Tables

Table 2-1. Demographic and Anthropometric Characteristics of Mother-Child Pairs in ELEMENT Cohort Study

Variable	N (N=202)	n(%) or μ(sd)	UCd Quartile 1 (0.026-0.116 μg/L)	UCd Quartile 2 (0.119-0.173 μg/L)	UCd Quartile 3 (0.177-0.260 μg/L)	UCd Quartile 4 (0.260-4.00 μg/L)	p-value*
Maternal Characteristics							
Maternal Age (years)	202	26.9(5.8)	26.2(5.7)	25.9(5.6)	26.4(5.0)	29.2(6.5)	0.01
Maternal SES[a]	190						
A/B		14(7.4%)	3(6.5%)	2(4.1%)	4(8.7%)	5(10.2%)	0.36
C		39(20.5%)	8(17.4%)	8(16.3%)	11(24.0%)	12(24.5%)	
C+		35(18.4%)	7(15.2%)	8(16.3%)	7(15.2%)	13(26.5%)	
D		11(5.8%)	2(4.4%)	2(4.1%)	2(4.4%)	5(10.2%)	
D+		69(36.3%)	17(37.0%)	24(49.0%)	18(39.1%)	10(20.4%)	
E		22(11.6%)	9(19.6%)	5(10.2%)	4(8.7%)	4(8.2%)	
Maternal history of smoking (% yes, 100+ packs in lifetime)	201	102(50.8%)	28(54.9%)	30(58.5%)	19(38.8%)	25(50.0%)	0.21
Parity	142	2.4(0.9)	2.7(0.9)	2.3(1.0)	2.3(0.9)	2.4(0.9)	0.26
Child Characteristics at Birth							
Gestational age (weeks)	199	38.9(1.5)	38.9(1.3)	38.7(1.8)	39.0(1.4)	38.9(1.6)	0.85
Birth weight (grams)	201	3145.5(426.9)	3255.6(373.2)	3145.1(450.0)	3096.6(438.5)	3081.4(433.0)	0.16
Low birthweight (%, <2500 grams)	201	9(4.5%)	1(2.0%)	1(2.0%)	2(4.1%)	5(10.0%)	0.17
Length (cm)	189	50.2(2.0)	50.5(1.9)	50.1(1.8)	50.4(2.1)	49.8(2.4)	0.39
Child Characteristics at Follow-up							
Age (years)	202	10.0(1.5)	9.5(1.0)	9.8(1.3)	10.3(1.7)	10.4(1.6)	<0.01
BMI	202	19.3(3.6)	18.7(3.2)	20.2(4.2)	19.4(3.5)	18.8(3.3)	0.13
Systolic BP[b] **(mmHg)**	202	102.3(10.2)	103.3(9.7)	100.7(11.4)	102.8(9.9)	102.5(9.9)	0.58
Diastolic BP[b]**(mmHg)**	202	65.0(7.3)	65.2(7.5)	63.8(7.5)	65.6(7.1)	65.2(7.2)	0.56
BP Status[c]							
Normal	202	163(80.7%)	38(74.5%)	43(84.3%)	41(82.0%)	41(82.0%)	0.33
Elevated		15(7.4%)	3(5.9%)	2(3.9%)	6(12.0%)	4(8.0%)	
Stage 1 hypertension		24(11.9%)	10(19.6%)	6(11.8%)	3(6.0%)	5(10.0%)	

Maternal UCd outlier has been removed (n=1), with a cut-off of unadjusted maternal UCd>7 μg/L, a value deemed implausible. Percentages are based on number of non-missing values. [a]Maternal SES is based on a standardized index from the Mexican Association of Marketing Research and Public Opinion Agencies (AMAI). [b]BP readings are the average of 3 measurements recorded at the same study visit. [c]Hypertension status was calculated using an macro package for R,(Sharma) which references the 2017 American Academy of Pediatrics (AAP) Guidelines.(Flynn et al., 2017) *Based on one-way ANOVA test for continuous variables and chi-squared test for categorical variables.

Table 2-2. Specific gravity adjusted concentration of third trimester urine cadmium (UCd, μg/L)

Population	N	Median (IQR)	Min, Max	p-value[a]
Mother (all)	202	0.17(0.12, 0.26)	0.03, 4.00	
Mother (ever-smokers)	102	0.16(0.11,0.24)	0.03, 1.69	0.13
Mother (never-smokers)	99	0.19(0.12, 0.27)	0.05, 4.00	
Offspring (all)	241	0.14(0.11,0.18)	0.01, 0.83	
Offspring (mother ever-smoker)	121	0.14(0.11, 0.17)	0.01, 0.78	0.94
Offspring (mother never-smoker)	114	0.14(0.11, 0.18)	0.02, 0.83	

[a]Based on Wilcoxon rank-sum test, comparing those with and without history of maternal smoking. Urinary cadmium concentration was adjusted for specific gravity using the following formula: Specific gravity adjusted urinary cadmium concentration=Urinary cadmium measurement for participant$_i$ *(median specific gravity for entire cohort-1)/(specific gravity measurement for participant$_i$-1) Ever and never smoking was defined as whether mother reported smoking 100 or more lifetime cigarettes at study recruitment. Maternal UCd outlier has been removed (n=1), with a cut-off of unadjusted maternal UCd>7 μg/L, a value deemed implausible.

Table 2-3. Associations of third trimester UCd with BP (mmHg) and birth weight(grams)

	Minimally Adjusted		Moderately Adjusted		Fully Adjusted	
Outcome	**N**	**β(95% CI)**	**N**	**β(95% CI)**	**N**	**β(95% CI)**
Birth weight[¶]	177	-41.47(-128.03, 45.09)[*]				
Birth weight[#]	177					
UCd Q1 (0.026-0.116 µg/L)		Ref				
UCd Q2 (0.119-0.173 µg/L)		-78.69(-243.61, 86.23)[*]				
UCd Q3 (0.177-0.260 µg/L)		-138.98(-307, 29.50)[*]				
UCd Q4 (0.260-4.00 µg/L)		-95.42(-266.04, 75.20)[*]				
Systolic BP[¶]	202	-0.80(-2.63, 1.02)[†]	194	-1.08(-2.92, 0.76)[‡]	181	-1.01(-3.04, 1.00)[§]
Systolic BP[#]	202		194		181	
UCd Q1 (0.026-0.116 µg/L)		Ref		Ref		Ref
UCd Q2 (0.119-0.173 µg/L)		**-4.12(-7.67, -0.56)**[†]		**-4.83(-8.42, -1.23)**[‡]		**-5.20(-9.03, -1.36)**[§]
UCd Q3 (0.177-0.260 µg/L)		-1.34(-4.96, 2.27)[†]		-2.04(-5.67, 1.59)[‡]		-2.23(-6.21, 1.73)[§]
UCd Q4 (0.260-4.00 µg/L)		-1.91(-5.57, 1.75)[†]		-2.48(-6.18, 1.22)[‡]		-2.31(-6.26, 1.64)[§]
Diastolic BP[¶]	202	-1.05(-2.41, 0.31)[†]	194	-1.12(-2.49, 0.26)[‡]	181	-1.22(-2.75, 0.29)[§]
Diastolic BP[#]	202		194		181	
UCd Q1 (0.026-0.116 µg/L)		Ref		Ref		Ref
UCd Q2 (0.119-0.173 µg/L)		-2.52(-5.19, 0.14)[†]		-2.67(-5.38, 0.05)[‡]		-2.82(-5.74, 0.10)[§]
UCd Q3 (0.177-0.260 µg/L)		-0.04(-2.75, 2.67)[†]		-0.18(-2.90, 2.58)[‡]		-0.33(-3.36, 2.70)[§]
UCd Q4 (0.260-4.00 µg/L)		-1.01(-3.76, 1.73)[†]		-1.18(-3.98, 1.62)[‡]		-1.07(-4.08, 1.94)[§]

[*]Adjusted for sex and maternal SES. [†]Adjusted for sex, child age, child height. [‡]Adjusted for sex, child age, child height, postnatal urinary cadmium burden. [§]Adjusted for sex, child age, child height, postnatal urinary cadmium, maternal ever-smoking, and maternal SES. [¶]Coefficients are the mean difference in BP (mmHg) or birthweight (grams) associated with a log-UCd (µg/L) increase in maternal urinary cadmium. [#]Coefficients are the mean difference in BP (mmHg) or birthweight (grams) compared to participants in the lowest quartile of maternal UCd (µg/L).

In birth weight models, only infants with normal birthweight (<2500 or >4500 grams) were included in the model. In all models, interactions for offspring sex were not significant at α=0.05.

Table 2-4. Associations of third trimester maternal urine cadmium and offspring high BP

	Minimally Adjusted*		Moderately Adjusted†		Fully Adjusted‡	
Exposure	**N**	**OR(95%CI)**	**N**	**OR(95%CI)**	**N**	**OR(95%CI)**
Log-UCd§	202	0.72(0.41, 1.27)	194	0.66(0.36, 1.22)	181	0.65(0.34, 1.23)
UCd Quartile¶	202		194		181	
Q1 (0.026-0.116 μg/L)		Ref		Ref		Ref
Q2 (0.119-0.173 μg/L)		0.45(0.16, 1.28)		0.36(.012, 1.12)		0.32(0.09,1.08)
Q3 (0.177-0.260 μg/L)		0.77(0.28, 2.12)		0.67(0.23, 1.91)		0.68(0.21, 2.16)
Q4 (0.260-4.00 μg/L)		0.74(0.27, 2.08)		0.65(0.22,1.92)		0.64(0.20, 2.00)

*Adjusted for sex, child age, child height. †Adjusted for sex, child age, child height, postnatal urinary cadmium burden. ‡Adjusted for sex, child age, child height, postnatal urinary cadmium, maternal ever-smoking, and maternal SES §Coefficients are the odds ratio for the association between BP (mmHg) and a log-UCd (μg/L) increase in maternal urinary cadmium. ¶Coefficients are the odds ratio (OR) for offspring elevated BP/hypertension, compared to participants in the lowest quartile of maternal UCd (μg/L).

Hypertension/elevated BP were defined based on the 2017 American Academy of Pediatrics guidelines for childhood hypertension.(Flynn et al., 2017) For Children aged 1–<13 years, BP categories were defined as: normal BP(<90th percentile), elevated BP (≥90th percentile to <95th percentile or 120/80 mm Hg to <95th percentile (whichever is lower)), stage 1 hypertension (≥95th percentile to <95th percentile + 12 mmHg, or 130/80 to 139/89 mm Hg (whichever is lower)), and stage 2 hypertension (Stage 2 HTN: ≥95th percentile + 12 mm Hg, or ≥140/90 mm Hg (whichever is lower)). For children ≥13 years, BP categories were defined as: normal BP (<120/<80 mmHg), elevated BP (120/<80 to 129/<80 mmHg), stage 1 hypertension (130/80 to 139/89 mmHg), and stage 2 hypertension (130/80 to 139/89 mmHg).

In all models, interactions for offspring sex were not significant at α=0.05

2.8 Supplemental Tables

Supplemental Table 2-1. Associations of third trimester urine cadmium with BP (mmHg) and birth weight (grams), limited to never-smoking mothers (n=99)

		Minimally Adjusted		Moderately Adjusted		Fully Adjusted
Outcome	**N**	**β(95% CI)**	**N**	**β(95% CI)**	**N**	**β(95% CI)**
Birth weight[¶]	89	-61.41(-189.38, 66.55)[*]				
Birth weight[#]	89					
UCd Q1 (0.026-0.116 µg/L)		Ref				
UCd Q2 (0.119-0.173 µg/L)		-58.24(-313.40, 196.91)[*]				
UCd Q3 (0.177-0.260 µg/L)		-119.49(-351.97, 113.00)[*]				
UCd Q4 (0.260-4.00 µg/L)		-197.18(-457.68, 63.32)[*]				
Systolic BP [¶]	99	-0.64(-3.33, 2.05)[†]	93	-1.15(-3.92, 1.62)[‡]	90	0.46(-2.59, 3.50)[§]
Systolic BP[#]	99		93		90	
UCd Q1 (0.026-0.116 µg/L)		Ref		Ref		Ref
UCd Q2 (0.119-0.173 µg/L)		-2.92(-8.67, 2.83)[†]		-3.45(-9.35, 2.44)[‡]		-2.17(-8.22, 3.88)[§]
UCd Q3 (0.177-0.260 µg/L)		-1.95(-7.31, 3.40)[†]		-2.94(-8.41, 2.54)[‡]		-0.40(-5.99, 5.18)[§]
UCd Q4 (0.260-4.00 µg/L)		-1.52(-7.43, 3.40)[†]		-2.70(-8.90, 3.50)[‡]		1.09(-5.44, 7.62)[§]
Diastolic BP[¶]	99	-1.30(-3.41 0.82)[†]	93	-1.46(-3.67, 0.76)[‡]	90	-1.21(-3.76, 1.03)[§]
Diastolic BP[#]	99		93		90	
UCd Q1 (0.026-0.116 µg/L)		Ref		Ref		Ref
UCd Q2 (0.119-0.173 µg/L)		-1.60(-6.16, 2.96)[†]		-1.07(-5.84, 3.70)[‡]		-0.27(-5.36, 4.82)[§]
UCd Q3 (0.177-0.260 µg/L)		-0.30(-3.95, 4.55)[†]		0.18(-4.25, 4.60)[‡]		1.59(-3.12, 6.29)[§]
UCd Q4 (0.260-4.00 µg/L)		-0.81(-5.50, 3.87)[†]		-1.10(-5.84, 3.70)[‡]		0.64(-4.86, 6.14)[§]

[*] Adjusted for sex and maternal SES (asset-based scale). [†]Adjusted for sex, child age, child height. [‡]Adjusted for sex, child age, child height, postnatal urinary cadmium burden. [§]Adjusted for sex, child age, child height, postnatal urinary cadmium, maternal ever-smoking, and maternal SES. [¶]Coefficients are the mean difference in BP (mmHg) or birthweight (grams) associated with a log-UCd (µg/L) increase in maternal urinary cadmium. [#]Coefficients are the mean difference in BP (mmHg) or birthweight (grams) compared to participants in the lowest quartile of maternal UCd (µg/L).

In birth weight models, only infants with normal birthweight (<2500 or >4500 grams) were included in the model. In all models, interactions for offspring sex were not significant at α=0.05.

Supplemental Table 2-2. Associations of third trimester maternal urine cadmium and offspring elevated BP/hypertension, limited to never-smoking mothers (n=99)

	Minimally Adjusted*		Moderately Adjusted†		Fully Adjusted‡	
Exposure	**N**	**OR(95%CI)**	**N**	**OR(95%CI)**	**N**	**OR(95%CI)**
Log-UCd§	99	0.72(0.31, 1.69)	93	0.62(0.24,1.62)	90	1.22(0.40, 3.72)
UCd Quartile¶	99		93		90	
Q1 (0.026-0.116 μg/L)		Ref		Ref		Ref
Q2 (0.119-0.173 μg/L)		0.31(0.05, 1.91)		0.35(0.06, 2.32)		0.59(0.07, 4.25)
Q3 (0.177-0.260 μg/L)		0.74(0.17, 3.13)		0.59(0.12, 2.82)		1.32(0.22, 7.88)
Q4 (0.260-4.00 μg/L)		0.85(0.17, 4.20)		0.70(0.12, 4.10)		2.07(0.25, 17.32)

*Adjusted for sex, child age, child height. †Adjusted for sex, child age, child height, postnatal urinary cadmium burden. ‡Adjusted for sex, child age, child height, postnatal urinary cadmium, and maternal SES. §Coefficients are the odds ratio for the association between BP (mmHg) and a log-UCd (μg/L) increase in maternal urinary cadmium. ¶Coefficients are the odds ratio (OR) for offspring elevated BP/hypertension, compared to participants in the lowest quartile of maternal UCd (μg/L).

Hypertension/elevated BP were defined based on the 2017 American Academy of Pediatrics guidelines for childhood hypertension.(Flynn et al., 2017) For Children aged 1–<13 years, BP categories were defined as: normal BP(<90th percentile), elevated BP (≥90th percentile to <95th percentile or 120/80 mm Hg to <95th percentile (whichever is lower)), stage 1 hypertension (≥95th percentile to <95th percentile + 12 mmHg, or 130/80 to 139/89 mm Hg (whichever is lower)), and stage 2 hypertension (Stage 2 HTN: ≥95th percentile + 12 mm Hg, or ≥140/90 mm Hg (whichever is lower)). For children ≥13 years, BP categories were defined as: normal BP (<120/<80 mmHg), elevated BP (120/<80 to 129/<80 mmHg), stage 1 hypertension (130/80 to 139/89 mmHg), and stage 2 hypertension (130/80 to 139/89 mmHg).

Supplemental Table 2-3. Associations of unadjusted third trimester urine cadmium with BP (mmHg) and birth weight (grams), controlling for specific gravity

	Minimally Adjusted		Moderately Adjusted		Fully Adjusted	
Outcome	**N**	**β(95% CI)**	**N**	**β(95% CI)**	**N**	**β(95% CI)**
Birth weight[¶]	177	-37.89(-130.54, 54.77)[*]				
Birth weight[#]						
UCd Q1 (0.026-0.116 µg/L)		Ref				
UCd Q2 (0.119-0.173 µg/L)		-19.25(-181.70, 143.21)[*]				
UCd Q3 (0.177-0.260 µg/L)		-2.21(-171.76, 167.34)[*]				
UCd Q4 (0.260-4.00 µg/L)		-68.51(-254.63, 117.62)[*]				
Systolic BP [¶]	202	-0.94(-2.91, 1.02)[†]	194	-1.53(-3.51, 0.46)[‡]	181	-1.35(-3.49, 0.80)[§]
Systolic BP[#]	202		194		181	
UCd Q1 (0.026-0.116 µg/L)		Ref		Ref		Ref
UCd Q2 (0.119-0.173 µg/L)		-2.56(-6.00,0.88)[†]		-2.48(-5.93, 0.97)[‡]		-2.83(-6.50, 0.84)[§]
UCd Q3 (0.177-0.260 µg/L)		**-4.09(-7.66, -0.52)**[†]		**-5.25(-8.92, -1.57)**[‡]		**-5.89(-9.83, -1.94))**[§]
UCd Q4 (0.260-4.00 µg/L)		-0.48(-4.45, 3.49)[†]		-1.82(-5.89, 2.24)[‡]		-1.92(-6.30, 2.47)[§]
Diastolic BP[¶]	202	-0.74(-2.21, 0.73)[†]	194	-1.00(02.50, 0.50)[‡]	181	-1.16(-2.78, 0.44)[§]
Diastolic BP[#]	202		194		181	
UCd Q1 (0.026-0.116 µg/L)		Ref		Ref		Ref
UCd Q2 (0.119-0.173 µg/L)		-1.78(-4.37, 0.81)[†]		-1.59(-4.21, 1.04)[‡]		-1.42(-4.25, 1.40)[§]
UCd Q3 (0.177-0.260 µg/L)		-2.41(-5.10, 0.28)[†]		-2.74(-5.54, 0.06)[‡]		-2.75(-5.78, 0.29)[§]
UCd Q4 (0.260-4.00 µg/L)		-0.14(-3.13, 2.84)[†]		-0.71(03.81, 2.38)[‡]		-0.92(-4.30, 2.46)[§]

[*]Adjusted for sex, maternal SES, and specific gravity. [†]Adjusted for sex, child age, child height, specific gravity [‡]Adjusted for sex, child age, child height, maternal urine specific gravity, postanal urine specific gravity, and unadjusted postnatal urinary cadmium [§]Adjusted for sex, child age, child height, postnatal urinary cadmium, maternal SES, maternal urine specific gravity, postanal urine specific gravity, and unadjusted postnatal urinary cadmium. [¶]Coefficients are the mean difference in BP (mmHg) or birthweight (grams) associated with 1 log-UCd µg/L increase in maternal urinary cadmium. [#]Coefficients are the mean difference in BP (mmHg) or birthweight (grams) compared to participants in the lowest quartile of unadjusted maternal UCd (µg/L).

In birth weight models, only infants with normal birthweight (<2500 or >4500 grams) were included in the model.

Supplemental Table 2-4. Associations of unadjusted third trimester maternal urine cadmium and offspring elevated BP/hypertension, controlling for specific gravity

Exposure	Minimally Adjusted*		Moderately Adjusted†		Fully Adjusted‡	
	N	**OR(95%CI)**	**N**	**OR(95%CI)**	**N**	**OR(95%CI)**
Log-UCd§	202	0.70(0.34, 1.27)	194	0.58(0.31, 1.12)	181	0.57(0.29, 1.13)
UCd Quintile¶	202		194		181	
Q1 (0.026-0.116 µg/L)		Ref		Ref		Ref
Q2 (0.119-0.173 µg/L)		0.59(0.23, 1.52)		0.47(0.17, 1.27)		0.47(0.16, 1.35)
Q3 (0.177-0.260 µg/L)		0.80(0.34, 1.93)		**0.22(0.06, 0.83)**		**0.21(0.05, 0.84)**
Q4 (0.260-4.00 µg/L)		1.14(0.49, 2.61)		0.87(0.31, 2.42)		0.72(0.24, 2.18)

*Adjusted for sex, child age, child height, specific gravity. †Adjusted for sex, child age, child height, postnatal urinary cadmium burden, and specific gravity. ‡Adjusted for sex, child age, child height, postnatal urinary cadmium, and maternal SES, and specific gravity. §Coefficients are the odds ratio for the association between BP (mmHg) and a 1 µg/L increase in maternal urinary cadmium. ¶Coefficients are the odds ratio (OR) for offspring elevated BP/hypertension, compared to participants in the lowest quartile of unadjusted maternal UCd (µg/L).

Hypertension/elevated BP were defined based on the 2017 American Academy of Pediatrics guidelines for childhood hypertension.(Flynn et al., 2017) For Children aged 1–<13 years, BP categories were defined as: normal BP(<90th percentile), elevated BP (≥90th percentile to <95th percentile or 120/80 mm Hg to <95th percentile (whichever is lower)), stage 1 hypertension (≥95th percentile to <95th percentile + 12 mmHg, or 130/80 to 139/89 mm Hg (whichever is lower)), and stage 2 hypertension (Stage 2 HTN: ≥95th percentile + 12 mm Hg, or ≥140/90 mm Hg (whichever is lower)). For children ≥13 years, BP categories were defined as: normal BP (<120/<80 mmHg), elevated BP (120/<80 to 129/<80 mmHg), stage 1 hypertension (130/80 to 139/89 mmHg), and stage 2 hypertension (130/80 to 139/89 mmHg).

Supplemental Table 2-5. Associations of specific-gravity adjusted third trimester urine cadmium with birthweight, including children with and without low birthweight, low birthweight N=9

Outcome	**N**	**β(95% CI)**
Birth weight¶	189	-77.60(-164.62, 9.42)†
Birth weight#		
UCd Q1 (0.026-0.116 µg/L)		Ref
UCd Q2 (0.119-0.173 µg/L)		-106.14(-281.87, 69.59)†
UCd Q3 (0.177-0.260 µg/L)		-166.98(-346.82, 12.86)†
UCd Q4 (0.260-4.00 µg/L)		-155.31(-334.93, 24.31)†

*Adjusted for sex, maternal SE, and specific gravity. ¶Coefficients are the mean difference in BP (mmHg) or birthweight (grams) associated with 1 log-UCd µg/L increase in maternal urinary cadmium. #Coefficients are the mean difference in BP (mmHg) or birthweight (grams) compared to participants in the lowest quartile of unadjusted maternal UCd (µg/L).

Supplemental Table 2-6. Associations of third trimester urine cadmium with BP (mmHg) and birth weight (grams), adjusting for parity

Outcome	N	β(95% CI)*
Birth weight[†]	125	-57.84(-162.89, 47.22)
Birth weight[§]	125	
UCd Q1 (0.026-0.116 μg/L)		Ref
UCd Q2 (0.119-0.173 μg/L)		-150.93(-356.25, 54.39)
UCd Q3 (0.177-0.260 μg/L)		**-306.08(-507.10, -105.06)**
UCd Q4 (0.260-4.00 μg/L)		142.42(-345.10, 60.25)

[*]Adjusted for sex and maternal SES, and parity. [†]Coefficient is the mean difference in birthweight (grams) associated with a log-UCd (μg/L) increase in maternal urinary cadmium. [§]Coefficients are the mean difference in BP (mmHg) or birthweight (grams) compared to participants in the lowest quartile of maternal UCd (μg/L).

Chapter 3. Is offspring genotype a modifier of the association between maternal cadmium burden and blood pressure?

3.1 Abstract

Background

Genetic variations are important in blood pressure (BP) regulation and cadmium (Cd) metabolism. Therefore, associations of maternal Cd burden with offspring BP may vary by offspring genotype. However, the role of offspring genetics in associations between prenatal Cd and offspring BP has not been investigated.

Objectives: We explored whether associations of maternal urinary cadmium (UCd) burden with offspring BP differ by offspring genotypes.

Methods: We analyzed data from 113 mother-child pairs recruited as part of the Early Life Exposure in Mexico to Environmental Toxicants (ELEMENT) study in Mexico City (1997-2000). Prenatal Cd burden was characterized using maternal urine collected in the third trimester of pregnancy. Offspring systolic blood pressure (SBP) and diastolic blood pressure (DBP) were measured at a single time point in middle childhood (age 7-15 years). Single nucleotide polymorphisms (SNPs) (N=39) characterizing variations in genes (N=15) related to cardiovascular function or heavy metal transport were genotyped in the offspring. Genotypes were coded in two ways: additive (number of minor alleles present as 0,1, or 2) and categorical (based on the presence or absence of a minor allele as 1 or 0, respectively). Specific gravity-adjusted maternal urinary Cd (UCd, μg/L) was log transformed and SBP and DBP were modeled as continuous (mmHg) variables. Effect modification by offspring genotype was assessed using interaction terms and stratified adjusted models. First, we fit multivariable linear regression models with specific-gravity adjusted Cd (log-UCd, μg/L) (exposure, continuous), genotypes

(modifier, coded either as additive or categorical), offspring BP (outcome, continuous), adjustment variables (sex, child age, child height, postnatal UCd, maternal ever-smoking, and maternal SES), and interaction terms (between maternal Cd and genotypes). For genotypes with interaction p-values that were < 0.10, we fit models (that include exposure, outcomes, and adjustment variables) stratified by genotype.

Results: Study participants included 55 males and 58 females, with a mean age of 10.4 (SD=1.5) years at blood pressure assessment. Median third trimester UCd concentration was 0.17 µg/L (IQR=0.13, 0.27). Overall, we did not observe a significant association between maternal UCd and offspring SBP (β=-1.71, 95% CI: -4.62, 1.19), however, there was a significant association between increasing maternal UCd and lower DBP (β=-2.48, 95% CI: -4.62, -0.35). Potential maternal UCd and offspring genotype interaction on SBP and DBP was observed for eight and two SNPs, respectively (interaction p<0.10). Maternal UCd was inversely associated with SBP among those with 0 copies of the *rs5909* (*HMGCR*) minor allele (β=-4.61 mmHg, 95%CI: -8.84, -0.38), at least one copy of the *rs10497900* (*PTH2R*) minor allele (β=-5.02 mmHg, 95%CI: -8.67, -1.38), and 0 copies of the *rs3771452* (*ADD2*) minor allele (β=-9.82 mmHg, 95%CI: -17.30, -2.35, p-value < 0.05) (interaction p-values 0.05, 0.05, and 0.02, respectively). There were suggestive (borderline) inverse associations of maternal UCd with lower DBP among those with 1 or 2 copies of the *rs208272* (*HMGCR*) minor allele (β=-5.57 mmHg, 95%CI: -11.17, 0.03, p-value=0.05) and 0 copies of the *rs4984* (*ADD2*) minor allele (β=-2.86 mmHg, 95%CI: -6.01, 0.29, p-value=0.07) (interaction p-values, 0.07 and 0.03, respectively). Similar associations were not observed for the other genotype groups.

Conclusion: Contrary to our hypothesis, we observed an inverse association between maternal UCd and SBP and DBP genotypes in some genotypes groups. Associations between maternal

UCd and offspring SBP and DBP may differ by variations in genes associated with oxidative stress and assembly of the spectrin-actin network in endothelial tissues. Associations between maternal UCd and offspring SBP may differ by variations in genes associated with calcium (Ca) homeostasis.

3.2 Introduction

Cadmium (Cd) is a ubiquitous, toxic heavy metal. Exposure to Cd is primarily from diet (Olsson et al., 2002) or tobacco smoke ("Substance Priority List | ATSDR," 2017). Cd is harmful to multiple organs and systems; it has been linked with numerous health outcomes in adults, including hypertension (Franceschini et al., 2017; Gallagher & Meliker, 2010; Garner & Levallois, 2017). Mounting evidence supports associations between maternal Cd burden and birth outcomes such as birth weight and infant growth (Johnston et al., 2014; Lin et al., 2011). Our previous analysis in a larger subset of the ELEMENT cohort, as well as several other studies, found no statistically significant relation between prenatal Cd burden and offspring blood pressure (BP) in childhood (Chatzi et al., 2019; Hawkesworth S, 2012; Howe et al., 2021; M. Zhang et al., 2021). Studies of prenatal Cd exposure have not considered potential effect modifications by genetic variations. However, gene-heavy metal interactions on cardiovascular outcomes, including BP, have been well-documented(Jhun et al., 2015; Wu et al., 2019; A. Zhang et al., 2010). Additionally, genetic variations are important in BP regulation and Cd metabolism (Joneidi et al., 2019; Tanira & Al Balushi, 2005). Therefore, associations of maternal Cd burden with offspring blood pressure (BP) may vary by offspring genotype related to BP regulation or Cd metabolism.

Cd metabolism involves several processes. Once ingested or inhaled, Cd binds to albumin and is transported through the bloodstream to the liver (Genchi et al., 2020). The liver and kidney synthesize metallothionein (MT), proteins that preferentially bind to heavy metals such as Cd, resulting in long-term accumulation of Cd in these organs (Genchi et al., 2020). While placental transfer of Cd is limited, Cd may have direct toxic effects on placental tissue, thereby disrupting fetal growth, development, and programming (Z. Chen et al., 2014). Mechanisms of toxicity include: induction of reactive oxidative species (ROS) production, reduced placental leptin synthesis, reduction in metabolism of placental glucocorticoids, impaired utero-placental blood flow, and maternal-fetal nutrient transport (Jaquet, Leger, Levy-Marchal, Oury, & Czernichow, 1998; Jones & Parer, 1983; Kippler, Hoque, Raqib, Ohrvik, Ekström, et al., 2010; J. Liu, Qu, & Kadiiska, 2009; Ronco, Urrutia, Montenegro, & Llanos, 2009; Stasenko et al., 2010; Valko, Jomova, Rhodes, Kuča, & Musílek, 2016). This may result in sub-optimal fetal growth or abnormal programming with potential consequences that lead to adverse cardiovascular outcomes later in life (Crispi et al., 2018; Neggers et al., 1990; H. Wang et al., 2015). Additionally, Cd may interfere with blood pressure regulation through direct vascular effects. Animal studies suggest that Cd may inhibit nitric oxide (NO) synthase which functions to suppress acetylcholine-induced vascular relaxation, (Yoopan et al., 2008), or increase oxidative stress (Almenara et al., 2013). Even at low levels, Cd has been linked with renal tubular damage and dysfunction and subsequently higher blood pressure in adults. (Satarug et al., 2005). Therefore, consideration of variations of genes involved in Cd metabolism is important in assessing relationships between maternal Cd and offspring blood pressure.

Previous studies have examined genetic variations in the context of Cd accumulation in tissues and toxicity. For example, matrix metalloproteinases (MMPs), zinc-dependent

proteinases, gene polymorphisms—particularly metallothionein-2A—have been shown to impact susceptibility to Cd (Kayaaltı, Aliyev, & Söylemezoğlu, 2011; Kita et al., 2006). In a cohort of Spanish adults, investigators found statistically significant interactions between urine Cd and polymorphisms of the zinc-transporter encoding gene *SLC30A7 rs3087816* in associations with albuminuria, a well-established marker of kidney damage related to hypertension (Grau-Perez et al., 2017). Evidence from human and experimental studies suggests the glutathione s-transferase (*GST*) family of genes may play a role in Cd toxicity (Khansakorn et al., 2012; Oberheitmann et al., 1999). Several studies, including one in the ELEMENT cohort, showed that variations in the hemochromatosis (*HFE*) iron regulatory genes modified absorption or health effects of heavy metals (lead and Cd) (Akesson, Stål, & Vahter, 2000; Cantonwine et al., 2010; Fan et al., 2014). Chinese lead-exposed workers with the *HFE H63D* variant had higher blood lead levels compared to those without and that the variant modified the association between blood iron stores and blood lead levels (Fan et al., 2014). In ELEMENT, infant carriers of the *HFE H63D* genotype had, on average, a 110.3 g (95% CI -216.1, -4.6) decrease in birthweight associated with increases in tibial lead (p for interaction<0.05) (Cantonwine et al., 2010).

To date, no study has examined offspring gene- prenatal Cd exposure interactions on childhood BP. This has limited uncovering potentially important associations among subgroups defined by genetic variations. In the present study, we explored whether associations of maternal urinary cadmium (UCd) burden with offspring BP differ by offspring genotypes. The present study has the potential to provide insights into the mechanisms associated with etiology of hypertension, as well as identifying genetic variations associated with differences in Cd toxicity.

3.3 Materials and Methods

Study Setting and Study Population

Our study used a subset of participants from the Early Life Exposure in Mexico to Environmental Toxicants (ELEMENT) study. This birth cohort study was initiated in Mexico City to study impacts of prenatal lead exposure on neurodevelopment, then later expanded to examine other environmental factors on child development (Perng et al., 2019). From 1994-2003, three sequential cohorts of healthy pregnant women were recruited from social security hospitals in Mexico City. At delivery, baseline demographic and health status information were collected from mothers and infants, followed by regular follow-up visits in infancy and early childhood (Perng et al., 2019). For the current study, we used data from the first cohort (recruited 1994-1995). DNA was extracted from 412 umbilical cord samples (67% of eligible participants). A detailed description of study procedures has been published elsewhere (Perng et al., 2019; Pilsner et al., 2010).

Between 2008-2011, when children were 8-15 years of age, mother-child dyads were contacted and recalled for a middle childhood/adolescent visit (49.6% participation). During this visit, information was collected on offspring health behaviors, a physical exam was performed, and blood and urine samples were collected. This analysis included 113 participants for whom genetic data, maternal prenatal Cd measures, and offspring BP were available.

The ethics and research committees of the Mexico National Institute of Public Health, the University of Michigan, and the University of Washington approved this research and all participants provided consent prior to enrollment.

Data Collection

Subjects were recruited at a regularly scheduled prenatal visit during their first trimester of pregnancy and followed up at each trimester of pregnancy. Baseline information on health status, and demographics was collected from all participants at delivery by a trained interviewer. Within twelve hours of delivery, anthropometric data from mother and newborn, venous, and cord blood samples were collected. DNA was extracted from umbilical cord blood samples collected at delivery. Gestational age, based on date of last menstrual period, was abstracted from medical records. During the follow-up visit when children were 8-15 years old, a physical exam, BP measurements, urine collection and venous blood sampling were performed on children.

Exposure

UCd was measured in mothers in the third trimester of pregnancy (fasting spot) and offspring (arbitrary spot) at the follow-up visit (Chatzi et al., 2019). UCd is a commonly used biomarker for Cd burden.(Caterina Vacchi-Suzzi & Meliker, 2016; Suwazono et al., 2005) Due to Cd's half-life of up to 38 years, it reflects long-term Cd exposure from dietary and environmental sources.(Caterina Vacchi-Suzzi & Meliker, 2016) Urine was collected in sterile cups, transferred to smaller containers for analysis within an hour, and then stored at -70 degrees Fahrenheit. Samples (0.5mL) were digested with equal volumes of nitric acid and left under a fume hood overnight. The digest was brought to 2% acid content with the addition of Milli-Q water. UCd concentration was analyzed using an Inductively Coupled Plasma Mass Spectrometer. Accuracy was determined using urinary reference material from Institut National de Santé Publique du Québec with a known concentration of Cd (2.1 ug/L). Samples were run in 25 separate batches of 20 and each included a reference material. The measured mean (SD) of

Cd in the reference material was 1.7 (0.1) ug/L, which indicates an accuracy of 82%. UCd was specific gravity-adjusted for dilution, using the following formula: UCd measurement for participant $_i$*(median specific gravity for entire cohort-1)/(specific gravity measurement for participant $_i$ -1) (Suwazono et al., 2005)

Outcome

BP readings were taken in the morning after subjects had rested quietly for 5 minutes and subjects were asked to abstain from caffeinated beverages for at least 12 hours before the follow-up visit. A single measurement of systolic BP (SBP) and diastolic BP (DBP) was measured on participants' left arm using a standard mercury column sphygmomanometer and an appropriately sized cuff.

In children 1-<13 years old, normal BP was defined as a SBP and/or DBP <90th percentile for age, sex, and height; elevated BP was defined as either 1)SBP and/or DBP ≥90th-<95th percentile for age, sex, and height, or 2) 120 mmHg SBP and/or 80 mmHg DBP to <95th percentile (whichever is lower); hypertension was defined as either: 1) a SBP and/or DBP ≥95th percentile for age, sex, and height, or 2) ≥130mmHg SBP and/or ≥ 80 mmHg SBP (whichever is lower). In children and adolescents ≥13 years old, normal blood pressure was defined as <120 mmHg SBP and/or <80 mmHg DBP; elevated BP was defined as 120-129 mmHg SBP and DBP <80 mmHg; hypertension was defined as ≥130 mmHg and/or SBP ≥80 mmHg. While information on hypertension and elevated blood pressure was available for the study population, due to the low prevalence of these outcomes, we were did not examine them in relation to Cd exposure or in effect modification analyses.

DNA Genotyping and SNP Selection

Offspring DNA was extracted using PureGene Kits (Gentra Systems, Minneapolis, MN) from whole white blood cells of archived umbilical blood cells collected at delivery. A TaqMan platform (Applied Biosystems, Carlsbad CA) was used to genotype single nucleotide polymorphisms (SNPs). We selected, *a priori,* SNPs at 88 chromosomal locations associated with 19 genes that have been previously associated with cardiovascular outcomes and heavy metal metabolism (see Supplemental Table 3-1). DNA extraction and genotyping was performed at the Harvard-Partners Center for Genetics and Genomics.

Hardy-Weinberg Equilibrium (HWE) p-value and minor allele frequency (MAF) were calculated for all SNPs. MAF was calculated as: [(Number of minor alleles)/Total number of major and minor alleles)]. Six SNPs could not be analyzed due to missing data or lab errors, leaving N=82 for analysis. We excluded 10 SNPs for which the X^2 test p-value for HWE was ≤0.05, indicating Hardy-Weinberg disequilibrium. Additionally, 17 SNPs were excluded due to low MAF (≤10%) and 4 SNPS were excluded due to lack of genotype variability (i.e., absence of minor alleles). Finally, we assessed linkage disequilibrium, a measure of non-random occurrence of two alleles at two loci using R^2, based on an external database of genetic data from Mexican Americans in Los Angeles (Machiela & Chanock, 2015). R^2 was calculated for each set of SNPs related to a common gene. If the R^2 exceeded 0.64, we retained the SNP with the higher MAF, excluding a total of 12 SNPs. Thus 39 SNPs related to 15 genes remained for further analyses.

Covariates

Covariates for adjustment were selected *a priori* based on previous literature. These included sex (male/female), child age (continuous), child height (continuous), offspring UCd

(continuous), maternal ever-smoking (former/current or never), maternal socioeconomic status (SES), and log-transformed child UCd concentration at the time of BP measurement. Maternal SES at study enrollment categorized into 7 levels, based on a standardized index from the Mexican Association of Marketing Research and Public Opinion Agencies (AMAI) that accounts for housing quality and household assets. ("Asociación Mexicana de Agencias de Inteligencia de Mercado y Opinión (AMAI) ")

Statistical Analysis

Univariate and bivariate summary statistics were used to summarize maternal and child demographic and anthropometric characteristics. Statistically significant difference in these characteristics by quartile of maternal UCd were examined with one-way ANOVA tests for continuous variables and Pearson's Chi-Square tests for categorical variables.

We initially conducted a main effect analysis by fitting multiple linear regression models to quantify relationships between maternal UCd and offspring BP (systolic or diastolic). Three sequential models were fitted: 1) a minimally adjusted model included sex, child age, and child height; 2) a partially adjusted model included variables in the minimally adjusted model and offspring log-transformed UCd; and 3) a fully adjusted model that included variables in the partially adjusted model and maternal ever-smoking, and maternal socioeconomic status (SES). The primary model was the fully adjusted model.

Next, we evaluated effect modification by offspring genotype in the association between maternal UCd and childhood BP using interaction terms. Four adjusted linear regression models, two for SBP and two for DBP, were run for each of the 39 SNPs; the first set had an interaction between log-transformed maternal UCd and number of copies of minor allele (0,1,2), and the

second set had an interaction between log-transformed maternal UCd and an indicator for the presence/absence of a minor allele (0,1). A Wald-based test was used to assess the statistical significance of multiplicative interactions. For this initial exploratory analysis, we used a threshold for statistical significance of $p<0.10$.

For SNPs with interactions that passed the threshold, we ran stratified, adjusted linear regression models. Exposures, outcomes, and covariates were the same as described above. Models were stratified by number of copies of minor allele (0,1,2) and/or presence of minor allele (yes/no). The threshold for statistical significance was $p<0.05$. All analyses were carried out using Stata 14.0 (Stata, 2015).

3.4 Results

Study participants included 55 male and 58 female children, with a mean age of 10.4 (SD=1.5) years at the follow up visit (**Table 3.7.1**). Maternal age, history of smoking, and SES did not vary significantly by quartile of maternal UCd (p=0.12, 0.25, 0.17, respectively) (**Table 3.7.1**). Median third trimester UCd concentration was 0.17 μg/L (IQR=0.13, 0.27) (Data not shown). The prevalence of elevated BP and stage 1 hypertension were 6.2% and 11.5%, respectively. This varied by quartile of maternal UCd, with children in the lowest quartile of maternal UCd having the greater prevalence of stage 1 hypertension, but children in 3rd and 4th quartiles of maternal UCd having a greater prevalence of elevated BP (**Table 3.1**). A summary of 39 SNPs that were evaluated, related genes (and gene symbols), functional class, MAF, and HWE p-values are shown in **Table 3.2.**

We did not observe a significant association between maternal UCd and offspring SBP, however, we observed a significant association between increasing maternal UCd and lower

DBP (β=2.48, 95% CI: -4.62, -0.35) **(Table 3.3)**. The maternal UCd and offspring genotype (either additive or indicator for minor allele) interaction on SBP passed our predetermined threshold (p<0.10) for 8 SNPs—*rs5909* (*HMGCR*)*, rs3761740* (*HMGCR*)*, rs7975232* (*VDR*)*, rs9288393* (*PTH2R*)*, r10497900* (*PTH2R*)*, rs4961* (*ADD1*)*, rs3771452* (*ADD2*)*,* and *rs1023421 (ATP1A2)* (**Table 3.4**). Similarly, the maternal UCd and offspring genotype multiplicative interaction on DBP passed our predetermined threshold for two SNPs—*rs3761740* (*HMGCR*) and *rs4984* (*ADD2*) (**Table 3.4**).

Tables 3.5 and **3.6** show the results of genotype-stratified models. Maternal UCd was inversely associated with SBP among those with 0 copies of the *rs5909* (*HMGCR*) minor allele (β=-4.61 mmHg, 95%CI: -8.84, -0.38), at least one copy of the *rs10497900* (*PTH2R*) minor allele (β=-5.02 mmHg, 95%CI: -8.67, -1.38), and 0 copies of the *rs3771452* (*ADD2*) minor allele (β=-9.82 mmHg, 95%CI: -17.30, -2.35, p-value < 0.05) (interaction p-values 0.05, 0.05, and 0.02, respectively). There were suggestive (borderline) inverse associations of maternal UCd with lower DBP among those with 1 or 2 copies of the *rs208272* (*HMGCR*) minor allele (β=-5.57 mmHg, 95%CI: -11.17, 0.03, p-value=0.05) and 0 copies of the *rs4984* (*ADD2*) minor allele (β=-2.86 mmHg, 95%CI: -6.01, 0.29, p-value=0.07) (interaction p-values, 0.07 and 0.03, respectively.)

3.5 Discussion

This novel study explored gene-environment interactions in the association of prenatal Cd and offspring childhood BP. In the current study, contrary to our hypothesis, we observed a small decrease in DBP associated with increasing maternal UCd. We found interactions of prenatal Cd with *HMGCR* (*rs5909)* and *PTH2R* (*rs10497900*) SNPs on SBP. Maternal UCd was

inversely associated with lower SBP in the absence of the minor alleles of *rs5909* and *rs3771452* and among those with at least one copy of the minor allele of *rs10497900*. In addition, we found interactions of prenatal Cd with *HMGCR* (*rs3761740)* and *ADD2* (*rs4984)* SNPs on DBP. There were borderline significant associations of maternal UCd and lower DBP among those with 1 or 2 copies of the minor allele *rs3761740* and 0 copies of the minor allele of *rs4984.*

In the current study, we found a small statistically significant decrease in DBP of -2.48 mmHg (95%CI -4.62, -0.35) associated with a log-μg/L increase in maternal UCd, which remained after adjustment for covariates. While this result differed in terms of significance from our previous analysis result (β=-1.22, 95%CI: -2.75, 0.29) in the larger cohort in ELEMENT, the direction of the estimates (i.e., inverse association) is similar. The magnitude of the estimate is slightly smaller for the previous study, although the 95% CI is relatively larger for the current study. Compared to the larger cohort from our previous analysis, participants in this analysis tended to be in a higher SES category and were slightly less likely to smoke. This, coupled with variability in sampling and slightly higher variation in maternal UCd concentration, could have explained the slight difference in our estimates. Further investigation of this question in a larger, diverse sample are warranted.

To our knowledge, this is the first study of prenatal Cd burden-offspring gene interactions on offspring BP. While multiple studies have examined gene-Cd interactions in relation to Cd transport and toxicity, few studies have examined gene-Cd interactions on BP outcomes, and none have explored it in the context of prenatal Cd burden. A study among Chinese adults found interactions of polymorphisms of the *MMP-2* and *MMP-9* genes with UCd on hypertension risk. Specifically, associations of UCd with hypertension risk were modified by *MMP-2* SNPs *rs14070* (p for interaction=0.022) and *rs7201* (p for interaction=0.009) (Wu et al., 2019). Our

study did not assess hypertension due to the small study size and the fact that *MMP-2* variations were not available for analysis in our study. In a study among Spanish adults, investigators found evidence of effect modification in Cd-albuminuria association by SNPs *rs3087816* (*SLC30A7*) ($p= 1.37 \cdot 10^{-5}$) and *rs4720672* (*RAC1*) ($p=1.37 \cdot 10^{-5}$) (Grau-Perez et al., 2017). Due to lack of data availability, we did not assess variations in *SLC30A7 or RAC1.* To our knowledge, only one other study has examined prenatal Cd-offspring gene interactions on offspring outcomes, though the outcome was neural tube defects (NTD) and not blood pressure (M. Liu et al., 2021). The authors found that the positive association of Cd with NTD was four-fold higher among those carrying the G allele of *rs4880* (*SOD2*) compared to those who were not. (M. Liu et al., 2021). Additionally, there was evidence of additive interaction by *rs1801133* (*SOD2*) in the association of Cd and NTDs (M. Liu et al., 2021). While we examined SNPs associated with *SOD2*, we observed no significant interactions by genotype in the associations of prenatal Cd with SBP or DBP.

We found an inverse relationship between maternal UCd and offspring SBP among those with no minor allele for *rs5909* (*HMGCR*) and at least one copy of the minor allele for r*s3761740* (*HMGCR*). As far as we know, this is the first study examining effect modification role of *HMGCR* genotypes in the association of Cd and BP. *HMGCR* codes for a rate-limiting enzyme for cholesterol synthesis (Friesen & Rodwell, 2004). Variations in *rs5909* have been and *rs3761740* have been linked to serum lipid levels (Y. C. Chen et al., 2009; Paththinige, Sirisena, & Dissanayake, 2017). In a study of 20,000 adults in the UK, possessing the G allele of rs17238540 (HMGCR) resulted in a stronger association between urinary sodium and blood pressure in men, while the opposite was observed in women (Freitas et al., 2009). Statins, or *HMGCR* inhibitors are commonly prescribed to lower serum cholesterol levels, though evidence

from randomized trials suggests that statin therapy may offer clinically-meaningful decreases in blood pressure (Strazzullo et al., 2007). Animal studies suggest that statins may improve endothelial function by increasing bioavailability of nitric oxide (NO) and inhibiting the production of reactive oxygen species (ROS) (Rikitake et al., 2001; Wolfrum, Jensen, & Liao, 2003). Reduced NO levels and increased ROS production are thought to be important factors in the pathogenesis of human hypertension (Hermann, Flammer, & Lüscher, 2006; Lassègue & Griendling, 2004). A possible mechanism for Cd-induced hypertension is through increased oxidative stress. Exposure to Cd may result in decreases in intracellular glutathione levels and subsequent increases in ROS production (J. Liu et al., 2009; Nemmiche, 2017). Thus, the results of our study suggest that variations in *HGMCR* may play a role in Cd toxicity related to blood pressure, possibly through a mechanism that involves oxidative stress.

We also found an inverse association between maternal UCd and offspring SBP among those possessing no copies of the minor allele for *rs3771452* (*ADD2*) and an inverse association between maternal UCd and offspring DBP in those with the at least one copy of the minor allele for *rs4984* (*ADD2*). A previous study in African American women found a significant interaction between BMI and *rs3771452* in associations with DBP(Taylor, Sun, Hunt, & Kardia, 2010). Another study found significant interactions with *rs3771452* and beta-blocker drugs in associations with SBP in hypertensive patients (Kardia et al., 2007). While our study is the first to examine interactions of Cd exposure and *ADD2* variation in relation to with blood pressure, dozens of animal and human studies have linked variations in *ADD2* to blood pressure (Bianchi, Ferrari, & Staessen, 2005; J. R. Zhang, Hu, & Li, 2019). For example, a study of 1583 hypertensive individuals in Minnesota, eight SNPs in *ADD2* were associated with SBP in untreated hypertensives (Kardia et al., 2007). Additionally, investigators observed a gene-drug

interaction (beta-blocker vs. diuretic) among those possessing the TT genotype at SNP *rs1541582* (Kardia et al., 2007). Another study of three European populations found that Slavic participants with a beta-adducin *C1797 T* allele had higher SBP compared to those with the CC allele (p=0.03) (Tikhonoff et al., 2003). *ADD2* codes for beta-adducin, membrane-skeletal proteins that maintain cellular shape and stability through promotion of binding of spectrin and actin filaments (Gilligan, Lozovatsky, & Silberfein, 1997). Importantly, adducin impacts ionic transport, particularly through epithelial NA^+ channels (Ferrandi et al., 1999). The *ADD2* gene regulates blood pressure mainly through control of Na^+-K^+-ATPase activity (Ferrandi et al., 1999). Stimulation of this enzyme may increase intracellular Na^+ and Ca^{2+} in the kidneys, which results in vasoconstriction and subsequently higher blood pressure (Jaitovich & Bertorello, 2010). Since Cd accumulates in the kidney, it is believed that Cd directly causes renal tubular damage (Satarug et al., 2005). This, taken together with the function of the *ADD2* gene in regulating K^+-ATPase activity, suggests that variations in ADD2 may modify Cd-induced kidney damage and subsequent hypertension.

In the current study, we found inverse associations of maternal UCd with offspring SBP among offspring with at least one copy of the minor allele of the *rs10497900* SNP in *PTH2R*. As far as we know, this is the first study of the *rs10497900* SNP related to BP risk or Cd. *PTH2R* codes for a protein that is expressed in endothelial cells and is a receptor for the parathyroid hormone (Dobolyi, Dimitrov, Palkovits, & Usdin, 2012). Hyperparathyroidism, a condition that results from the parathyroid glands releasing too much parathyroid hormone into the blood stream, is a known cause of hypertension (Fisher & Perrier, 2020). Thyroid hormone insufficiencies may result in blood pressure changes through pathways of impaired renal function, hormonal changes, or obesity (Fisher & Perrier, 2020). Importantly, Parathyroid

hormone regulates calcium (Ca) homeostasis, which plays a crucial role in vascular disorders and development of cardiovascular disease (Goettsch, Iwata, & Aikawa, 2014). One of the proposed mechanisms of Cd toxicity is through disruption of Ca absorption. Since Cd and Ca share similar physiochemical properties, Cd utilizes Ca transport pathways, mediating cellular uptake of Ca (Choong, Liu, & Templeton, 2014; Thevenod, 2010). Additionally, Cd may bind to Ca receptors on cell surfaces, therefore changing the function of these cells (Choong et al., 2014). Our results suggest that genetic variations in *PTH2R* might play a role in Cd-related BP variations through mechanisms that potentially involve disruptions in Ca absorption.

Interestingly, contrary to our hypothesis, we found a statistically significant inverse association between maternal UCd and DBP in our study sample. Additionally, in genotype-stratified analyses, the statistically significant relationships we observed between UCd and SBP/DBP were all inverse. This observation could be due to uncontrolled confounding, possibly by diet or socioeconomic status. In the ELEMENT cohort, it was observed that maternal intake of fruit and vegetables predicted maternal UCd (Moynihan et al., 2017). Consumption of these types of foods has been linked to lower BP (Ndanuko, Tapsell, Charlton, Neale, & Batterham, 2016). Additionally, small increases in maternal UCd levels may lead to potential regulatory feedback mechanisms (such as those that involve increased production of enzymes that counteract reactive oxygen species and reduce oxidative stress) that protect from long term risk (Jan et al., 2015). These are potential areas that need to be addressed in future studies.

A strength of this study is the novel assessment of the interactions of prenatal Cd burden and genetic variations on offspring BP, which may shed light on the developmental origins of hypertension. Additionally, our study uses maternal UCd as a biomarker of prenatal Cd exposure, rather than alternatives such as cord blood. UCd is a relatively temporally stable

biomarker of long-term Cd body burden (S. Adams & P. Newcomb, 2014; Vacchi-Suzzi et al., 2016). Furthermore, use of maternal UCd accounts for the limited transfer of Cd to the fetus and the fact that maternal body burden, and not fetal dose, may be most important when measuring offspring health effects (Kippler, Hoque, Raqib, Ohrvik, Ekstrom, et al., 2010). Our study has several limitations. Importantly, our study size was small and our study may be underpowered to adequately detect interactions. Therefore, larger studies are needed. Second, while we did attempt to gate our analyses by limiting our SNP selection based on MAF, R^2, and potentially significant interactions, there is a risk of type 1 error due to multiple testing. Additionally, despite a standardized protocol, a single measurement of BP is susceptible to measurement error. We would expect this misclassification of the outcome to be non-differential with respect to UCd, and thus attenuate our estimates. While we adjusted for a number of confounders, unmeasured and residual confounding—including by diet—is a potential limitation. Functional roles of the identified SNPs in relation to Cd and/or BP regulation need to be demonstrated. Finally, generalizability of our observations to non-Mexican populations may be limited. Future studies in diverse cohorts are needed.

In conclusion, our study found potential evidence that variations in *HMGCR rs5909* SNP, *PTH2R rs10497900* SNP, and *ADD2 rs3771452* SNP modified associations of maternal Cd burden and childhood SBP. Our findings also suggest that variations in *HMGCR rs3761740* SNP and *ADD2 rs4984* SNP modified associations of maternal Cd burden with DBP. If replicated, our findings could improve the understanding of the relationships between prenatal Cd burden and offspring BP. It could also help identify populations at higher risk for detrimental effect of prenatal Cd on BP. These will help the effort to improve health outcomes throughout the life course.

3.6 References

Adams, S., & Newcomb, P. (2014). Cadmium blood and urine concentrations as measures of exposure: NHANES 1999–2010. *Journal of Exposure Science and Environmental Epidemiology, 24*(2), 163-170.

Akesson, A., Stål, P., & Vahter, M. (2000). Phlebotomy increases cadmium uptake in hemochromatosis. *Environ Health Perspect, 108*(4), 289-291. doi:10.1289/ehp.108-1638026

Almenara, C. C., Broseghini-Filho, G. B., Vescovi, M. V., Angeli, J. K., Faria, T. e. O., Stefanon, I., . . . Padilha, A. S. (2013). Chronic cadmium treatment promotes oxidative stress and endothelial damage in isolated rat aorta. *PLoS One, 8*(7), e68418. doi:10.1371/journal.pone.0068418

Asociación Mexicana de Agencias de Inteligencia de Mercado y Opinión (AMAI) Retrieved from https://nse.amai.org/niveles-socio-economicos-amai/

Bianchi, G., Ferrari, P., & Staessen, J. A. (2005). Adducin polymorphism: detection and impact on hypertension and related disorders. *Hypertension, 45*(3), 331-340. doi:10.1161/01.HYP.0000156497.39375.37

Cantonwine, D., Hu, H., Téllez-Rojo, M. M., Sánchez, B. N., Lamadrid-Figueroa, H., Ettinger, A. S., . . . Wright, R. O. (2010). HFE gene variants modify the association between maternal lead burden and infant birthweight: a prospective birth cohort study in Mexico City, Mexico. *Environ Health, 9*, 43. doi:10.1186/1476-069X-9-43

Caterina Vacchi-Suzzi, D. K., James Harrington, Keith Levine, and Jaymie R., & Meliker. (2016). Is Urinary Cadmium a Biomarker of Long-term Exposure in Humans? A Review. *Curr Environ Health Rep, 3*(4), 450-458.

Chatzi, L., Ierodiakonou, D., Margetaki, K., Vafeiadi, M., Chalkiadaki, G., Roumeliotaki, T., . . . Kippler, M. (2019). Associations of Prenatal Exposure to Cadmium With Child Growth, Obesity, and Cardiometabolic Traits. *Am J Epidemiol, 188*(1), 141-150. doi:10.1093/aje/kwy216

Chen, Y. C., Chen, Y. D., Li, X., Post, W., Herrington, D., Polak, J. F., . . . Taylor, K. D. (2009). The HMG-CoA reductase gene and lipid and lipoprotein levels: the multi-ethnic study of atherosclerosis. *Lipids, 44*(8), 733-743. doi:10.1007/s11745-009-3314-6

Chen, Z., Myers, R., Wei, T., Bind, E., Kassim, P., Wang, G., . . . Wang, X. (2014). Placental transfer and concentrations of cadmium, mercury, lead, and selenium in mothers, newborns, and young children. *J Expo Sci Environ Epidemiol, 24*(5), 537-544. doi:10.1038/jes.2014.26

Choong, G., Liu, Y., & Templeton, D. M. (2014). Interplay of calcium and cadmium in mediating cadmium toxicity. *Chem Biol Interact, 211*, 54-65. doi:10.1016/j.cbi.2014.01.007

Crispi, F., Miranda, J., & Gratacós, E. (2018). Long-term cardiovascular consequences of fetal growth restriction: biology, clinical implications, and opportunities for prevention of adult disease. *Am J Obstet Gynecol, 218*(2S), S869-S879. doi:10.1016/j.ajog.2017.12.012

Dobolyi, A., Dimitrov, E., Palkovits, M., & Usdin, T. B. (2012). The neuroendocrine functions of the parathyroid hormone 2 receptor. *Front Endocrinol (Lausanne), 3*, 121. doi:10.3389/fendo.2012.00121

Fan, G., Du, G., Li, H., Lin, F., Sun, Z., Yang, W., . . . Zhou, F. (2014). The effect of the hemochromatosis (HFE) genotype on lead load and iron metabolism among lead smelter workers. *PLoS One, 9*(7), e101537. doi:10.1371/journal.pone.0101537

Ferrandi, M., Salardi, S., Tripodi, G., Barassi, P., Rivera, R., Manunta, P., . . . Karlish, S. J. (1999). Evidence for an interaction between adducin and Na(+)-K(+)-ATPase: relation to genetic hypertension. *Am J Physiol, 277*(4), H1338-1349. doi:10.1152/ajpheart.1999.277.4.H1338

Fisher, S. B., & Perrier, N. D. (2020). Primary hyperparathyroidism and hypertension. *Gland Surg, 9*(1), 142-149. doi:10.21037/gs.2019.10.21

Franceschini, N., Fry, R. C., Balakrishnan, P., Navas-Acien, A., Oliver-Williams, C., Howard, A. G., . . . Tellez-Plaza, M. (2017). Cadmium body burden and increased blood pressure in middle-aged American Indians: the Strong Heart Study. *J Hum Hypertens, 31*(3), 225-230. doi:10.1038/jhh.2016.67

Freitas, R. N., Khaw, K. T., Wu, K., Bowman, R., Jeffery, H., Luben, R., . . . Bingham, S. A. (2009). A HMGCR polymorphism is associated with relations between blood pressure and urinary sodium and potassium ratio in the Epic-Norfolk Study. *J Am Soc Hypertens, 3*(4), 238-244. doi:10.1016/j.jash.2009.05.001

Friesen, J. A., & Rodwell, V. W. (2004). The 3-hydroxy-3-methylglutaryl coenzyme-A (HMG-CoA) reductases. *Genome Biol, 5*(11), 248. doi:10.1186/gb-2004-5-11-248

Gallagher, C. M., & Meliker, J. R. (2010). Blood and urine cadmium, blood pressure, and hypertension: a systematic review and meta-analysis. *Environ Health Perspect, 118*(12), 1676-1684. doi:10.1289/ehp.1002077

Garner, R. E., & Levallois, P. (2017). Associations between cadmium levels in blood and urine, blood pressure and hypertension among Canadian adults. *Environ Res, 155*, 64-72. doi:10.1016/j.envres.2017.01.040

Genchi, G., Sinicropi, M. S., Lauria, G., Carocci, A., & Catalano, A. (2020). The Effects of Cadmium Toxicity *Int J Environ Res Public Health, 17*(11), 3782. doi:10.3390/ijerph17113782

Gilligan, D. M., Lozovatsky, L., & Silberfein, A. (1997). Organization of the human beta-adducin gene (ADD2). *Genomics, 43*(2), 141-148. doi:10.1006/geno.1997.4802

Goettsch, C., Iwata, H., & Aikawa, E. (2014). Parathyroid hormone: critical bridge between bone metabolism and cardiovascular disease. *Arterioscler Thromb Vasc Biol, 34*(7), 1333-1335. doi:10.1161/ATVBAHA.114.303637

Grau-Perez, M., Pichler, G., Galan-Chilet, I., Briongos-Figuero, L. S., Rentero-Garrido, P., Lopez-Izquierdo, R., . . . Tellez-Plaza, M. (2017). Urine cadmium levels and albuminuria in a general population from Spain: A gene-environment interaction analysis. *Environ Int, 106*, 27-36. doi:10.1016/j.envint.2017.05.008

Hawkesworth S, W. Y., Kippler M, Fulford AJC, Arifeen SE, Persson L, Moore SE, Vahter M. (2012). Early exposure to toxic metals has a limited effect on blood pressure or kidney function in later childhood, rural Bangladesh. *International Journal of Epidemiology*(42), 176-185.

Hermann, M., Flammer, A., & Lüscher, T. F. (2006). Nitric oxide in hypertension. *J Clin Hypertens (Greenwich), 8*(12 Suppl 4), 17-29. doi:10.1111/j.1524-6175.2006.06032.x

Howe, C. G., Margetaki, K., Vafeiadi, M., Roumeliotaki, T., Karachaliou, M., Kogevinas, M., . . . Chatzi, L. (2021). Prenatal metal mixtures and child blood pressure in the Rhea mother-child cohort in Greece. *Environ Health, 20*(1), 1. doi:10.1186/s12940-020-00685-9

Jaitovich, A., & Bertorello, A. M. (2010). Salt, Na+,K+-ATPase and hypertension. *Life Sci, 86*(3-4), 73-78. doi:10.1016/j.lfs.2009.10.019

Jan, A., Azam, M., Siddiqui, K., Ali, A., Choi, I., & Haq, A. (2015). Heavy Metals and Human Health: Mechanistic Insight into Toxicity and Counter Defense System of Antioxidants. *Int J Mol Sci, 16*(12), 29592-29630.

Jaquet, D., Leger, J., Levy-Marchal, C., Oury, J. F., & Czernichow, P. (1998). Ontogeny of leptin in human fetuses and newborns: effect of intrauterine growth retardation on serum leptin concentrations. *J Clin Endocrinol Metab, 83*(4), 1243-1246. doi:10.1210/jcem.83.4.4731

Jhun, M. A., Hu, H., Schwartz, J., Weisskopf, M. G., Nie, L. H., Sparrow, D., . . . Park, S. K. (2015). Effect modification by vitamin D receptor genetic polymorphisms in the association between cumulative lead exposure and pulse pressure: a longitudinal study. *Environ Health, 14*, 5. doi:10.1186/1476-069X-14-5

Johnston, J. E., Valentiner, E., Maxson, P., Miranda, M. L., & Fry, R. C. (2014). Maternal cadmium levels during pregnancy associated with lower birth weight in infants in a North Carolina cohort. *PLoS One, 9*(10), e109661. doi:10.1371/journal.pone.0109661

Joneidi, Z., Mortazavi, Y., Memari, F., Roointan, A., Chahardouli, B., & Rostami, S. (2019). The impact of genetic variation on metabolism of heavy metals: Genetic predisposition? *Biomed Pharmacother, 113*, 108642. doi:10.1016/j.biopha.2019.108642

Jones, C. T., & Parer, J. T. (1983). The effect of alterations in placental blood flow on the growth of and nutrient supply to the fetal guinea-pig. *J Physiol, 343*, 525-537.

Kardia, S. L., Sun, Y. V., Hamon, S. C., Barkley, R. A., Boerwinkle, E., & Turner, S. T. (2007). Interactions between the adducin 2 gene and antihypertensive drug therapies in determining blood pressure in people with hypertension. *BMC Med Genet, 8*, 61. doi:10.1186/1471-2350-8-61

Kayaaltı, Z., Aliyev, V., & Söylemezoğlu, T. (2011). The potential effect of metallothionein 2A -5A/G single nucleotide polymorphism on blood cadmium, lead, zinc and copper levels. *Toxicol Appl Pharmacol, 256*(1), 1-7. doi:10.1016/j.taap.2011.06.023

Khansakorn, N., Wongwit, W., Tharnpoophasiam, P., Hengprasith, B., Suwannathon, L., Chanprasertyothin, S., . . . Sirivarasai, J. (2012). Genetic variations of glutathione s-transferase influence on blood cadmium concentration. *J Toxicol, 2012*, 356126. doi:10.1155/2012/356126

Kippler, M., Hoque, A. M., Raqib, R., Ohrvik, H., Ekstrom, E. C., & Vahter, M. (2010). Accumulation of cadmium in human placenta interacts with the transport of micronutrients to the fetus. *Toxicol Lett, 192*(2), 162-168. doi:10.1016/j.toxlet.2009.10.018

Kippler, M., Hoque, A. M., Raqib, R., Ohrvik, H., Ekström, E. C., & Vahter, M. (2010). Accumulation of cadmium in human placenta interacts with the transport of micronutrients to the fetus. *Toxicol Lett, 192*(2), 162-168. doi:10.1016/j.toxlet.2009.10.018

Kita, K., Miura, N., Yoshida, M., Yamazaki, K., Ohkubo, T., Imai, Y., & Naganuma, A. (2006). Potential effect on cellular response to cadmium of a single-nucleotide A --> G polymorphism in the promoter of the human gene for metallothionein IIA. *Hum Genet, 120*(4), 553-560. doi:10.1007/s00439-006-0238-6

Lassègue, B., & Griendling, K. K. (2004). Reactive oxygen species in hypertension; An update. *Am J Hypertens, 17*(9), 852-860. doi:10.1016/j.amjhyper.2004.02.004

Lin, C.-M., Doyle, P., Wang, D., Hwang, Y.-H., & Chen, P.-C. (2011). Does prenatal cadmium exposure affect fetal and child growth? *Occupational and Environmental Medicine, 68*, 641-646

Liu, J., Qu, W., & Kadiiska, M. B. (2009). Role of oxidative stress in cadmium toxicity and carcinogenesis. *Toxicol Appl Pharmacol, 238*(3), 209-214. doi:10.1016/j.taap.2009.01.029

Liu, M., Yu, J., Su, Z., Sun, Y., Liu, Y., Xie, Q., . . . Ren, A. (2021). Associations between prenatal exposure to cadmium and lead with neural tube defect risks are modified by single-nucleotide polymorphisms of fetal M*THFR* and S*OD2* : a case–control study. *Environmental Health, 20*(66). doi:https://doi.org/10.1186/s12940-021-00752-9

Machiela, M. J., & Chanock, S. J. (2015). LDlink: a web-based application for exploring population-specific haplotype structure and linking correlated alleles of possible functional variants. *Bioinformatics, 31*(21), 3555-3557. doi:10.1093/bioinformatics/btv402

Moynihan, M., Peterson, K. E., Cantoral, A., Song, P. X. K., Jones, A., Solano-González, M., . . . Téllez-Rojo, M. M. (2017). Dietary predictors of urinary cadmium among pregnant women and children. *Sci Total Environ, 575*, 1255-1262. doi:10.1016/j.scitotenv.2016.09.204

Ndanuko, R. N., Tapsell, L. C., Charlton, K. E., Neale, E. P., & Batterham, M. J. (2016). Dietary Patterns and Blood Pressure in Adults: A Systematic Review and Meta-Analysis of Randomized Controlled Trials. *Adv Nutr, 7*(1), 76-89. doi:10.3945/an.115.009753

Neggers, Y. H., Cutter, G. R., Acton, R. T., Alvarez, J. O., Bonner, J. L., Goldenberg, R. L., . . . Roseman, J. M. (1990). A Positive Association between Maternal Serum Zinc Concentration and Birth-Weight. *American Journal of Clinical Nutrition, 51*(4), 678-684.

Nemmiche, S. (2017). Oxidative Signaling Response to Cadmium Exposure. *Toxicol Sci, 156*(1), 4-10. doi:10.1093/toxsci/kfw222

Oberheitmann, B., Schäfer, J., Dally, H., Garms, A., Frentzel-Beyme, R., & Hoffmann, W. (1999). The chromosome-based challenge assay using fluorescence in situ hybridization: a possible test for increased cancer susceptibility. *Mutat Res, 428*(1-2), 157-164. doi:10.1016/s1383-5742(99)00043-5

Olsson, I., Bensryd, I., Lundh, T., Ottosson, H., Skerfvin, S., & Oskarsson, A. (2002). Cadmium in blood and urine--impact of sex, age, dietary intake, iron status, and former smoking--association of renal effects. *Environmental Health Perspectives, 110*, 1185-1190.

Paththinige, C. S., Sirisena, N. D., & Dissanayake, V. (2017). Genetic determinants of inherited susceptibility to hypercholesterolemia - a comprehensive literature review. *Lipids Health Dis, 16*(1), 103. doi:10.1186/s12944-017-0488-4

Perng, W., Tamayo-Ortiz, M., Tang, L., Sanchez, B. N., Cantoral, A., Meeker, J. D., . . . Peterson, K. E. (2019). Early Life Exposure in Mexico to ENvironmental Toxicants (ELEMENT) Project. *BMJ Open, 9*(8), e030427. doi:10.1136/bmjopen-2019-030427

Pilsner, J. R., Hu, H., Wright, R. O., Kordas, K., Ettinger, A. S., Sánchez, B. N., . . . Hernández-Avila, M. (2010). Maternal MTHFR genotype and haplotype predict deficits in early cognitive development in a lead-exposed birth cohort in Mexico City. *Am J Clin Nutr, 92*(1), 226-234. doi:10.3945/ajcn.2009.28839

Rikitake, Y., Kawashima, S., Takeshita, S., Yamashita, T., Azumi, H., Yasuhara, M., . . . Yokoyama, M. (2001). Anti-oxidative properties of fluvastatin, an HMG-CoA reductase

inhibitor, contribute to prevention of atherosclerosis in cholesterol-fed rabbits. *Atherosclerosis, 154*(1), 87-96. doi:10.1016/s0021-9150(00)00468-8

Ronco, A. M., Urrutia, M., Montenegro, M., & Llanos, M. N. (2009). Cadmium exposure during pregnancy reduces birth weight and increases maternal and foetal glucocorticoids. *Toxicol Lett, 188*(3), 186-191. doi:10.1016/j.toxlet.2009.04.008

Satarug, S., Nishijo, M., Ujjin, P., Vanavanitkun, Y., & Moore, M. R. (2005). Cadmium-induced nephropathy in the development of high blood pressure. *Toxicol Lett, 157*(1), 57-68. doi:10.1016/j.toxlet.2005.01.004

Stasenko, S., Bradford, E. M., Piasek, M., Henson, M. C., Varnai, V. M., Jurasović, J., & Kusec, V. (2010). Metals in human placenta: focus on the effects of cadmium on steroid hormones and leptin. *J Appl Toxicol, 30*(3), 242-253. doi:10.1002/jat.1490

Stata, C. (2015). *Stata Statistical Software: Release 14*. College Station, TX: StataCorp LP.

Strazzullo, P., Kerry, S. M., Barbato, A., Versiero, M., D'Elia, L., & Cappuccio, F. P. (2007). Do statins reduce blood pressure?: a meta-analysis of randomized, controlled trials. *Hypertension, 49*(4), 792-798. doi:10.1161/01.HYP.0000259737.43916.42

Substance Priority List | ATSDR. (2017, October 3 2017). Retrieved from https://www.atsdr.cdc.gov/spl/index.html

Suwazono, Y., Akesson, A., Alfven, T., Jarup, L., & Vahter, M. (2005). Creatinine versus specific gravity-adjusted urinary cadmium concentrations. *Biomarkers, 10*(2-3), 117-126. doi:10.1080/13547500500159001

Tanira, M. O., & Al Balushi, K. A. (2005). Genetic variations related to hypertension: a review. *J Hum Hypertens, 19*(1), 7-19. doi:10.1038/sj.jhh.1001780

Taylor, J. Y., Sun, Y. V., Hunt, S. C., & Kardia, S. L. (2010). Gene-environment interaction for hypertension among African American women across generations. *Biol Res Nurs, 12*(2), 149-155. doi:10.1177/1099800410371225

Thevenod, F. (2010). Catch me if you can! Novel aspects of cadmium transport in mammalian cells. *Biometals, 23*, 857-875.

Tikhonoff, V., Kuznetsova, T., Stolarz, K., Bianchi, G., Casiglia, E., Kawecka-Jaszcz, K., . . . Staessen, J. A. (2003). beta-Adducin polymorphisms, blood pressure, and sodium excretion in three European populations. *Am J Hypertens, 16*(10), 840-846. doi:10.1016/s0895-7061(03)00975-0

Vacchi-Suzzi, C., Kruse, D., Harrington, J., Levin, K., & Meliker, J. R. (2016). Is Urinary Cadmium a Biomarker of Long-term Exposure in Humans? A Review. *Curr Environ Health Rep, 3*(4), 450-458.

Valko, M., Jomova, K., Rhodes, C. J., Kuča, K., & Musílek, K. (2016). Redox- and non-redox-metal-induced formation of free radicals and their role in human disease. *Arch Toxicol, 90*(1), 1-37. doi:10.1007/s00204-015-1579-5

Wang, H., Hu, Y. F., Hao, J. H., Chen, Y. H., Su, P. Y., Wang, Y., . . . Xu, D. X. (2015). Maternal zinc deficiency during pregnancy elevates the risks of fetal growth restriction: a population-based birth cohort study. *Sci Rep, 5*, 11262. doi:10.1038/srep11262

Wolfrum, S., Jensen, K. S., & Liao, J. K. (2003). Endothelium-dependent effects of statins. *Arterioscler Thromb Vasc Biol, 23*(5), 729-736. doi:10.1161/01.ATV.0000063385.12476.A7

Wu, W., Liu, D., Jiang, S., Zhang, K., Zhou, H., & Lu, Q. (2019). Polymorphisms in gene MMP-2 modify the association of cadmium exposure with hypertension risk. *Environ Int, 124*, 441-447. doi:10.1016/j.envint.2019.01.041

Yoopan, N., Watcharasit, P., Wongsawatkul, O., Piyachaturawat, P., & Satayavivad, J. (2008). Attenuation of eNOS expression in cadmium-induced hypertensive rats. *Toxicol Lett, 176*(2), 157-161. doi:10.1016/j.toxlet.2007.11.002

Zhang, A., Park, S. K., Wright, R. O., Weisskopf, M. G., Mukherjee, B., Nie, H., . . . Hu, H. (2010). HFE H63D polymorphism as a modifier of the effect of cumulative lead exposure on pulse pressure: the Normative Aging Study. *Environ Health Perspect, 118*(9), 1261-1266. doi:10.1289/ehp.1002251

Zhang, J. R., Hu, W. N., & Li, C. Y. (2019). A Review of the Epidemiological Evidence for Adducin Family Gene Polymorphisms and Hypertension. *Cardiol Res Pract, 2019*, 7135604. doi:10.1155/2019/7135604

Zhang, M., Liu, T., Wang, G., Buckley, J. P., Guallar, E., Hong, X., . . . Mueller, N. T. (2021). Exposure to Heavy Metals and Trace Elements and Childhood Blood Pressure in a U.S. Urban, Low-Income, Minority Birth Cohort. *Environ Health Perspect, 129*(6), 67005. doi:10.1289/EHP8325

3.7 Tables

Table 3-1. Demographic and Anthropometric Characteristics of Mother-Child Pairs in ELEMENT Cohort Study

Variable	N	n(%) or μ(sd)	UCd Quartile 1 (0.03-0.11 μg/L) N=29	UCd Quartile 2 (0.12-0.18 μg/L) N=28	UCd Quartile 3 (0.18-0.27 μg/L) N=28	UCd Quartile 4 (μg/L) N=28	p-value*
Maternal Characteristics							
Maternal Age (years)	113	27.0(5.3)	26.3(5.3)	26.4(4.2)	26.4(4.9)	27.1(4.9)	0.12
Maternal SES[a]	113						
A/B		10(9.1%)	3(11.1%)	1(3.6%)	3(11.1%)	3(10.7%)	0.17
C		19(17.3%)	2(7.4%)	7(25.0%)	5(18.5%)	5(17.9%)	
C+		23(20.9%)	5(18.5%)	2(7.1%)	6(22.2%)	10(35.7%)	
D		5(4.6%)	1(3.7%)	0(0.0%)	1(3.7%)	3(10.7%)	
D+		39(35.5%)	11(40.7%)	14(50.0%)	10(37.0%)	4(14.3%)	
E		14(12.7%)	5(18.5%)	4(14.3%)	2(7.4%)	3(10.7%)	
Maternal history of smoking (% yes)	113	61(54.0%)	19(65.5%)	16(57.1%)	11(40.3%)	15(53.6%)	0.25
Parity	113	2.4(1.0)	2.6(1.0)	2.5(1.2)	2.2(0.9)	2.3(1.0)	0.46
Child Characteristics at Birth							
Gestational age (weeks)	111	39.1(1.1)	40.0(1.0)	39.1(1.1)	39.1(1.3)	39.1(1.0)	0.43
Birth weight (grams)	112	3187.1(422.6)	3262.9(354.1)	3193(471.5)	3157.0(509.4)	3131.6(348.8)	0.11
Low birthweight (%, <2500 grams)	112	3(2.7%)	0(0.0%)	1(3.6%)	1(3.7%)	1(3.6%)	0.78
Length (cm)	105	50.5(2.0)	50.9(1.8)	50.2(1.9)	50.6(2.4)	50.5(1.9)	0.52
Child Characteristics at Follow-up							
Age (years)	113	10.4(1.5)	9.7(1.0)	10.1(1.3)	11.2(1.5)	10.7(1.6)	0.08
BMI	113	20.1(3.8)	19.3(3.1)	21.2(4.9)	20.8(3.4)	19.2(3.5)	0.06
Systolic BP[b] **(mmHg)**	113	102.5(10.8)	104.0(9.8)	100.1(12.8)	104.2(10.1)	101.8(10.4)	0.54
Diastolic BP[b]**(mmHg)**	113	64.8(7.5)	66.4(8.1)	62.9(8.3)	65.3(6.8)	65.2(7.2)	0.46
BP Status[c]	113						
Normal		93(82.3%)	21(72.4%)	23(82.1%)	24(85.7%)	25(89.3%)	
Elevated		7(6.2%)	1(3.5%)	0(0.0%)	3(10.7%)	3(10.7%)	0.03
Stage 1 hypertension		13(11.5%)	7(24.1%)	5(17.9%)	1(3.6%)	0(0.00%)	

Maternal UCd outlier has been removed (n=1), with a cut-off of unadjusted maternal UCd>7 μg/L, a value deemed implausible. Percentages are based on number of non-missing values. [a]Maternal SES is based on a standardized index from the Mexican Association of Marketing Research and Public Opinion Agencies (AMAI). [b]BP readings are the average of 3 measurements recorded at the same study visit. [c]Hypertension status was calculated using a macro package for R, (Sharma) which references the 2017 American Academy of Pediatrics (AAP) Guidelines. (Flynn et al., 2017) *Based on one-way ANOVA test for continuous variables and chi-squared test for categorical variables.

Table 3-2. Genetic Variations in Offspring

SNP	Related Gene Name	Related Gene Symbol	Functional Class	Number of Minor Allele (0/1/2)	MAF *	HWE *P* value†
rs708272	cholesteryl ester transfer protein	CETP	intron	29/43/21	0.46	0.53
rs1800775			2KB upstream	28/42/17	0.44	0.83
rs3846662	3-hydroxy-3-methylglutaryl-CoA reductase	HMGCR	intron	28/32/21	0.46	0.07
rs5909			3 prime UTR	58/19/4	0.17	0.22
rs3761740			2KB upstream	47/19/3	0.18	0.68
rs715948	LDL receptor related protein 1	LRP1	intron	28/39/5	0.34	0.12
rs4367982			intron	24/48/20	0.48	0.83
rs2306692			intron	60/17/2	0.62	0.13
rs10876966			intron	43/46/4	0.29	0.08
rs6581124			intron	32/19/14	0.39	0.82
rs34574998			synonymous	22/10/1	0.18	1.00
rs1800159			intron	33/44/10	0.37	0.49
rs7978567			intron	21/29/5	0.35	0.38
rs180016			missense	30/34/11	0.37	0.81
rs1454626	very low density lipoprotein receptor	VLDLR	intron; 2KB upstream	42/22/7	0.25	0.13
rs1695	glutathione S-transferase pi 1	GSTP1	missense	31/32/11	0.36	0.62
rs749174			intron	46/22/2	0.19	1.00
rs7975232	vitamin D receptor	VDR	intron	30/44/14	0.41	0.83
rs1544410			intron	40/27/5	0.26	1.00
rs10735810			initiator codon	29/34/17	0.43	0.26
rs1801197	calcitonin receptor	CALCR	missense	29/40/17	0.43	0.66
rs6442037	parathyroid hormone 1 receptor	PTHR1	intron	62/27/3	0.18	1.00
rs724449			intron	25/45/14	0.42	0.51
rs9288393	parathyroid hormone 2 receptor	PTH2R	intron	43/28/4	0.24	1.00
rs10497900			intron	23/50/16	0.46	0.29
rs7121	GNAS complex locus	GNAS	missense	28/41/9	0.38	0.35
rs4961	adducin 1	ADD1	missense	44/28/0	0.19	0.06
rs3771452	adducin 2	ADD2	intron	30/44/13	0.40	0.82
rs1541582			intron	47/42/12	0.33	0.65
rs4984			synonymous	55/21/1	0.15	1.00
rs17698193			3 prime UTR	55/28/3	0.20	1.00
rs12695877	angiotensin II receptor type 1	AGTR1	intron	50/34/6	0.26	1.00
rs12721241			intron	32/39/14	0.39	0.82
rs2675511			intron	66/29/4	0.19	0.74
rs6801836			intron	52/18/0	0.13	0.59
rs3798215	superoxide dismutase 2	SOD2	intron	56/29/3	0.20	1.00
rs1967802			3 prime UTR	11/1/01	0.12	0.12
rs1023421	ATPase Na+/K+ transporting subunit alpha 2	ATP1A2	intron	22/33/21	0.49	0.26
rs12410866			intron	67/22/0	0.12	0.35

*Minor allele frequency (MAF) was calculated as: [(Number of minor alleles)/Total number of major and minor alleles)]; †Hardy-Weinberg test of SNP distribution; significance indicates lack of equilibrium. N=17 SNPs were excluded due to a minor allele frequency ≤0.1, N=10 SNPs were excluded due to HWE p≤0.05, and N=12 SNPs were excluded due to $R^2 \geq 0.64$. N=4 SNPS were excluded due to lack of genotype variability, 7 SNPs were missing from the dataset.

Table 3-3. Associations of maternal third trimester urine cadmium with offspring BP (mmHg

Outcome	Minimally Adjusted*		Moderately Adjusted)†		Fully Adjusted‡	
	N	β(95% CI)	N	β(95% CI)	N	β(95% CI)
Systolic BP¶	113	-0.66(-3.24, 1.91)†	110	-0.99(-3.65, 1.68)‡	107	-1.71(-4.62, 1.19)§
Diastolic BP¶	113	**-2.10(-3.97, -0.22)**†	110	**-2.24(-4.15, -0.33)**‡	107	**-2.49(-4.63, -0.35)**§

Coefficients are the mean difference in BP (mmHg) associated with a log-UCd (μg/L) increase in maternal urinary cadmium. *Adjusted for sex, child age, child height· †Adjusted for sex, child age, child height, postnatal urinary cadmium burden. ‡Adjusted for sex, child age, child height, postnatal urinary cadmium, maternal ever-smoking, and maternal SES

Table 3-4. Interaction between maternal cadmium burden and blood-pressure related genotypes on offspring blood pressure

SNP	Related Gene	Systolic		Diastolic	
		Additive*	Indicator†	Additive *	Indicator†
rs708272	CETP	0.43	0.35	0.80	0.78
rs1800775		0.20	0.21	0.69	0.94
rs3846662	HMGCR	0.60	0.87	0.75	0.65
rs5909		***0.07***	***0.05***	0.36	0.83
rs3761740		***0.09***	***0.07***	***0.07***	0.28
rs715948	LRP1	0.45	0.61	0.04	0.10
rs4367982		0.81	0.97	0.58	0.27
rs2306692		0.12	0.15	0.86	0.26
rs10876966		0.89	0.94	0.19	0.89
rs6581124		0.81	0.90	0.47	0.25
rs34574998		0.69	0.61	0.70	0.51
rs1800159		0.84	0.45	0.24	0.78
rs7978567		0.48	0.56	0.88	0.71
rs180016		0.84	0.84	0.74	0.59
rs1454626	VLRLR	0.31	0.62	0.95	0.40
rs1695	GSTP1	0.24	0.17	0.27	0.62
rs749174		0.38	0.36	0.85	0.60
rs7975232	VDR	***0.06***	0.14	0.15	0.79
rs1544410		0.99	0.89	0.32	0.29
rs10735810		0.23	0.20	0.10	0.29
rs1801197	CGRP	0.15	0.14	0.41	0.77
rs6442037	PTHR1	0.31	0.37	0.82	0.57
rs724449		0.10	0.15	0.70	0.88
rs9288393	PTH2R	***0.07***	0.13	0.60	0.73
rs10497900		***0.07***	***0.05***	0.50	0.42
rs7121	GNAS1	0.14	0.23	0.50	0.87
rs4961	ADD1	***0.09***	***0.09***	0.61	0.44
rs3771452	ADD2	0.11	***0.02***	0.32	0.58
rs1541582		0.95	0.98	0.72	0.47
rs4984		0.36	0.37	0.62	***0.03***
rs17698193		0.15	0.13	0.62	0.60
rs12695877	AGTR1	0.26	0.88	0.92	0.91
rs12721241		0.68	0.77	0.19	0.73
rs2675511		0.30	0.45	0.37	0.17
rs6801836		0.45	0.45	0.30	0.27
rs3798215	SOD2	0.42	0.48	0.38	0.30
rs1967802		--	--	--	0.31
rs1023421	ATP1A2	0.11	***0.03***	0.68	0.85
rs12410866		0.92	0.92	0.72	0.95

*Additive coding (0,1,2) by number of minor alleles. Number of minor alleles was included as a continuous interaction term with maternal UCd. †2 Category indictor (0,1) based on any minor allele present. Number of minor alleles was included as an indicator interaction term with maternal UCd. P-values for interaction were generated with a Wald-based test. Interaction could not be assessed for SNP rs1967802 due to lack of variability of genotypes in study population

Table 3-5. Association between cadmium and systolic blood pressure, stratified by number of minor alleles present and genotype

Copies of Minor Allele	Genotype	N	β (95%CI)*	p-value
HMGCR (rs5909)				
0	GG	56	***-4.61(-8.84, -0.38)***	***0.03***
1	AG	19	4.78(-5.68, 15.24)	0.32
2	AA	4	--	--
1 or 2	AG or AA	23	2.61(-5.17, 10.38)	0.48
(rs3761740)				
0	AA	47	-4.35(-9.74, 1.04)	0.11
1	CA	19	4.31(-5.98, 14.61)	0.36
2	CC	3	--	--
1 or 2	CA or CC	22	0.50(-7.27, 8.27)	0.89
VDR (rs7975232)				
0	AA	29	2.00(-4.64, 8.63)	0.52
1	CA	42	-1.38(-6.11, 3.35)	0.56
2	CC	14	-1.59(-28.26, 26.07)	0.87
1 or 2	CA or CC	56	-2.58(-6.73, 1.57)	0.22
PTH2R (rs9288393)				
0	GG	43	5.00(-1.41, 11.40)	0.12
1	CG	27	-2.63(-9.52, 4.26)	0.43
2	CC	4	--	--
1 or 2	CG or CC	31	-3.19(-9.44, 3.07)	0.30
(rs10497900)				
0	TT	23	1.20(-10.16, 12.57)	0.82
1	GT	49	-4.25(-8.87, 0.37)	0.07
2	GG	15	-7.86(-20.02, 4.31)	0.15
1 or 2	GT or GG	64	***-5.02(-8.67, -1.38)***	***0.0008***
ADD1 (rs4961)				
0	TT	43	-4.64(-10.21, 0.94)	0.10
1	GT	28	2.19(-2.92, 7.29)	0.38
2	GG	0	--	--
1 or 2	GT or GG	28	2.19(-2.92, 7.29)	0.38
ADD2 (rs3771452)				
0	GG	28	***-9.82(-17.30, -2.35)***	***0.01***
1	GA	44	0.00(-4.40, 4.40)	1.00
2	AA	13	-6.66(-25.32, 12.00)	0.34
1 or 2	GA or AA	57	-0.24(-3.89, 3.41)	0.90
ATP1A2 (rs1023421)				
0	AA	22	5.29(-4.20, 13.78)	0.25
1	GA	32	-4.13(-11.22, 2.95)	0.24
2	GG	20	-2.66(-16.03, 10.71)	0.66
1 or 2	GA or GG	52	-4.04(-8.53, 0.46)	0.90

*All models adjusted for child age at BP measurement, socioeconomic status, height, maternal smoking status, and log-transformed postnatal urinary cadmium concentration. Includes SNPs for which interaction term was significant. Some estimates could not be generated due to a small N. These cells are marked with a dashed line.

Table 3-6. Association between cadmium and diastolic blood pressure, stratified by number of minor alleles present and genotype

Copies of Minor Allele	Genotype	N	β (95%CI)	p-value
HMGCR (rs3761740)				
0	AA	47	-0.45(-4.32, 3.43)	0.82
1	CA	19	-3.07(-9.86, 3.73)	0.33
2	CC	3	--	--
1 or 2	**CA or CC**	**22**	**-5.57(-11.17, 0.03)**	**0.05**
ADD2 (rs4984)				
0	**TT**	**54**	**-2.86(-6.01, 0.29)**	**0.07**
1	TC	20	-2.91(-9.80, 3.98)	0.36
2	CC	1	--	--
1 or 2	TC or CC	21	-3.11(-9.36, 3.14)	0.29

*All models adjusted for child age at BP measurement, socioeconomic status, height, maternal smoking status, and log-transformed postnatal urinary cadmium concentration. Includes SNPs for which interaction term was significant. Some estimates could not be generated due to a small N. These cells are marked with a dashed line.

3.8 Supplemental Tables

Supplemental Table 3-1. SNPS and associated genes considered for this analysis (n=88)

SNP	Related Gene Name	Related Gene Symbol
rs5882 rs708272 rs5882	Cholesteryl Ester Transfer Protein	CETP
rs3846662 rs2303151 rs3846663 rs5909 rs3921914 rs3761740	3-Hydroxy-3-Methylglutaryl-Coenzyme A Reductase	HMGCR
rs1801177 rs328 rs268	lipoprotein lipase	LPL
rs4759275 rs715948 rs4759044 rs4367982 rs2306692 rs10876966 rs6581124 rs34574998 rs6581128 rs1800159 rs7978567 rs180016	Low Density Lipoprotein-Related Protein 1	LRP1
rs1454626	Very Low Density Lipoprotein Receptor	VLDR
rs1695 rs1138272 rs6591255 rs6591256 rs749174 rs947895	Glutathione s-transferase pi 1	GSTP1
rs7975232 rs1544410 rs10735810 rs731236	Vitamin D receptor	VDR
rs1801725 rs1042636 rs1801726	Calcium sensing receptor	CaSR
rs1801197	Calcitonin gene-related peptide-receptor	CGRP
rs6442037 rs724449	Parathyroid hormone receptor-1	PTHR1

rs7652849		
rs9288393	Parathyroid hormone receptor-2	PTHR2
rs10497900		
rs897083		
rs7121	Stimulatory G protein alpha subunit	GNAS1
rs4961	Adducin alpha	ADD1
rs2024458	Adducin beta	ADD2
rs3771452		
rs1541582		
rs2110981		
rs4852700		
rs3755375		
rs2072246		
rs2270042		
rs4984		
rs740387		
rs17698193		
rs12695877	Angiotensin II type1 receptor	AGTR1
rs4681444		
rs1492130		
rs718858		
rs1492099		
rs12721241		
rs12721331		
rs2675511		
rs12695902		
rs6801836		
rs1800766		
s5193	Angiotensin II type 2 receptor	AGTR2
rs1403543		
rs5051	Angiotensinogen preproprotein	AGT
rs4880	Superoxide dismutase 2	SOD2
rs3798215		
rs1967802		
rs1016732	Sodium Potassium ATPase alpha 2	ATP1A2
rs2753267		
rs1023421		
rs11265329		
rs16831388		
rs17846714		
rs10494336		
rs12410866		
rs2070704		

Chapter 4. Associations of Cadmium Burden with Blood Pressure and Hypertension among Young Adults: the CARDIA Study

4.1 Abstract

Introduction

Accumulating evidence has linked cadmium (Cd) exposure and burden, even at low levels, to adverse health outcomes. Findings from studies investigating associations of Cd with blood pressure and hypertension have been inconsistent and have largely relied on cross-sectional data. Further, potential effect modifiers such as sex or heavy metals (e.g., zinc, Zn) in the relationships are not clear.

Objectives

We sought to determine the association between toenail Cd in young adulthood and longitudinal changes in blood pressure, as well as risk of hypertension into middle adulthood. Additionally, we examined whether toenail Zn concentration, sex, body mass index, or smoking status were effect modifiers of these associations.

Methods

We analyzed data from 3497 participants in the Coronary Artery Risk Development in Young Adults (CARDIA) Study without prevalent hypertension, aged 18-35 at recruitment in 1985-1986. Toenail heavy metal samples were collected in year 2 (1987-1988) and Cd was assessed with collision-cell-coupled plasma mass spectrometry. Systolic blood pressure (SBP) and diastolic blood (DBP) were measured sequentially during up to eight study visits occurring over 28 years (until 2015/2016). Hypertension was defined based on the American Heart Association

clinical definition-- a blood pressure reading of ≥130 mmHg systolic blood pressure and/or ≥80 mmHg diastolic blood pressure or self-reported use of antihypertensive medication(s). Generalized estimating equations (GEE) in multi-variable adjusted models were used to estimate the association between log-transformed Cd (continuous and quartiles) and SBP/DBP. Multi-variable adjusted Cox proportional hazards models were used to estimate the association between log-transformed Cd (continuous and quartiles) and risk of hypertension. Models with interaction terms and stratified models were used to examine effect modification by Zn (μg/g), sex (male/female), body mass index (BMI, kg/m^3), physical activity (exercise units), and smoking status (never/current/former smoker).

Results

Mean age of participants was 26.8(SD=3.6) years at toenail Cd measurement. Median toenail Cd concentration was 0.0085 μg/g (IQR=0.0044, 0.0197). During the 28-year follow-up, there were 1703 hypertension cases. After adjustment for covariates, toenail Cd concentration (log-μg/g) was not associated with SBP (β=0.09, 95%CI: -0.18, 0.35), DBP (β=-0.06, 95%CI: -0.26, 0.15), or risk of hypertension (HR=1.02, 95%CI: 0.98,1.07). We observed evidence of effect modification by sex in the association of Cd with hypertension (p-value for interaction=0.05) and some suggestion of effect modification by sex in the association with DBP (p-value for interaction=0.08). Toenail Cd was marginally associated with the risk of hypertension among females (HR=1.06, 95%CI: 0.99, 1.13) but not males (HR=0.98, 95%CI: 0.90, 1.05). There was an indication of potential effect modification by smoking status in the association of Cd and DBP (p-value for interaction=0.06). We did not observe significant interactions between toenail Cd and Zn, or BMI on any of the outcomes we considered.

Conclusions

Overall, we did not find associations of toenail Cd with longitudinal BP measures or hypertension among adults. We found some evidence of effect modification by sex in associations of toenail Cd with hypertension and DBP, and of effect modification by smoking status in associations of toenail Cd with DBP. Associations of toenail Cd with hypertension were marginally significant among females but not males. Findings of this study contribute to the understanding of sex-specific differences in Cd toxicity and hypertension risk.

4.2 Introduction

Cadmium (Cd) is a ubiquitous, toxic heavy metal. In the general population, exposure is mainly through diet and tobacco smoking (Jarup et al., 1998; "Substance Priority List | ATSDR," 2017). Higher Cd body burden, even at low levels, has been related to high blood pressure and hypertension, though results have been inconsistent across studies (Gallagher & Meliker, 2010; Martins et al., 2021). Mechanisms for this association have not been postulated and are largely based on animal studies. One mechanism suggests that Cd depletes the antioxidant glutathione, inducing oxidative stress, a key component of the pathogenesis of hypertension (Wolf & Baynes, 2007; Yiin et al., 1999). Cd-induced hypertension may also result from kidney damage, and subsequent salt retention and volume overload in blood vessels (Satarug, Nishijo, Lasker, Edwards, & Moore, 2006).

While some epidemiologic studies found significant positive associations between Cd and BP (An et al., 2017; Franceschini et al., 2017; Satarug et al., 2005; Tellez-Plaza et al., 2008), other studies have found no association (Mordukhovich et al., 2012; Schutte et al., 2008). Furthermore, associations of Cd with clinical hypertension have not been thoroughly studied and

studies have relied mostly on cross sectional data. Besides the inconsistencies, most studies have been cross-sectional and thus the effect of Cd on longitudinal BP and incident hypertension have not been thoroughly investigated. Further, the role of potential effect modifiers of the associations, such as heavy metals (e.g., Zn), is not clear.

Heavy metals like Zn may be important effect modifiers by antagonizing Cd toxicity (D. Zhang et al., 2019). These metals compete with Cd for metallothionein (MT), proteins with a high affinity for metal ions while playing an important role in heavy metal detoxification and essential element absorption (Brzoska & Moniuszko-Jakoniuk, 2001; Powell, 2000; Ruttkay-Nedecky et al., 2013). Other factors such as sex and body mass index (BMI) have linked to differential Cd absorption and toxicity (Tinkov et al., 2017; Vahter, Akesson, Lidén, Ceccatelli, & Berglund, 2007) as well as blood pressure regulation and incident hypertension (Gillis & Sullivan, 2016; Ji et al., 2020; Sandberg & Ji, 2012). Additionally, smoking is an established risk factor for hypertension and is associated with increased Cd body burden, due to the high uptake of Cd by tobacco plants (S. V. Adams & P. A. Newcomb, 2014; Gao, Shi, & Wang, 2017; Satarug & Moore, 2004). Some research suggestions that regular physical activity reduces oxidative stress (Cui et al., 2016; Simioni et al., 2018). A likely mechanism of Cd toxicity is through increasing oxidative stress, and plausibly physical activity could mitigate these harmful effects (Wolf & Baynes, 2007; Yiin et al., 1999). Therefore, these factors can play significant roles in the relationships between Cd, blood pressure, and risk of hypertension.

We sought to determine the association between toenail Cd in young adulthood and longitudinal changes in blood pressure, as well as risk of hypertension among participants of the Coronary Artery Risk Development in Young Adults (CARDIA) Study and the CARDIA Trace

Element sub study. Secondarily, we tested whether sex, toenail Zn concentration, BMI, physical smoking status, and physical activity are effect modifiers of these associations.

4.3 Methods

Study Setting and Study Population

The Coronary Artery Risk Development in Young Adults (CARDIA) study is a multi-center, longitudinal study, designed to examine cardiovascular risk factors in a multi-ethnic population. Detailed methods for recruitment and data collection have been published previously (Friedman et al., 1988). Briefly, CARDIA was initiated in 1985 at four centers in the United States—Birmingham, AL; Chicago, IL; Minneapolis, MN; and Oakland, CA. The study sample comprised of 5116 young adults aged 18-30, with roughly equal distribution of sex and ethnicity (black and white). Follow-up examinations were conducted at up to eight time points—year 2 (1987/1988), year 5(1990/1991), year 7(1992/1993), year 10(1995/1996), year 15(2000/2001), year 20(2005/2006), year 25(2010/2011), and year 30(2015/2016). Participation ranged from 90% at year 2 to 71% at year 30.

The current analysis is based on data from the CARDIA Trace Elements sub-study which was designed to evaluate links between trace elements and cardiovascular disease (CVD) risk factors in young adults. Heavy metal concentrations were measured in toenail clippings collected from 94.3% attending participants (4362/4624) at the year 2 (Y2) study visit (Yang et al., 2019). After exclusion of participants with unavailable data on Cd (N=11), prevalent hypertension (N=865), or both (N=2), our study included N=3497 participants for whom toenail Cd concentration was available and who were without prevalent hypertension at Y2. Informed

consent was obtained from all participants and the ethics and research committees of all participating institutions approved study protocols.

Data Collection

At each study visit, trained study staff performed a physical examination and administered a series of structured questionnaires to obtain socio-demographic and anthropometric data. Information collected included SBP/DBP, medication use, educational attainment, health risk behaviors (i.e., smoking and drinking), BMI, diet, and psychosocial indicators. For our analyses, we considered Y2 as baseline since it was during this examination that Cd and Zn were measured.

Assessment of Toenail Cadmium and Zinc

Toenail clippings are considered a valid measure of heavy metal exposure, reflecting exposure in the past 3-12 months (Grashow et al., 2014; He, 2011). Toenail clippings from all 10 toes were collected during the Y2 clinical examination using stainless steel clippers. Toenails were stored at room temperature until processing, then washed in a sonicator with deionized water. Toenail samples were analyzed at the University of Missouri-Columbia Research Reactor Center. Toenail Cd was measured using collision-cell inductively coupled-plasma mass-spectrometry (CC-ICP-MIS). Instrumental neutron-activation analysis (INAA) was used for Zn. (T.-p. Cheng, Morris, S.R., Sate, & Baskett, 1994; Li et al., 2021). Processing of toenails was done in random order by blinded laboratory personnel. We retained one toenail Cd value that was below the limit of detection and a single value of Zn (1060 μg/g) was deemed implausible and changed to a missing for our analyses.

Blood Pressure Outcomes

Blood pressure (BP) was measured using a standardized protocol at each visit (Y2, Y5, Y7, Y10, Y15, Y20, Y25, Y30). SBP and DBP (in mmHg) were collected by trained study staff at each follow-up visit. Prior to BP measurement, participants were asked to sit quietly for five minutes with their feet flat on the floor and right arm circumference was measured to ensure proper cuff size. Three readings were taken, at least 30 seconds apart, and rounded to the nearest whole number. In our analysis, we took the average of the second and third SBP and DBP readings. BP was recorded using a Hawksley random-zero sphygmomanometer (W.A. Baum Co., Copiague, NY) in years 2, 5, 7, 10, and 15. In years 25 and 30, an automated BP measurement monitor was used. To ensure consistency between measurements in earlier and later years, Y25 and Y30 values were calibrated to a random-zero sphygmomanometer, based on measurements from both instruments in a subset (N=906) of CARDIA participants (Jacobs et al., 2012).

We defined hypertension based on the 2017 American Heart Association (AHA) guidelines and considered participants as hypertensive at a given study visit if they had SBP ≥130 mmHg and/or DBP ≥80 mmHg (Whelton et al., 2018). Additionally, we considered a patient as hypertensive if they self-reported use of antihypertensive medication(s), regardless of their BP reading. Once a participant was defined as hypertensive at a given study visit, we defined them as hypertensive for all subsequent visits. The AHA changed their clinical guidelines for treatment of hypertension in 2017, after all CARDIA study visits occurred. Thus, we conducted a sensitivity analysis, repeating hypertension analyses with the former clinical definition of hypertension--SBP ≥140 mmHg and/or a DBP ≥90 mmHg or self-reported use of antihypertensive medication (Whelton et al., 2018).

Covariates

Covariates for adjustment were selected *a priori,* based on previous literature. We considered the following covariates: sex, race (black, white, Hispanic), study center (Birmingham, AL, Chicago, IL, Minneapolis, MN, Oakland, CA), BMI at Y2 (kg/m^3) smoking history at Y2 (former, current, never), alcohol use at Y2 (never, light, moderate, at risk), years of educational attainment at Y2, and exercise at Y2 (exercise units as described below).

A structured questionnaire and pre-defined criteria were used to categorize smoking status (Whooley, Boyd, Gardin, & Williams, 2002). Participants were asked if they have ever regularly smoked cigarettes, at least 5 cigarettes per week, most weeks. Those who responded never were considered "never smokers,", those who had in the past 3 months were considered "current smokers," and those who had, but not in the past 3 months, were considered "former smokers." Data collection methods in CARDIA for self-report alcohol use has been described elsewhere (Halanych et al., 2010). Gender-specific categorization of weekly drinking frequency at Y2 was based on categories suggested by the National Institute on Alcohol Abuse and Alcoholism (NIAA) (Halanych et al., 2010). Light drinking was defined as <7 drinks for men and <4 drinks for women. Moderate drinking was defined as 7-14 drinks for men and 4-7 drinks for women. At-risk drinking was defined as >14 drinks for men and >7 drinks for women. Never drinkers reported no current alcohol consumption (Halanych et al., 2010).

To quantify physical activity, we used exercise units (EU). EUs are continuous scores calculated based on the CARDIA Physical Activity History Questionnaire, a validated questionnaire that ascertains duration and intensity of activity over the past 12-months (Jacobs, Hahn, Haskell, Pirie, & Sidney, 1989). For reference, an EU score of 100 would be equivalent to

approximately 2-3 hours of vigorous physical activity per week during 6 months of the year (Xun et al., 2010).

Statistical Analysis

We calculated univariate and bivariate summary statistics for sociodemographic and anthropometric characteristics of participants at Y2 visit. Statistically significant differences by quartiles of toenail Cd were examined with one-way ANOVA tests for continuous variables and Pearson's Chi-square tests for categorical variables. Additionally, we compared these characteristics between those who did and did not attend the year 30 follow-up visit, to assess extent of selection (due to loss to follow-up) bias using the same statistical tests. Median, interquartile range (IQR), and range were calculated for toenail Cd and Zn.

Toenail Cd was modeled as continuous ($\log_{10}$ transformed to normalize the right skewed distribution) and quartile groups. Generalized estimating equations (GEE) were used to quantify relationships between toenail Cd and changes in SBP and DBP between Y2 and Y30. We assumed an identity link and an exchangeable correlation structure. Three sequential models were fit using the data: 1) a minimally adjusted model which included: sex, race, and study center; 2) a partially adjusted model which included the covariates in the minimally adjusted model plus BMI at Y2, smoking history at Y2, and alcohol use at Y2; 3) a fully adjusted model which included covariates in the partially adjusted model plus exercise at Y2, and years of education at Y2. Additionally, all GEE models were adjusted for time (in years) since Y2, to account for differences in BP changes over time. The fully adjusted model was considered as the primary model.

A Cox proportional hazards model was used to calculate the risk of hypertension associated with toenail Cd concentration. Similar, three sequential models were fit using the data as described above. A sensitivity analysis was conducted, repeating the same analyses, but using the pre-2017 clinical definition of hypertension.

Effect modification was assessed as follows. In fully adjusted models, effect modification by toenail Zn concentration, sex, BMI at Y2, and smoking status at Y2 were assessed using models that included respective multiplicative interaction terms of the exposure (continuous Cd) and effect modifier, along with the other adjustment variables. Statistical significance of multiplicative interactions was assessed based on a Wald-based test. If the interaction term was significant ($p<0.05$), we conducted a stratified analysis, by fitting fully adjusted models among strata defined by respective effect modifiers.

Statistical significance was determined using the p-value < 0.05 cutoff. All analyses were carried out using Stata 14.0 (Stata, 2015)

4.4 Results

Socio-demographic and anthropometric characteristics of the analytic study population at Y2 are shown in **Table 4.1**. Our sample included 1408 males (40.3%) and 2089 females (59.7%); it comprised of 53.3% white, 46.4% black, and 0.4% Hispanic participants; and. The mean age of participants at baseline was 26.8years (SD=3.6). Twenty-nine (N=1012) percent of the population reported current smoking, 14.3% (N=498) were former smokers, and 56.7% (N=1980) were never-smokers. Factors associated with being in a lower quartile of toenail Cd exposure included male sex ($p<0.001$), higher age ($p<0.001$), more years of education ($p<0.001$), white race ($p<0.001$), living in Birmingham or Oakland ($p<0.001$), being a never-smoker

(p=0.04) and being a light or moderate drinker (p=0.017). Additionally, those in the highest quartile of toenail Cd concentrations had a higher Zn concentration (p<0.001), a slightly higher SBP (p=0.004), but a slightly lower DBP (p=0.002). BMI at Y2 (p=0.17), and physical activity (p=0.16) did not vary significantly between Cd quartiles. Median Cd concentration was 0.0085 μg/g (IQR=0.004, 0.0197) and mean Zn concentration was 55.4 μg/g (SD=21.5).

In the fully adjusted GEE model, toenail Cd (in a log-μg/g) at Y2 was not associated with SBP (β=0.09, 95%CI: -0.18, 0.35) or DBP (β=-0.06, 95%CI: -0.26, 0.15) **(Table 4.2**). SBP and DBP of participants in the first quartile of Cd were not different to SBP and DBP of participants in quartiles 2, 3, or 4**(Table 4.2).** Similarly, Cd was not associated with risk of hypertension (HR=1.02, 95%CI: 0.98, 1.07), nor did the risk of hypertension differ when comparing participants in the first quartile of Cd to participants in quartiles 2,3, or 4**(Table 4.3).** When the definition of hypertension was changed to the pre-2017 clinical definition, results of analyses examining Cd and risk of hypertension associations were similar (HR=0.98, 95% CI=0.93, 1.04) **(Supplemental Table 4.1**). **Supplemental Table 4.2** compares baseline characteristics of participants who attended the Y30 visit to those who did not, showing that most covariates differed between these groups. Loss-to-follow-up was associated with being male, black, and a smoker.

In models that included interaction terms, we did find that, after adjustment for covariates, there was a marginally significant interaction between sex and Cd on hypertension risk (p=0.05) **(Table 4.4)**. In stratified analyses, the positive association of Cd with the risk of hypertension was marginally significant among females (HR=1.06, 95%CI: 0.99, 1.13) but not males (HR=0.98, 95%CI: 0.90, 1.05) **(Table 4.5).** While interaction between smoking and Cd with DBP was marginally insignificant (p=0.06) **(Table 4.4),** there were no significant

associations between Cd and DBP within any smoking stratum **(Table 4.5)**. There was no indication of interaction by Zn status, BMI, or physical activity **(Table 4.4)** (all interaction p-values > 0.10)**.**

4.5 Discussion

In this large prospective cohort study of adults free of hypertension at baseline, we found no significant association between toenail Cd concentration and longitudinal differences in BP or risk of hypertension. However, we found evidence for effect modification by sex of the association of between toenail Cd and risk of hypertension and DBP. The positive association of Cd with hypertension was marginally significant among females, but not males. We also observed some evidence of effect modification by smoking status in the association of toenail Cd and DBP. We did not observe effect modification of Cd and longitudinal BP changes by toenail Zn, BMI, or physical activity.

Median toenail Cd concentration in our population was 0.0085 μg/g, which was relatively low compared to that in other populations. Median concentrations in other non-occupationally exposed populations ranged from 0.005 μg/g to 0.048 μg/g (Mordukhovich et al., 2012; Platz et al., 2002; Vinceti et al., 2007; White, O'Brien, Jackson, & Karagas, 2018). Our results are consistent with some, but not all studies of Cd and BP. Our results are similar to a cross-sectional analysis of toenail Cd and BP in older male United States Veterans that reported no association of Cd with SBP (β=0.22, 95% CI=-0.10, 0.54) or DBP (β=0.12, 95% CI=-0.05, 0.28). On the other hand, several epidemiologic studies have found statistically significant associations of Cd with higher BP (An et al., 2017; Franceschini et al., 2017; Satarug et al., 2005; Tellez-Plaza et

al., 2008). For example, in older Native Americans living in the Southwest United States, log-UCd was associated with higher SBP (β=1.64, p=0.002). However, none of these studies relied on toenail Cd as a biomarker for exposure, instead using blood Cd or UCd. Importantly, no previous studies, to our knowledge have examined Cd in relation to longitudinal changes in BP or risk of incident hypertension.

We observed a marginally significant positive association of Cd with the risk of hypertension among females (HR=1.06, 95%CI: 0.99, 1.13) but not males (HR=0.98, 95%CI: 0.90, 1.05). This exploratory finding suggests that females may be more vulnerable to the toxic impacts of Cd. Compared to men living in similar areas, women tend to have a higher Cd concentrations in blood, urine, and kidney cortex, suggesting that women may be more susceptible to the toxic effects of Cd (Vahter et al., 2007). Proposed mechanisms for sex differences in Cd toxicity include: genetic factors (e.g. such as higher expression of MT genes) (Björkman, Vahter, & Pedersen, 2000; Kwon et al., 2007), lower iron body stores leading to increased intestinal absorption of Cd (Vahter et al., 2007), and iron deficiency during pregnancy that facilitates Cd absorption (Akesson et al., 2002).

We also observed potential effect modification by smoking status in associations of Cd with DBP. Cd body burden is generally much higher in smokers and smoking is an independent risk factor for hypertension (S. V. Adams & P. A. Newcomb, 2014; W. Wang et al., 2006). While not significant in any smoking strata, the point estimate showed a positive association in never-smokers (β=0.14, 95% CI=-0.13, 0.41), but inverse associations in former smokers (β=-0.15, 95% CI=-0.72, 0.42) and current smokers (β=-0.25, 95% CI=-0.63, 0.13). Similarly, other studies found stronger associations of Cd and BP among never smokers compared to ever smokers (Franceschini et al., 2017; Tellez-Plaza et al., 2008). This could be explained by

potential differences in sources and frequency of Cd exposure, co-exposures related to smoking status (unadjusted confounding), or random variability(Tellez-Plaza et al., 2008).

A key strength of this study is the utilization of a large, diverse existing cohort specifically designed to examine cardiovascular risk development including BP and hypertension. Therefore, a standardized protocol was followed to obtain BP measurements, and measurements were taken consistently at each study visit. Additionally, our relatively large sample size and large number of cases of hypertension allowed us to examine potential effect modifiers with adequate statistical power. Importantly, unlike previous studies of Cd, BP or hypertension that have relied on cross-sectional data, we were able to examine BP and risk of hypertension longitudinally. The large and comprehensive nature of this dataset enabled us to control for many important confounders, including lifestyle factors (e.g., smoking, alcohol use, and physical activity), as well as demographic factors (e.g., sex, race, and education).

Our study has several limitations. First, toenail Cd was measured at a single time point in Y2, and we were consequently unable to account for changes in Cd exposure over time. Likewise, effect modifiers (Zn, BMI, smoking status, physical activity) were only considered at Y2, and likely changed during 28 years of follow-up. Additionally, while toenail Cd reflects exposure over the past 3-12 months, it is unclear whether toenail Cd concentrations are an accurate measure of chronic Cd exposure or Cd burden (Grashow et al., 2014). Due to its long half-life, Cd accumulates in the kidney, likely continuously producing toxic effects on the kidneys and blood vessels long after initial exposure (Jarup et al., 1998). Additionally, there is concern of selection bias due to loss-to-follow-up, suggested by the differences in baseline characteristics between those who dropped out of the study and those who did not. Compared to individuals who were present for the Y30 follow-up, those not present were more likely to be

male, black, and a smoker—all risk factors for hypertension and higher BP (W. Wang et al., 2006). Next, the clinical definition of hypertension changed in 2017, lowering the cut-point for hypertension from 140/90 mmHg to 130/80 mmHg. Clearly this had an impact on prescribing practices for anti-hypertensive medication, which was part of our definition for hypertension. However, in sensitivity analyses that used the pre-2017 definition of hypertension, results were very similar in magnitude. Although BP was measured at each study visit, we relied on measurements from a specific day and time to reflect BP over the entire time frame between study visits. This meant that we did not have highly granular information about the timing of hypertension development. However, we would expect any misclassification of the outcome to be non-differential with respect to toenail Cd concentration, and therefore bias effect estimates toward the null. Future studies could utilize marginal structural models (MSM) to address measurement error in BP measurements (Kyle, Moodie, Klein, & Abrahamowicz, 2016; Robins, Hernán, & Brumback, 2000). Finally, while we adjusted for a number of confounders, the potential for unmeasured or residual confounding exists.

Our findings suggest that associations of Cd and hypertension may be limited to females. This adds to the understanding of mechanisms of Cd toxicity and identifies subgroups most susceptible to the cardiovascular toxicity of Cd. If replicated, our findings are a tool in targeting BP screening and treatment.

4.6 References

(NCD-RisC), N. R. F. C. (2021). Worldwide trends in hypertension prevalence and progress in treatment and control from 1990 to 2019: a pooled analysis of 1201 population-representative studies with 104 million participants. *Lancet*. doi:10.1016/S0140-6736(21)01330-1

Adams, S., & Newcomb, P. (2014). Cadmium blood and urine concentrations as measures of exposure: NHANES 1999–2010. *Journal of Exposure Science and Environmental Epidemiology, 24*(2), 163-170.

Adams, S. V., & Newcomb, P. A. (2014). Cadmium blood and urine concentrations as measures of exposure: NHANES 1999-2010. *J Expo Sci Environ Epidemiol, 24*(2), 163-170. doi:10.1038/jes.2013.55

Akesson, A., Berglund, M., Schütz, A., Bjellerup, P., Bremme, K., & Vahter, M. (2002). Cadmium exposure in pregnancy and lactation in relation to iron status. *Am J Public Health, 92*(2), 284-287. doi:10.2105/ajph.92.2.284

Akesson, A., Stål, P., & Vahter, M. (2000). Phlebotomy increases cadmium uptake in hemochromatosis. *Environ Health Perspect, 108*(4), 289-291. doi:10.1289/ehp.108-1638026

Almenara, C. C., Broseghini-Filho, G. B., Vescovi, M. V., Angeli, J. K., Faria, T. e. O., Stefanon, I., . . . Padilha, A. S. (2013). Chronic cadmium treatment promotes oxidative stress and endothelial damage in isolated rat aorta. *PLoS One, 8*(7), e68418. doi:10.1371/journal.pone.0068418

An, H. C., Sung, J. H., Lee, J., Sim, C. S., Kim, S. H., & Kim, Y. (2017). The association between cadmium and lead exposure and blood pressure among workers of a smelting industry: a cross-sectional study. *Ann Occup Environ Med, 29*, 47. doi:10.1186/s40557-017-0202-z

Asociación Mexicana de Agencias de Inteligencia de Mercado y Opinión (AMAI) Retrieved from https://nse.amai.org/niveles-socio-economicos-amai/

Barker, D. J., Osmond, C., Golding, J., Kuh, D., & Wadsworth, M. E. (1989). Growth in utero, blood pressure in childhood and adult life, and mortality from cardiovascular disease. *BMJ, 298*(6673), 564-567.

Berntein, I., Mongeon, J., Badger, G., Solomon, L., Heil, S., & Higgins, S. (2005). Maternal smoking and its association with birth weight. *Obstetrics and Gynecology, 106*(5), 986-991.

Bianchi, G., Ferrari, P., & Staessen, J. A. (2005). Adducin polymorphism: detection and impact on hypertension and related disorders. *Hypertension, 45*(3), 331-340. doi:10.1161/01.HYP.0000156497.39375.37

Björkman, L., Vahter, M., & Pedersen, N. L. (2000). Both the environment and genes are important for concentrations of cadmium and lead in blood. *Environ Health Perspect, 108*(8), 719-722. doi:10.1289/ehp.108-1638287

Borgi, L., Muraki, I., Satija, A., Willett, W. C., Rimm, E. B., & Forman, J. P. (2016). Fruit and Vegetable Consumption and the Incidence of Hypertension in Three Prospective Cohort Studies. *Hypertension, 67*(2), 288-293. doi:10.1161/HYPERTENSIONAHA.115.06497

Brzoska, M. M., & Moniuszko-Jakoniuk, J. (2001). Interactions between cadmium and zinc in the organism. *Food and Chemical Toxicology, 39*(10), 967-980. doi:Doi 10.1016/S0278-6915(01)00048-5

Bulka, C. M., Mabila, S. L., Lash, J. P., Turyk, M. E., & Argos, M. (2017). Arsenic and Obesity: A Comparison of Urine Dilution Adjustment Methods. *Environ Health Perspect, 125*(8), 087020. doi:10.1289/EHP1202

Burganowski, R., Vahter, M., Queirolo, E. I., Peregalli, F., Baccino, V., Barcia, E., . . . Kordas, K. (2019). A cross-sectional study of urinary cadmium concentrations in relation to dietary intakes in Uruguayan school children. *Sci Total Environ, 658*, 1239-1248. doi:10.1016/j.scitotenv.2018.12.220

Cantonwine, D., Hu, H., Téllez-Rojo, M. M., Sánchez, B. N., Lamadrid-Figueroa, H., Ettinger, A. S., . . . Wright, R. O. (2010). HFE gene variants modify the association between maternal lead burden

and infant birthweight: a prospective birth cohort study in Mexico City, Mexico. *Environ Health, 9*, 43. doi:10.1186/1476-069X-9-43

Caterina Vacchi-Suzzi, D. K., James Harrington, Keith Levine, and Jaymie R., & Meliker. (2016). Is Urinary Cadmium a Biomarker of Long-term Exposure in Humans? A Review. *Curr Environ Health Rep, 3*(4), 450-458.

Chatzi, L., Ierodiakonou, D., Margetaki, K., Vafeiadi, M., Chalkiadaki, G., Roumeliotaki, T., . . . Kippler, M. (2019). Associations of Prenatal Exposure to Cadmium With Child Growth, Obesity, and Cardiometabolic Traits. *Am J Epidemiol, 188*(1), 141-150. doi:10.1093/aje/kwy216

Chen, Y. C., Chen, Y. D., Li, X., Post, W., Herrington, D., Polak, J. F., . . . Taylor, K. D. (2009). The HMG-CoA reductase gene and lipid and lipoprotein levels: the multi-ethnic study of atherosclerosis. *Lipids, 44*(8), 733-743. doi:10.1007/s11745-009-3314-6

Chen, Z., Myers, R., Wei, T., Bind, E., Kassim, P., Wang, G., . . . Wang, X. (2014). Placental transfer and concentrations of cadmium, mercury, lead, and selenium in mothers, newborns, and young children. *J Expo Sci Environ Epidemiol, 24*(5), 537-544. doi:10.1038/jes.2014.26

Cheng, L., Zhang, B., Zheng, T., Hu, J., Zhou, A., Bassig, B. A., . . . Li, Y. (2017). Critical Windows of Prenatal Exposure to Cadmium and Size at Birth. *Int J Environ Res Public Health, 14*(1). doi:10.3390/ijerph14010058

Cheng, T.-p., Morris, J. S., S.R., K., Sate, V. L., & Baskett, C. K. (1994). The analysis of human nails for 24 elements via k0 and cyclic neutron activation analysis. *Nuclear Instruments and Methods in Physics Research Section A: Accelerators, Spectrometers, Detectors and Associated Equipment, 352*, 457–460.

Choong, G., Liu, Y., & Templeton, D. M. (2014). Interplay of calcium and cadmium in mediating cadmium toxicity. *Chem Biol Interact, 211*, 54-65. doi:10.1016/j.cbi.2014.01.007

Ciesielski, T., Weuve, J., Bellinger, D. C., Schwartz, J., Lanphear, B., & Wright, R. O. (2012). Cadmium exposure and neurodevelopmental outcomes in U.S. children. *Environ Health Perspect, 120*(5), 758-763. doi:10.1289/ehp.1104152

Crispi, F., Miranda, J., & Gratacós, E. (2018). Long-term cardiovascular consequences of fetal growth restriction: biology, clinical implications, and opportunities for prevention of adult disease. *Am J Obstet Gynecol, 218*(2S), S869-S879. doi:10.1016/j.ajog.2017.12.012

Cui, X., Ohtsu, M., Mise, N., Ikegami, A., Mizuno, A., Sakamoto, T., . . . Kayama, F. (2016). Heavy metal exposure, in combination with physical activity and aging, is related with oxidative stress in Japanese women from a rural agricultural community. *Springerplus, 5*(1), 885. doi:10.1186/s40064-016-2430-z

Dobolyi, A., Dimitrov, E., Palkovits, M., & Usdin, T. B. (2012). The neuroendocrine functions of the parathyroid hormone 2 receptor. *Front Endocrinol (Lausanne), 3*, 121. doi:10.3389/fendo.2012.00121

Ehret, G. B., Ferreira, T., Chasman, D. I., Jackson, A. U., Schmidt, E. M., Johnson, T., . . . Consortium, W. T. C. C. (2016). The genetics of blood pressure regulation and its target organs from association studies in 342,415 individuals. *Nat Genet, 48*(10), 1171-1184. doi:10.1038/ng.3667

Falkner, B. (2002). Birth weight as a predictor of future hypertension. *American Journal of Hypertension, 15*(S2), 43S-45S.

Fan, G., Du, G., Li, H., Lin, F., Sun, Z., Yang, W., . . . Zhou, F. (2014). The effect of the hemochromatosis (HFE) genotype on lead load and iron metabolism among lead smelter workers. *PLoS One, 9*(7), e101537. doi:10.1371/journal.pone.0101537

Ferrandi, M., Salardi, S., Tripodi, G., Barassi, P., Rivera, R., Manunta, P., . . . Karlish, S. J. (1999). Evidence for an interaction between adducin and Na(+)-K(+)-ATPase: relation to genetic hypertension. *Am J Physiol, 277*(4), H1338-1349. doi:10.1152/ajpheart.1999.277.4.H1338

Fisher, S. B., & Perrier, N. D. (2020). Primary hyperparathyroidism and hypertension. *Gland Surg, 9*(1), 142-149. doi:10.21037/gs.2019.10.21

Flynn, J. T., Kaelber, D. C., Baker-Smith, C. M., Blowey, D., Carroll, A. E., Daniels, S. R., . . . Management, S. S. (2017). Clinical Practice Guideline for Screening and Management of High Blood Pressure in Children and Adolescents. *Pediatrics, 140*(3). doi:ARTN e20171904

10.1542/peds.2017-1904

Franceschini, N., Fry, R. C., Balakrishnan, P., Navas-Acien, A., Oliver-Williams, C., Howard, A. G., . . . Tellez-Plaza, M. (2017). Cadmium body burden and increased blood pressure in middle-aged American Indians: the Strong Heart Study. *J Hum Hypertens, 31*(3), 225-230. doi:10.1038/jhh.2016.67

Freitas, R. N., Khaw, K. T., Wu, K., Bowman, R., Jeffery, H., Luben, R., . . . Bingham, S. A. (2009). A HMGCR polymorphism is associated with relations between blood pressure and urinary sodium and potassium ratio in the Epic-Norfolk Study. *J Am Soc Hypertens, 3*(4), 238-244. doi:10.1016/j.jash.2009.05.001

Friedman, G. D., Cutter, G. R., Donahue, R. P., Hughes, G. H., Hulley, S. B., Jacobs, D. R., . . . Savage, P. J. (1988). CARDIA: study design, recruitment, and some characteristics of the examined subjects. *J Clin Epidemiol, 41*(11), 1105-1116. doi:10.1016/0895-4356(88)90080-7

Friesen, J. A., & Rodwell, V. W. (2004). The 3-hydroxy-3-methylglutaryl coenzyme-A (HMG-CoA) reductases. *Genome Biol, 5*(11), 248. doi:10.1186/gb-2004-5-11-248

Gallagher, C. M., & Meliker, J. R. (2010). Blood and urine cadmium, blood pressure, and hypertension: a systematic review and meta-analysis. *Environ Health Perspect, 118*(12), 1676-1684. doi:10.1289/ehp.1002077

Gao, K., Shi, X., & Wang, W. (2017). The life-course impact of smoking on hypertension, myocardial infarction and respiratory diseases. *Sci Rep, 7*(1), 4330. doi:10.1038/s41598-017-04552-5

Garner, R. E., & Levallois, P. (2017). Associations between cadmium levels in blood and urine, blood pressure and hypertension among Canadian adults. *Environ Res, 155*, 64-72. doi:10.1016/j.envres.2017.01.040

Genchi, G., Sinicropi, M. S., Lauria, G., Carocci, A., & Catalano, A. (2020). The Effects of Cadmium Toxicity *Int J Environ Res Public Health, 17*(11), 3782. doi:10.3390/ijerph17113782

Gilligan, D. M., Lozovatsky, L., & Silberfein, A. (1997). Organization of the human beta-adducin gene (ADD2). *Genomics, 43*(2), 141-148. doi:10.1006/geno.1997.4802

Gillis, E. E., & Sullivan, J. C. (2016). Sex Differences in Hypertension: Recent Advances. *Hypertension, 68*(6), 1322-1327. doi:10.1161/HYPERTENSIONAHA.116.06602

Goettsch, C., Iwata, H., & Aikawa, E. (2014). Parathyroid hormone: critical bridge between bone metabolism and cardiovascular disease. *Arterioscler Thromb Vasc Biol, 34*(7), 1333-1335. doi:10.1161/ATVBAHA.114.303637

Grashow, R., Zhang, J. M., Fang, S. C., Weisskopf, M. G., Christiani, D. C., & Cavallari, J. M. (2014). Toenail Metal Concentration as a Biomarker of Occupational Welding Fume Exposure. *Journal of Occupational and Environmental Hygiene, 11*(6), 397-405. doi:10.1080/15459624.2013.875182

Grau-Perez, M., Pichler, G., Galan-Chilet, I., Briongos-Figuero, L. S., Rentero-Garrido, P., Lopez-Izquierdo, R., . . . Tellez-Plaza, M. (2017). Urine cadmium levels and albuminuria in a general population from Spain: A gene-environment interaction analysis. *Environ Int, 106*, 27-36. doi:10.1016/j.envint.2017.05.008

Halanych, J. H., Safford, M. M., Kertesz, S. G., Pletcher, M. J., Kim, Y. I., Person, S. D., . . . Kiefe, C. I. (2010). Alcohol consumption in young adults and incident hypertension: 20-year follow-up from the Coronary Artery Risk Development in Young Adults Study. *Am J Epidemiol, 171*(5), 532-539. doi:10.1093/aje/kwp417

Hao, G., Wang, X., Treiber, F. A., Harshfield, G., Kapuku, G., & Su, S. (2017). Blood Pressure Trajectories From Childhood to Young Adulthood Associated With Cardiovascular Risk: Results From the 23-Year Longitudinal Georgia Stress and Heart Study. *Hypertension, 69*(3), 435-442. doi:10.1161/HYPERTENSIONAHA.116.08312

Hawkesworth S, W. Y., Kippler M, Fulford AJC, Arifeen SE, Persson L, Moore SE, Vahter M. (2012). Early exposure to toxic metals has a limited effect on blood pressure or kidney function in later childhood, rural Bangladesh. *International Journal of Epidemiology*(42), 176-185.

He, K. (2011). Trace elements in nails as biomarkers in clinical research. *Eur J Clin Invest, 41*(1), 98-102. doi:10.1111/j.1365-2362.2010.02373.x

Hermann, M., Flammer, A., & Lüscher, T. F. (2006). Nitric oxide in hypertension. *J Clin Hypertens (Greenwich), 8*(12 Suppl 4), 17-29. doi:10.1111/j.1524-6175.2006.06032.x

Howe, C. G., Margetaki, K., Vafeiadi, M., Roumeliotaki, T., Karachaliou, M., Kogevinas, M., . . . Chatzi, L. (2021). Prenatal metal mixtures and child blood pressure in the Rhea mother-child cohort in Greece. *Environ Health, 20*(1), 1. doi:10.1186/s12940-020-00685-9

Jacobs, D. R., Hahn, L. P., Haskell, W. L., Pirie, P., & Sidney, S. (1989). Validity and Reliability of Short Physical Activity History: Cardia and the Minnesota Heart Health Program. *J Cardiopulm Rehabil, 9*(11), 448-459. doi:10.1097/00008483-198911000-00003

Jacobs, D. R., Yatsuya, H., Hearst, M. O., Thyagarajan, B., Kalhan, R., Rosenberg, S., . . . Duprez, D. A. (2012). Rate of decline of forced vital capacity predicts future arterial hypertension: the Coronary Artery Risk Development in Young Adults Study. *Hypertension, 59*(2), 219-225. doi:10.1161/HYPERTENSIONAHA.111.184101

Jaitovich, A., & Bertorello, A. M. (2010). Salt, Na+,K+-ATPase and hypertension. *Life Sci, 86*(3-4), 73-78. doi:10.1016/j.lfs.2009.10.019

Jan, A., Azam, M., Siddiqui, K., Ali, A., Choi, I., & Haq, A. (2015). Heavy Metals and Human Health: Mechanistic Insight into Toxicity and Counter Defense System of Antioxidants. *Int J Mol Sci, 16*(12), 29592-29630.

Jaquet, D., Leger, J., Levy-Marchal, C., Oury, J. F., & Czernichow, P. (1998). Ontogeny of leptin in human fetuses and newborns: effect of intrauterine growth retardation on serum leptin concentrations. *J Clin Endocrinol Metab, 83*(4), 1243-1246. doi:10.1210/jcem.83.4.4731

Jarup, L., Berglund, M., Elinder, C. G., Nordberg, G., & Vahter, M. (1998). Health effects of cadmium exposure--a review of the literature and a risk estimate. *Scand J Work Environ Health, 24 Suppl 1*, 1-51.

Jhun, M. A., Hu, H., Schwartz, J., Weisskopf, M. G., Nie, L. H., Sparrow, D., . . . Park, S. K. (2015). Effect modification by vitamin D receptor genetic polymorphisms in the association between cumulative lead exposure and pulse pressure: a longitudinal study. *Environ Health, 14*, 5. doi:10.1186/1476-069X-14-5

Ji, H., Kim, A., Ebinger, J. E., Niiranen, T. J., Claggett, B. L., Bairey Merz, C. N., & Cheng, S. (2020). Sex Differences in Blood Pressure Trajectories Over the Life Course. *JAMA Cardiol, 5*(3), 19-26. doi:10.1001/jamacardio.2019.5306

Johnston, J. E., Valentiner, E., Maxson, P., Miranda, M. L., & Fry, R. C. (2014). Maternal cadmium levels during pregnancy associated with lower birth weight in infants in a North Carolina cohort. *PLoS One, 9*(10), e109661. doi:10.1371/journal.pone.0109661

Joneidi, Z., Mortazavi, Y., Memari, F., Roointan, A., Chahardouli, B., & Rostami, S. (2019). The impact of genetic variation on metabolism of heavy metals: Genetic predisposition? *Biomed Pharmacother, 113*, 108642. doi:10.1016/j.biopha.2019.108642

Jones, C. T., & Parer, J. T. (1983). The effect of alterations in placental blood flow on the growth of and nutrient supply to the fetal guinea-pig. *J Physiol, 343*, 525-537.

Juarez-Rojas, J. G., Cardoso-Saldana, G. C., Posadas-Sanchez, R., Medina-Urrutia, A. X., Yamamoto-Kimura, L., & Posadas-Romero, C. (2008). Blood pressure and associated cardiovascular risk factors in adolescents of Mexico City. *Arch Cardiol Mex, 78*(4), 384-391.

Kardia, S. L., Sun, Y. V., Hamon, S. C., Barkley, R. A., Boerwinkle, E., & Turner, S. T. (2007). Interactions between the adducin 2 gene and antihypertensive drug therapies in determining blood pressure in people with hypertension. *BMC Med Genet, 8*, 61. doi:10.1186/1471-2350-8-61

Kayaaltı, Z., Aliyev, V., & Söylemezoğlu, T. (2011). The potential effect of metallothionein 2A -5A/G single nucleotide polymorphism on blood cadmium, lead, zinc and copper levels. *Toxicol Appl Pharmacol, 256*(1), 1-7. doi:10.1016/j.taap.2011.06.023

Khansakorn, N., Wongwit, W., Tharnpoophasiam, P., Hengprasith, B., Suwannathon, L., Chanprasertyothin, S., . . . Sirivarasai, J. (2012). Genetic variations of glutathione s-transferase influence on blood cadmium concentration. *J Toxicol, 2012*, 356126. doi:10.1155/2012/356126

Kippler, M., Hoque, A. M., Raqib, R., Ohrvik, H., Ekstrom, E. C., & Vahter, M. (2010). Accumulation of cadmium in human placenta interacts with the transport of micronutrients to the fetus. *Toxicol Lett, 192*(2), 162-168. doi:10.1016/j.toxlet.2009.10.018

Kippler, M., Hoque, A. M., Raqib, R., Ohrvik, H., Ekström, E. C., & Vahter, M. (2010). Accumulation of cadmium in human placenta interacts with the transport of micronutrients to the fetus. *Toxicol Lett, 192*(2), 162-168. doi:10.1016/j.toxlet.2009.10.018

Kippler, M., Nermell, B., Hamadani, J., Tofail, F., Moore, S., & Vahter, M. (2010). Burden of cadmium in early childhood: longitudinal assessment of urinary cadmium in rural Bangladesh. *Toxicol Lett, 198*(1), 20-25. doi:10.1016/j.toxlet.2010.04.029

Kippler, M., Tofail, F., Gardner, R., Rahman, A., Hamadani, J., Bottai, M., & Vahter, M. (2012). Maternal cadmium exposure during pregnancy and size at birth: a prospective cohort study. *Environ Health Perspect, 120*(2), 284-289.

Kita, K., Miura, N., Yoshida, M., Yamazaki, K., Ohkubo, T., Imai, Y., & Naganuma, A. (2006). Potential effect on cellular response to cadmium of a single-nucleotide A --> G polymorphism in the promoter of the human gene for metallothionein IIA. *Hum Genet, 120*(4), 553-560. doi:10.1007/s00439-006-0238-6

Kwon, C. S., Kountouri, A. M., Mayer, C., Gordon, M. J., Kwun, I. S., & Beattie, J. H. (2007). Mononuclear cell metallothionein mRNA levels in human subjects with poor zinc nutrition. *Br J Nutr, 97*(2), 247-254. doi:10.1017/S0007114507328614

Kyle, R. P., Moodie, E. E., Klein, M. B., & Abrahamowicz, M. (2016). Correcting for Measurement Error in Time-Varying Covariates in Marginal Structural Models. *Am J Epidemiol, 184*(3), 249-258. doi:10.1093/aje/kww068

Lassègue, B., & Griendling, K. K. (2004). Reactive oxygen species in hypertension; An update. *Am J Hypertens, 17*(9), 852-860. doi:10.1016/j.amjhyper.2004.02.004

Li, Y., Chen, C., Lu, L., Guo, W., VanWagner, L. B., Shikany, J. M., . . . Kahe, K. (2021). Cadmium Exposure in Young Adulthood Is Associated with Risk of Nonalcoholic Fatty Liver Disease in Midlife. *Dig Dis Sci*. doi:10.1007/s10620-021-06869-8

Lin, C.-M., Doyle, P., Wang, D., Hwang, Y.-H., & Chen, P.-C. (2011). Does prenatal cadmium exposure affect fetal and child growth? *Occupational and Environmental Medicine, 68*, 641-646

Liu, J., Qu, W., & Kadiiska, M. B. (2009). Role of oxidative stress in cadmium toxicity and carcinogenesis. *Toxicol Appl Pharmacol, 238*(3), 209-214. doi:10.1016/j.taap.2009.01.029

Liu, M., Yu, J., Su, Z., Sun, Y., Liu, Y., Xie, Q., . . . Ren, A. (2021). Associations between prenatal exposure to cadmium and lead with neural tube defect risks are modified by single-nucleotide polymorphisms of fetal M*THFR* and S*OD2* : a case–control study. *Environmental Health, 20*(66). doi:https://doi.org/10.1186/s12940-021-00752-9

Machiela, M. J., & Chanock, S. J. (2015). LDlink: a web-based application for exploring population-specific haplotype structure and linking correlated alleles of possible functional variants. *Bioinformatics, 31*(21), 3555-3557. doi:10.1093/bioinformatics/btv402

Martins, A. C., Almeida Lopes, A. C. B., Urbano, M. R., Carvalho, M. F. H., Silva, A. M. R., Tinkov, A. A., . . . Paoliello, M. M. B. (2021). An updated systematic review on the association between Cd exposure, blood pressure and hypertension. *Ecotoxicol Environ Saf, 208*, 111636. doi:10.1016/j.ecoenv.2020.111636

Miyashita, Y., Flynn, J. T., & Hanevold, C. D. (2017). Diagnosis and management of white-coat hypertension in children and adolescents: A Midwest Pediatric Nephrology Consortium study. *J Clin Hypertens (Greenwich), 19*(9), 884-889. doi:10.1111/jch.13006

Mordukhovich, I., Wright, R. O., Hu, H., Amarasiriwardena, C., Baccarelli, A., Litonjua, A., . . . Schwartz, J. (2012). Associations of toenail arsenic, cadmium, mercury, manganese, and lead with blood pressure in the normative aging study. *Environ Health Perspect, 120*(1), 98-104. doi:10.1289/ehp.1002805

Moynihan, M., Peterson, K. E., Cantoral, A., Song, P. X. K., Jones, A., Solano-González, M., . . . Téllez-Rojo, M. M. (2017). Dietary predictors of urinary cadmium among pregnant women and children. *Sci Total Environ, 575*, 1255-1262. doi:10.1016/j.scitotenv.2016.09.204

Murphy, M. M., Stettler, N., Smith, K. M., & Reiss, R. (2014). Associations of consumption of fruits and vegetables during pregnancy with infant birth weight or small for gestational age births: a systematic review of the literature. *Int J Womens Health, 6*, 899-912. doi:10.2147/IJWH.S67130

Ndanuko, R. N., Tapsell, L. C., Charlton, K. E., Neale, E. P., & Batterham, M. J. (2016). Dietary Patterns and Blood Pressure in Adults: A Systematic Review and Meta-Analysis of Randomized Controlled Trials. *Adv Nutr, 7*(1), 76-89. doi:10.3945/an.115.009753

Neggers, Y. H., Cutter, G. R., Acton, R. T., Alvarez, J. O., Bonner, J. L., Goldenberg, R. L., . . . Roseman, J. M. (1990). A Positive Association between Maternal Serum Zinc Concentration and Birth-Weight. *American Journal of Clinical Nutrition, 51*(4), 678-684.

Nemmiche, S. (2017). Oxidative Signaling Response to Cadmium Exposure. *Toxicol Sci, 156*(1), 4-10. doi:10.1093/toxsci/kfw222

O'Sullivan, J., Wright, C., Pearce, M. S., & Parker, L. (2002). The influence of age and gender on the relationship between birth weight and blood pressure in childhood: a study using 24-hour and casual blood pressure. *European Journal of Pediatrics, 161*(8), 423-427. doi:10.1007/s00431-002-0985-x

Oberheitmann, B., Schäfer, J., Dally, H., Garms, A., Frentzel-Beyme, R., & Hoffmann, W. (1999). The chromosome-based challenge assay using fluorescence in situ hybridization: a possible test for increased cancer susceptibility. *Mutat Res, 428*(1-2), 157-164. doi:10.1016/s1383-5742(99)00043-5

Olsson, I., Bensryd, I., Lundh, T., Ottosson, H., Skerfvin, S., & Oskarsson, A. (2002). Cadmium in blood and urine--impact of sex, age, dietary intake, iron status, and former smoking--association of renal effects. *Environmental Health Perspectives, 110*, 1185-1190.

Organization, W. H. (2004). *World Health Organization, International Classification of Diseases, 10th Revision* (2 ed.). Geneva: World Health Organization.

Paththinige, C. S., Sirisena, N. D., & Dissanayake, V. (2017). Genetic determinants of inherited susceptibility to hypercholesterolemia - a comprehensive literature review. *Lipids Health Dis, 16*(1), 103. doi:10.1186/s12944-017-0488-4

Perng, W., Tamayo-Ortiz, M., Tang, L., Sanchez, B. N., Cantoral, A., Meeker, J. D., . . . Peterson, K. E. (2019). Early Life Exposure in Mexico to ENvironmental Toxicants (ELEMENT) Project. *BMJ Open, 9*(8), e030427. doi:10.1136/bmjopen-2019-030427

Pilsner, J. R., Hu, H., Wright, R. O., Kordas, K., Ettinger, A. S., Sánchez, B. N., . . . Hernández-Avila, M. (2010). Maternal MTHFR genotype and haplotype predict deficits in early cognitive development in a lead-exposed birth cohort in Mexico City. *Am J Clin Nutr, 92*(1), 226-234. doi:10.3945/ajcn.2009.28839

Platz, E. A., Helzlsouer, K. J., Hoffman, S. C., Morris, J. S., Baskett, C. K., & Comstock, G. W. (2002). Prediagnostic toenail cadmium and zinc and subsequent prostate cancer risk. *Prostate, 52*(4), 288-296. doi:10.1002/pros.10115

Powell, S. R. (2000). The antioxidant properties of zinc. *J Nutr, 130*(5S Suppl), 1446S-1454S.

Protano, C., Astolfi, M. L., Canepari, S., & Vitali, M. (2016). Urinary levels of trace elements among primary school-aged children from Italy: The contribution of smoking habits of family members. *Sci Total Environ, 557-558*, 378-385. doi:10.1016/j.scitotenv.2016.03.073

Prozialeck, W. C., & Edwards, J. R. (2010). Early biomarkers of cadmium exposure and nephrotoxicity. *Biometals, 23*(5), 793-809. doi:10.1007/s10534-010-9288-2
Rikitake, Y., Kawashima, S., Takeshita, S., Yamashita, T., Azumi, H., Yasuhara, M., . . . Yokoyama, M. (2001). Anti-oxidative properties of fluvastatin, an HMG-CoA reductase inhibitor, contribute to prevention of atherosclerosis in cholesterol-fed rabbits. *Atherosclerosis, 154*(1), 87-96. doi:10.1016/s0021-9150(00)00468-8
Robins, J. M., Hernán, M. A., & Brumback, B. (2000). Marginal structural models and causal inference in epidemiology. *Epidemiology, 11*(5), 550-560. doi:10.1097/00001648-200009000-00011
Rodrigo, R., González, J., & Paoletto, F. (2011). The role of oxidative stress in the pathophysiology of hypertension. *Hypertens Res, 34*(4), 431-440. doi:10.1038/hr.2010.264
Romano, M., Enquobahrie, D., Simpson, C., Checkoway, H., & Williams, M. (2016). Maternal body burden of cadmium and offspring size at birth. *Environ Res, 147*, 461-468.
Ronco, A. M., Urrutia, M., Montenegro, M., & Llanos, M. N. (2009). Cadmium exposure during pregnancy reduces birth weight and increases maternal and foetal glucocorticoids. *Toxicol Lett, 188*(3), 186-191. doi:10.1016/j.toxlet.2009.04.008
Ruttkay-Nedecky, B., Nejdl, L., Gumulec, J., Zitka, O., Masarik, M., Eckschlager, T., . . . Kizek, R. (2013). The role of metallothionein in oxidative stress. *Int J Mol Sci, 14*(3), 6044-6066. doi:10.3390/ijms14036044
Sandberg, K., & Ji, H. (2012). Sex differences in primary hypertension. *Biol Sex Differ, 3*(1), 7. doi:10.1186/2042-6410-3-7
Satarug, S., & Moore, M. R. (2004). Adverse health effects of chronic exposure to low-level cadmium in foodstuffs and cigarette smoke. *Environ Health Perspect, 112*(10), 1099-1103. doi:10.1289/ehp.6751
Satarug, S., Nishijo, M., Lasker, J. M., Edwards, R. J., & Moore, M. R. (2006). Kidney dysfunction and hypertension: role for cadmium, p450 and heme oxygenases? *Tohoku J Exp Med, 208*(3), 179-202.
Satarug, S., Nishijo, M., Ujjin, P., Vanavanitkun, Y., & Moore, M. R. (2005). Cadmium-induced nephropathy in the development of high blood pressure. *Toxicol Lett, 157*(1), 57-68. doi:10.1016/j.toxlet.2005.01.004
Schutte, R., Nawrot, T., Richart, T., Thijs, L., Roels, H. A., Van Bortel, L. M., . . . Staessen, J. A. (2008). Arterial structure and function and environmental exposure to cadmium. *Occup Environ Med, 65*(6), 412-419. doi:10.1136/oem.2007.035576
Shah, P. S., & births, K. S. G. o. D. o. L. P. (2010). Parity and low birth weight and preterm birth: a systematic review and meta-analyses. *Acta Obstet Gynecol Scand, 89*(7), 862-875. doi:10.3109/00016349.2010.486827
Sharma, A. M., Dan L. Calculator: AAP 2017 pediatric blood pressure percentiles. Retrieved from https://apps.cpeg-gcep.net/BPz_cpeg/
Simioni, C., Zauli, G., Martelli, A. M., Vitale, M., Sacchetti, G., Gonelli, A., & Neri, L. M. (2018). Oxidative stress: role of physical exercise and antioxidant nutraceuticals in adulthood and aging. *Oncotarget, 9*(24), 17181-17198. doi:10.18632/oncotarget.24729
Song, P., Zhang, Y., Yu, J., Zha, M., Zhu, Y., Rahimi, K., & Rudan, I. (2019a). Global Prevalence of Hypertension in Children: A Systematic Review and Meta-analysis. *JAMA Pediatr*, 1-10. doi:10.1001/jamapediatrics.2019.3310
Song, P., Zhang, Y., Yu, J., Zha, M., Zhu, Y., Rahimi, K., & Rudan, I. (2019b). Global Prevalence of Hypertension in Children: A Systematic Review and Meta-analysis. *JAMA Pediatr, 173*(12), 1154-1163. doi:10.1001/jamapediatrics.2019.3310
Stasenko, S., Bradford, E. M., Piasek, M., Henson, M. C., Varnai, V. M., Jurasović, J., & Kusec, V. (2010). Metals in human placenta: focus on the effects of cadmium on steroid hormones and leptin. *J Appl Toxicol, 30*(3), 242-253. doi:10.1002/jat.1490
Stata, C. (2015). *Stata Statistical Software: Release 14*. College Station, TX: StataCorp LP.

Strazzullo, P., Kerry, S. M., Barbato, A., Versiero, M., D'Elia, L., & Cappuccio, F. P. (2007). Do statins reduce blood pressure?: a meta-analysis of randomized, controlled trials. *Hypertension, 49*(4), 792-798. doi:10.1161/01.HYP.0000259737.43916.42

Substance Priority List | ATSDR. (2017, October 3 2017). Retrieved from https://www.atsdr.cdc.gov/spl/index.html

Suwazono, Y., Akesson, A., Alfven, T., Jarup, L., & Vahter, M. (2005). Creatinine versus specific gravity-adjusted urinary cadmium concentrations. *Biomarkers, 10*(2-3), 117-126. doi:10.1080/13547500500159001

Tanira, M. O., & Al Balushi, K. A. (2005). Genetic variations related to hypertension: a review. *J Hum Hypertens, 19*(1), 7-19. doi:10.1038/sj.jhh.1001780

Taylor, J. Y., Sun, Y. V., Hunt, S. C., & Kardia, S. L. (2010). Gene-environment interaction for hypertension among African American women across generations. *Biol Res Nurs, 12*(2), 149-155. doi:10.1177/1099800410371225

Tellez-Plaza, M., Navas-Acien, A., Criniceanu, C., & Guallar, E. (2008). Cadmium Exposure and Hypertension in the 1999–2004 National Health and Nutrition Examination Survey (NHANES). *Environmental Health Perspectives, 116*(1).

Theodore, R. F., Broadbent, J., Nagin, D., Ambler, A., Hogan, S., Ramrakha, S., . . . Poulton, R. (2015). Childhood to Early-Midlife Systolic Blood Pressure Trajectories: Early-Life Predictors, Effect Modifiers, and Adult Cardiovascular Outcomes. *Hypertension, 66*(6), 1108-1115. doi:10.1161/HYPERTENSIONAHA.115.05831

Thevenod, F. (2010). Catch me if you can! Novel aspects of cadmium transport in mammalian cells. *Biometals, 23*, 857-875.

Tikhonoff, V., Kuznetsova, T., Stolarz, K., Bianchi, G., Casiglia, E., Kawecka-Jaszcz, K., . . . Staessen, J. A. (2003). beta-Adducin polymorphisms, blood pressure, and sodium excretion in three European populations. *Am J Hypertens, 16*(10), 840-846. doi:10.1016/s0895-7061(03)00975-0

Tinkov, A. A., Filippini, T., Ajsuvakova, O. P., Aaseth, J., Gluhcheva, Y. G., Ivanova, J. M., . . . Skalny, A. V. (2017). The role of cadmium in obesity and diabetes. *Sci Total Environ, 601-602*, 741-755. doi:10.1016/j.scitotenv.2017.05.224

Vacchi-Suzzi, C., Kruse, D., Harrington, J., Levin, K., & Meliker, J. R. (2016). Is Urinary Cadmium a Biomarker of Long-term Exposure in Humans? A Review. *Curr Environ Health Rep, 3*(4), 450-458.

Vahter, M., Akesson, A., Lidén, C., Ceccatelli, S., & Berglund, M. (2007). Gender differences in the disposition and toxicity of metals. *Environ Res, 104*(1), 85-95. doi:10.1016/j.envres.2006.08.003

Valko, M., Jomova, K., Rhodes, C. J., Kuča, K., & Musílek, K. (2016). Redox- and non-redox-metal-induced formation of free radicals and their role in human disease. *Arch Toxicol, 90*(1), 1-37. doi:10.1007/s00204-015-1579-5

Vinceti, M., Venturelli, M., Sighinolfi, C., Trerotoli, P., Bonvicini, F., Ferrari, A., . . . Vivoli, G. (2007). Case-control study of toenail cadmium and prostate cancer risk in Italy. *Sci Total Environ, 373*(1), 77-81. doi:10.1016/j.scitotenv.2006.11.005

Wai, K. M., Mar, O., Kosaka, S., Umemura, M., & Watanabe, C. (2017). Prenatal Heavy Metal Exposure and Adverse Birth Outcomes in Myanmar: A Birth-Cohort Study. *Int J Environ Res Public Health, 14*(11). doi:10.3390/ijerph14111339

Wang, H., Hu, Y. F., Hao, J. H., Chen, Y. H., Su, P. Y., Wang, Y., . . . Xu, D. X. (2015). Maternal zinc deficiency during pregnancy elevates the risks of fetal growth restriction: a population-based birth cohort study. *Sci Rep, 5*, 11262. doi:10.1038/srep11262

Wang, H., Liu, L., Hu, Y. F., Hao, J. H., Chen, Y. H., Su, P. Y., . . . Xu, D. X. (2016). Maternal serum cadmium level during pregnancy and its association with small for gestational age infants: a population-based birth cohort study. *Sci Rep, 6*, 22631. doi:10.1038/srep22631

Wang, W., Lee, E. T., Fabsitz, R. R., Devereux, R., Best, L., Welty, T. K., & Howard, B. V. (2006). A longitudinal study of hypertension risk factors and their relation to cardiovascular disease: the Strong Heart Study. *Hypertension, 47*(3), 403-409. doi:10.1161/01.HYP.0000200710.29498.80

Whelton, P. K., Carey, R. M., Aronow, W. S., Casey, D. E., Collins, K. J., Dennison Himmelfarb, C., . . . Wright, J. T. (2018). 2017 ACC/AHA/AAPA/ABC/ACPM/AGS/APhA/ASH/ASPC/NMA/PCNA Guideline for the Prevention, Detection, Evaluation, and Management of High Blood Pressure in Adults: A Report of the American College of Cardiology/American Heart Association Task Force on Clinical Practice Guidelines. *Circulation, 138*(17), e484-e594. doi:10.1161/CIR.0000000000000596

White, A. J., O'Brien, K. M., Jackson, B. P., & Karagas, M. R. (2018). Urine and toenail cadmium levels in pregnant women: A reliability study. *Environ Int, 118*, 86-91. doi:10.1016/j.envint.2018.05.030

Whooley, M. A., Boyd, A. L., Gardin, J. M., & Williams, D. R. (2002). Religious involvement and cigarette smoking in young adults: the CARDIA study (Coronary Artery Risk Development in Young Adults)study (*Arch Intern Med, 162*(14), 1604-1610. doi:10.1001/archinte.162.14.1604

Willey, J. Z., Moon, Y. P., Kahn, E., Rodriguez, C. J., Rundek, T., Cheung, K., . . . Elkind, M. S. (2014). Population attributable risks of hypertension and diabetes for cardiovascular disease and stroke in the northern Manhattan study. *J Am Heart Assoc, 3*(5), e001106. doi:10.1161/JAHA.114.001106

Wolf, M. B., & Baynes, J. W. (2007). Cadmium and mercury cause an oxidative stress-induced endothelial dysfunction. *Biometals, 20*(1), 73-81. doi:10.1007/s10534-006-9016-0

Wolfrum, S., Jensen, K. S., & Liao, J. K. (2003). Endothelium-dependent effects of statins. *Arterioscler Thromb Vasc Biol, 23*(5), 729-736. doi:10.1161/01.ATV.0000063385.12476.A7

Wu, W., Liu, D., Jiang, S., Zhang, K., Zhou, H., & Lu, Q. (2019). Polymorphisms in gene MMP-2 modify the association of cadmium exposure with hypertension risk. *Environ Int, 124*, 441-447. doi:10.1016/j.envint.2019.01.041

Xun, P., Liu, K., Morris, J. S., Daviglus, M. L., Stevens, J., Jacobs, D. R., & He, K. (2010). Associations of toenail selenium levels with inflammatory biomarkers of fibrinogen, high-sensitivity c-reactive protein, and interleukin-6: The CARDIA Trace Element Study. *Am J Epidemiol, 171*(7), 793-800. doi:10.1093/aje/kwq001

Yang, K., Xun, P., Carnethon, M., Carson, A. P., Lu, L., Zhu, J., & He, K. (2019). Low to moderate toenail arsenic levels in young adulthood and incidence of diabetes later in life: findings from the CARDIA Trace Element study. *Environ Res, 171*, 321-327. doi:10.1016/j.envres.2019.01.035

Yiin, S. J., Chern, C. L., Sheu, J. Y., Tseng, W. C., & Lin, T. H. (1999). Cadmium-induced renal lipid peroxidation in rats and protection by selenium. *J Toxicol Environ Health A, 57*(6), 403-413.

Yoopan, N., Watcharasit, P., Wongsawatkul, O., Piyachaturawat, P., & Satayavivad, J. (2008). Attenuation of eNOS expression in cadmium-induced hypertensive rats. *Toxicol Lett, 176*(2), 157-161. doi:10.1016/j.toxlet.2007.11.002

Zhang, A., Hu, H., Sánchez, B. N., Ettinger, A. S., Park, S. K., Cantonwine, D., . . . Tellez-Rojo, M. M. (2012). Association between prenatal lead exposure and blood pressure in children. *Environ Health Perspect, 120*(3), 445-450. doi:10.1289/ehp.1103736

Zhang, A., Park, S. K., Wright, R. O., Weisskopf, M. G., Mukherjee, B., Nie, H., . . . Hu, H. (2010). HFE H63D polymorphism as a modifier of the effect of cumulative lead exposure on pulse pressure: the Normative Aging Study. *Environ Health Perspect, 118*(9), 1261-1266. doi:10.1289/ehp.1002251

Zhang, D., Zhang, T., Liu, J., Chen, J., Li, Y., Ning, G., . . . Ma, H. (2019). Zn Supplement-Antagonized Cadmium-Induced Cytotoxicity in Macrophages In Vitro: Involvement of Cadmium Bioaccumulation and Metallothioneins Regulation. *J Agric Food Chem, 67*(16), 4611-4622. doi:10.1021/acs.jafc.9b00232

Zhang, J. R., Hu, W. N., & Li, C. Y. (2019). A Review of the Epidemiological Evidence for Adducin Family Gene Polymorphisms and Hypertension. *Cardiol Res Pract, 2019*, 7135604. doi:10.1155/2019/7135604

Zhang, M., Liu, T., Wang, G., Buckley, J. P., Guallar, E., Hong, X., . . . Mueller, N. T. (2021). Exposure to Heavy Metals and Trace Elements and Childhood Blood Pressure in a U.S. Urban, Low-Income, Minority Birth Cohort. *Environ Health Perspect, 129*(6), 67005. doi:10.1289/EHP8325

4.7 Tables

Table 4-1. Selected characteristics of analytic population at Y2

Variable	N	n(%), μ(sd)	Cd Q1 0.0000-0.0044 μg/g N=893	Cd Q2 0.0045-0.0085 μg/g N=865	Cd Q3 0.0086-0.0197 μg/g N=870	Cd Q4 0.0198-4.1334 μg/g N=869	p-value*
Male Sex	3497	1408(40.3)	412(46.1)	300(34.7)	343(39.4)	353(40.6)	<0.001
Age	3494	26.8(3.6)	27.2(3.4)	27.0(3.6)	26.7(3.8)	26.4(3.7)	<0.001
Education (years)	3491	14.2(2.4)	14.6(2.3)	14.4(2.3)	14.0(2.4)	13.9(2.3)	<0.001
Race	3497						
White		1862(53.3)	525(58.8)	492(56.9)	417(47.9)	428(49.3)	
Black		1622(46.4)	366(41.0)	370(42.8)	450(51.7)	436(50.2)	<0.001
Hispanic		13(0.4)	2(0.2)	43(0.4)	3(0.3)	5(0.6)	
Study Center							
Birmingham, AL		776(22.2)	279(31.2)	261(30.2)	157(18.1)	78(9.0)	
Chicago, IL	3497	771(22.1)	97(10.9)	153(17.7)	195(22.4)	326(37.5)	<0.001
Minneapolis, MN		1015(29.0)	211(23.6)	213(24.6)	257(29.5)	334(38.4)	
Oakland, CA		936(26.8)	306(34.3)	238(27.5)	261(30.0)	131(15.1)	
Smoking Status[a]							
Never smoker	3490	1980(56.7)	508(57.1)	496(57.5)	497(57.5)	475(55.0)	0.042
Former smoker		498(14.3)	150(16.9)	121(14.0)	115(13.3)	110(12.7)	
Current smoker		1012(29.0)	231(26.0)	245(28.4)	252(29.2)	279(32.3)	
Alcohol use[b]							
None		874(29.6)	214(28.5)	222(20.8)	212(29.1)	226(30.9)	
Light	2957	1089(36.8)	286(28.0)	292(39.2)	246(33.7)	265(36.3)	0.017
Moderate		613(20.7)	174(23.1)	146(19.6)	150(20.6)	143(19.6)	
At-risk		381(12.9)	78(10.4)	85(11.4)	121(16.6)	97(13.3)	
Physical Activity (Exercise units)[c]	3486	380.6(286.8)	392.8(293.4)	364(274.3)	387.9(290.0)	376.9(288.7)	0.162
BMI (kg/m^3)							
Underweight (<18.5)		129(3.7)	33(3.7)	28(3.2)	37(3.6)	31(3.6)	
Normal (18.5-24.9)	3497	2080(59.5)	531(59.5)	537(62.1)	498(57.2)	514(59.2)	0.17
Overweight (25-29.9)		860(24.6)	237(26.5)	199(23.0)	207(23.8)	217(25.0)	
Obese (30.0+)		428(12.2)	92(10.3)	101(11.7)	128(14.7)	107(12.3	
Systolic Blood pressure (mmHg)	3494	105.1(8.9)	105.1(8.6)	104.5(8.9)	104.9(9.1)	106.0(8.8)	0.004
Diastolic Blood pressure (mmHg)	3494	64.9(7.7)	65.7(7.4)	64.8(7.6)	64.5(7.5)	64.4(8.2)	0.002
Zinc (μg/g)	3496	55.4(21.5)	50.5(19.1)	51.4(18.1)	54.4(19.4)	65.5(25.4)	<0.001

Study population excludes individuals with missing toenail Cd (N=13) or prevalent hypertension at Y2 (N=867). N=2 participants met both conditions. N=3 individuals with missing SBP and DBP were retained in the analysis set.

Median cadmium concentration was 0.0085 μg/g, IQR=0.004, 0.0197.

*P-value is based on chi^2 test for categorical variables and one-way ANOVA for continuous variables.

[a]Participants were asked if they have ever smoked cigarettes regularly (5+ cigarettes/week, most weeks). Those who responded no were considered non-smokers, those who responded yes were categorized as current smokers, former smokers smoked regularly in the past, but longer smoke.

Table 4-2. Associations between toenail cadmium concentration and longitudinal blood pressure measurements

	Minimally adjusted* **β(95%CI)**	**Moderately adjusted†** **β(95%CI)**	**Fully adjusted ‡** **β(95%CI)**
SBP			
Log Cd μg/g	0.12(-0.14, 0.37)	0.13(-0.14, 0.39)	0.09(-0.18, 0.35)
Cd Q1	Ref	Ref	Ref
Cd Q2	0.41(-0.37, 1.18)	0.53(-0.27, 1.33)	0.50(-0.30, 1.30)
Cd Q3	0.34(-0.44, 1.12)	0.33(-0.49, 1.15)	0.26(-0.55, 1.08)
Cd Q4	0.54(-0.27, 1.36)	0.49(-0.37, 1.35)	0.37(-0.49, 1.22)
DBP			
Log Cd μg/g	-0.02(-0.21, 0.17)	-0.02(-0.23, 0.18)	-0.06(-0.26, 0.15)
Cd Q1	Ref	Ref	Ref
Cd Q2	0.17(-0.42, 0.77)	0.15(-0.48, 0.77)	0.12(-0.50, 0.74)
Cd Q3	-0.19(-0.79, 0.41)	-0.21(-0.84, 0.43)	-0.25(-0.89, 0.38)
Cd Q4	-0.09(-0.72, 0.53)	-0.20(-0.86, 0.46)	-0.30(-0.96, 0.37)

*GEE models adjusted for sex, race, time(years) since toenail Cd measurement, and study center

†GEE models adjusted for sex, race, time(years) since toenail Cd measurement, study center, BMI at Y2, smoking at Y2, and alcohol use at Y2.

‡ GEE models adjusted for sex, race, time(years) since toenail Cd measurement, study center, BMI at Y2, smoking at Y2, alcohol use at Y2, exercise at Y2, years of education at Y2.

Table 4-3. Associations between toenail cadmium concentration and risk of hypertension

Exposure	**Minimally adjusted*** **HR (95%CI)**	**Moderately adjusted†** **HR(95%CI)**	**Fully adjusted‡** **HR(95%CI)**
Log Cd μg/g	1.02(0.98, 1.07)	1.02(0.97, 1.07)	1.02(0.98, 1.07)
Cd Q1	Ref	Ref	Ref
Cd Q2	0.95(0.83, 1.09)	0.94(0.81, 1.10)	0.95(0.81, 1.10)
Cd Q3	1.02(0.89, 1.18)	1.01(0.86, 1.17)	1.00(0.86, 1.17)
Cd Q4	1.05(0.91, 1.21)	1.02(0.87, 1.20)	1.01(0.86, 1.18)

*Adjusted for sex, race, and study center

†Adjusted for sex, race, study center, BMI at Y2, smoking at Y2, and alcohol use at Y2.

‡Adjusted for sex, race, study center, BMI at Y2, smoking at Y2, alcohol use at Y2, exercise at Y2, and years of education at Y2.

Table 4-4. P-values of interaction terms in the association between toenail Cd concentration and blood pressure variables

	Outcome					
Effect Modifier	Hypertension		SBP		DBP	
	Cd(μg/g)	Cd Quartiles	Cd(μg/g)	Cd Quartiles	Cd(μg/g)	Cd Quartiles
Zinc	0.72	0.49	0.80	0.98	0.53	0.69
Smoking	0.67	0.86	0.26	0.91	**0.06**	0.31
BMI	0.47	0.34	0.80	0.70	0.43	0.58
Sex	**0.05**	0.28	0.68	0.63	**0.08**	0.33

All models adjusted for sex, race, study center, BMI at Y2, smoking at Y2, alcohol use at Y2, exercise at Y2, and years of education at Y2.
Cd quartiles coded as continuous 1,2,3,4
Zinc coded as continuous (μg/g); Smoking coded as continuous 1,2,3, for never, former, and current smoker; BMI coded as continuous 1,2,3,4, for underweight, normal, overweight, and obese; sex coded as male/female

Table 4-5. Sex and smoking-specific associations between toenail cadmium concentration and longitudinal blood pressure measurements

	Male β(95%CI)	**Female β(95%CI)**	**Never Smoker β(95%CI)**	**Former Smoker β(95%CI)**	**Current Smoker β(95%CI)**
SBP					
Log Cd μg/g	0.04(-0.37, 0.44)	0.17(-0.18, 0.53)	0.20(-0.14, 0.55)	0.11(-0.63 0.86)	-0.12(-0.62, 0.39)
Cd Q1	Ref	Ref	Ref	Ref	Ref
Cd Q2	**1.19(0.02, 2.37)**	0.04(-1.04, 1.13)	0.05(-0.99, 1.09)	1.37(-0.62, 3.37)	1.11(-0.48, 2.70)
Cd Q3	0.47(-0.71, 1.65)	0.17(-0.95, 1.29)	-0.36(-1.43, 0.71)	0.51(-1.51, 2.53)	0.52(-1.09, 2.13)
Cd Q4	0.72(-0.52, 1.96)	0.13(-1.04, 1.30)	0.52(-0.62, 1.66)	0.19(-1.96, 2.33)	0.39(-1.25, 2.03)
DBP					
Log Cd μg/g	-0.21(-0.53, 0.11)	0.07(-0.20, 0.34)	0.14(-0.13, 0.41)	-0.15(-0.72, 0.42)	-0.25(-0.63, 0.13)
Cd Q1	Ref	Ref	Ref	Ref	Ref
Cd Q2	0.17(-0.42, 0.77)	0.61(-0.31, 1.54)	0.05(-0.99, 1.09)	1.37(-0.62, 3.37)	1.11(-0.48, 2.70)
Cd Q3	-0.19(-0.79, 0.41)	-0.28(-1.21, 0.66)	-0.36(-2.43, 0.71)	0.51(-1.51, 2.53)	0.52(-1.09, 2.13)
Cd Q4	-0.09(-0.72, 0.53)	-0.36(-1.35, 0.62)	0.52(-0.62, 1.66)	0.19(-1.96, 2.34)	0.39(-1.25, 2.03)

GEE models adjusted for sex, race, time(years) since toenail Cd measurement, study center, BMI at Y2, smoking at Y2, alcohol use at Y2, exercise at Y2, years of education at Y2.

Table 4.6. Sex-specific associations between toenail cadmium concentration and risk of hypertension

Exposure	Males HR (95%CI)	Females HR (95%CI)
Log Cd μg/g	0.98(0.90, 1.05)	**1.06(0.99, 1.13)**
Cd Quartile 1	Ref	Ref
Cd Quartile 2	0.96(0.77, 1.20)	0.91(0.75, 1.12)
Cd Quartile 3	1.07(0.86, 1.33)	0.98 (0.79, 1.21)
Cd Quartile 4	0.94(0.74, 1.19)	1.06(0.86, 1.32)

P-value for sex*continuous Cd interaction term=0.05 and sex*Cd quartile interaction term=0.08
All models adjusted for race, study center, BMI at Y2, smoking at Y2, alcohol use at Y2, exercise at Y2, and years of education at Y2

4.8 Supplemental Tables

Supplemental Table 4-1. Associations between toenail cadmium concentration and risk of hypertension, using pre-2017 hypertension definition (N=4191)

Exposure	Minimally adjusted* HR (95%CI)	Moderately adjusted† HR(95%CI)	Fully adjusted‡ HR(95%CI)
Log Cd μg/g	1.00(0.95, 1.05)	0.99(0.94, 1.05)	0.98(0.93, 1.04)
Cd Quartile 1	Ref	Ref	Ref
Cd Quartile 2	0.92(0.80, 1.06)	0.90(0.77, 1.06)	0.90(0.77, 1.05)
Cd Quartile 3	1.00(0.87, 1.16)	0.95(0.81, 1.11)	0.93(0.80, 1.10)
Cd Quartile 4	0.97(0.83, 1.12)	0.92(0.78, 1.09)	0.90(0.76, 1.07)

These models exclude individuals with missing toenail Cd (N=13) or prevalent hypertension at Y2 (N=184). N=1 participants met both conditions. N=3 individuals with missing SBP and DBP were retained in the analysis set. These exclusions differ from the main analysis, due to the differing clinical definition of hypertension.
*Adjusted for sex, race, and study center
†Adjusted for sex, race, study center, BMI at Y2, smoking at Y2, and alcohol use at Y2.
‡Adjusted for sex, race, study center, BMI at Y2, smoking at Y2, alcohol use at Y2, exercise at Y2, and years of education at Y2.

Supplemental Table 4-2. Baseline (Y2) characteristics of CARDIA participants, comparing those in the study at Y30, to those not in study at Y30

Variable	N	All n(%), μ(sd)	Attended Y30 (N=2476)	Did not Attend Y 30 (N=1021)	p-value*
Male Sex	3497	1408(40.3)	957(38.7)	451(44.2)	0.002
Age	3494	26.8(3.6)	27.0(3.6)	26.4(3.7)	<0.001
Education (years)	3491	14.2(2.4)	14.5(2.3)	13.6(2.3)	<0.001
Race					
White	3497	1862(53.3)	1387(56.0)	475(46.5)	<0.001
Black		1622(46.4)	1080(43.6)	542(53.1)	
Hispanic		13(0.4)	9(0.4)	4(0.4)	
Study Center					
Birmingham, AL	3497	776(22.2)	529(21.4)	246(24.1)	<0.001
Chicago, IL		771(22.1)	565(22.8)	206(20.2)	
Minneapolis, MN		1015(29.0)	672(27.1)	343(33.6)	
Oakland, CA		936(26.8)	710(28.7)	226(22.1)	
Smoking Status[a]					
Never smoker	3490	1980(56.7)	1473(59.8)	503(49.5)	<0.001
Former smoker		498(14.3)	363(14.7)	133(13.1)	
Current smoker		1012(29.0)	628(25.5)	379(37.3)	
Alcohol use[b]					
None	2957	874(29.6)	610(28.9)	264(31.1)	0.017
Light		1089(36.8)	781(37.1)	308(36.3)	
Moderate		613(20.7)	455(21.6)	158(18.6)	
At-risk		381(12.9)	262(12.4)	119(14.0)	
Physical Activity (Exercise units)[c]	3486	380.6(286.8)	386.5(284.4)	366.0(292.3)	0.054
BMI (kg/m^3)					
Underweight (<18.5)	3497	129(3.7)	84(3.4)	45(4.4)	0.125
Normal (18.5-24.9)		2080(59.5)	1482(59.9)	598(58.6)	
Overweight (25-29.9)		860(24.6)	622(25.1)	238(23.3)	
Obese (30.0+)		428(12.2)	288(11.6)	140(13.7)	
Systolic Blood pressure (mmHg)	3494	105.1(8.9)	104.9(8.9)	105.6(8.8)	0.022
Diastolic Blood pressure (mmHg)	3494	64.9(7.7)	65.0(7.5)	64.6(8.1)	0.266
Zinc (μg/g)	3496	55.4(21.5)	55.9(21.9)	54.1(20.6)	0.024
Cadmium (μg/g)[†]	3497	0.03(0.10)	0.02(0.08)	0.03(0.14)	0.030

*P-value is based on chi^2 test for categorical variables and one-way ANOVA for continuous variables.

[†]Variable was log transformed before one-way ANOVA was performed, however untransformed means are shown

[a]Participants were asked if they have ever smoked cigarettes regularly (5+ cigarettes/week, most weeks). Those who responded no were considered non-smokers, those who responded yes were categorized as current smokers, former smokers smoked regularly in the past, but longer smoke.

[b]Light drinking was defined at <7 drinks for men and <4 drinks for women, moderate drinking was defined as 7-14 drinks for men and 4-7 drinks for women, and at-risk drinking was defined as >14 drinks for men and >7 drinks for women.

Chapter 5. Discussion and Recommendations

5.1 Overall Findings

We found no association of prenatal Cd with birthweight (β=-41.47 grams, 95%CI: -128.03, 45.08), offspring SBP (β=-1.01 mmHg, 95%CI: -3.04, 1.00), DBP (β=-1.22 mmHg, 95%CI: -2.75, 0.29), or high BP (OR=0.65, 95%CI: 0.34, 1.23). We found evidence of maternal UCd and offspring genotype interaction on SBP and DBP for eight and two SNPs, respectively (interaction p<0.10). Maternal UCd was inversely associated with SBP among those with 0 copies of the *rs5909* (*HMGCR*) minor allele (β=-4.61 mmHg, 95%CI: -8.84, -0.38), at least one copy of the *rs10497900* (*PTH2R*) minor allele (β=-5.02 mmHg, 95%CI: -8.67, -1.38), and 0 copies of the *rs3771452* (*ADD2*) minor allele (β=-9.82 mmHg, 95%CI: -17.30, -2.35, p-value < 0.05) (interaction p-values 0.05, 0.05, and 0.02, respectively). There were suggestive (borderline) inverse associations of maternal UCd with lower DBP among those with at least one copy of the *rs208272* (*HMGCR*) minor allele (β=-5.57 mmHg, 95%CI: -11.17, 0.03, p-value=0.05) and 0 copies of the *rs4984* (*ADD2*) minor allele (β=-2.86 mmHg, 95%CI: -6.01, 0.29, p-value=0.07) (interaction p-values, 0.07 and 0.03, respectively). In CARDIA, toenail Cd concentration (log-μg/g) was not associated with SBP (β=0.09, 95%CI: -0.18, 0.35), DBP (β=-0.06, 95%CI: -0.26, 0.15), or risk of hypertension (HR=1.02, 95%CI: 0.98,1.07). We observed evidence of effect modification by sex in Cd-hypertension associations (p-value for interaction=0.05). Associations of Cd with risk of hypertension was marginally significant among females (HR=1.06, 95%CI: 0.99, 1.13) but not males (HR=0.98, 95%CI: 0.90, 1.05).

5.2 Conclusions

We did not observe a statistically significant association between maternal UCd and middle childhood BP in all ELEMENT participations, however, contrary to our hypothesis, we observed an inverse association between maternal UCd and SBP and DBP among subgroups defined by offspring genotypes for genes associated with oxidative stress and calcium (Ca) homeostasis. We did not find an association between toenail Cd concentration and longitudinal BP changes or risk of hypertension in adults, however, there was some indication of effect modification by sex. More specifically, a positive association of toenail Cd with risk of hypertension was observed among females, but not males.

5.3 Strengths and Limitations

This dissertation helps fill the gaps in literature regarding the impacts of prenatal Cd or adult Cd on BP outcomes. This was the first study to examine the role of genetic variations in prenatal Cd-BP associations, findings which may elucidate mechanisms of Cd cardiovascular toxicity. Additionally, our study was one of the first to examine Cd burden with longitudinal BP outcomes, including incident hypertension. The rich datasets available in ELEMENT and CARDIA allowed us to control for important potential confounders as well as assess important potential effect modifiers.

While detailed limitations of each study are outlined in the respective chapters describing the studies, some overall limitations of the project deserve mention. A key limitation to this research was the relatively small sample size of analytic study population from the ELEMENT cohort. This meant that we were likely underpowered to detect small, but potentially important

associations between maternal prenatal Cd burden and offspring BP. Additionally, the small sample size made it difficult to adequately assess effect modification by sex and genetic variation. Another limitation of this dissertation is potential misclassification of BP outcomes. In our analyses, we relied on BP measurements taken in clinical settings, and used single measurements to represent BP over a longer time period in the ELEMENT Study. We used biomarkers of Cd exposure, urine and toenail concentration, to estimate prenatal exposure and young adult body burden, respectively. It is unclear to what extent these measurements reflect the etiologically relevant time periods of exposure/burden and/or accurate biologically important doses of Cd exposure/burden. These measurements are subject to non-differential misclassification, which likely would have attenuated our effect estimates. While our study included adjustment for many important confounders, there was likely uncontrolled confounding by factors such as diet and residual confounding by SES. Finally, our results may not be generalizable to populations with different levels of Cd exposure, risk factors for hypertension, demographic make-up, or genetic susceptibility factors. Therefore, generalizability of our findings in the current study requires careful considerations.

5.4 Future Research Directions and Public Health Implications

Our findings warrant replication in large, diverse study populations. Future studies should examine sex-Cd interactions with BP outcomes and investigate the mechanisms associated with sex-specific differences related to Cd toxicity on cardiovascular health. There should be further investigation of genetic susceptibility to Cd toxicity and research of gene-Cd interactions on BP.

Importantly, analyses should be conducted with larger study populations to ensure adequate power to conduct interaction analyses.

Since exposure to heavy metal does not occur in isolation, studies should employ methods that allow for analysis of heavy metal mixtures (e.g., lead, mercury, arsenic, and iron) in association with BP outcomes. Studies should also quantify interactions of Cd with other heavy metals. For example, higher body iron stores may mitigate Cd toxicity. Thus, examining serum ferratin levels—a biomarker of body iron stores—in the association with Cd body burden and blood pressure could give valuable insights into the mechanisms of Cd toxicity.

Efforts should also be made to adequately control for dietary characteristics, particularly those that indicate a healthy diet (e.g., fruit and vegetable consumption). Previous analyses of our population linked higher maternal UCd with fruit and vegetable consumption, food items that may be associated with a healthier diet overall, as well as lower blood pressure, and a lower risk of low birthweight. However, adjusting for consumption of individual food items may be inadequate to control for confounding; thus, quantifying dietary patterns associated with both Cd exposure and cardiovascular health outcomes would be useful.

If replicated, findings our study can be used to identify subgroups at highest risk of Cd cardiovascular toxicity, which may serve as a valuable tool to clinicians and policy makers for targeting interventions and BP screening efforts. Additionally, our findings of sex and genotype-specific associations contribute to the understanding of mechanisms of Cd toxicity and can generate hypotheses for further research.