Maternal body burden of cadmium: impacts on gestational diabetes and fetal growth

Megan E. Romano

A dissertation

submitted in partial fulfillment of the

requirements for the degree of

Doctor of Philosophy

University of Washington

2013

Reading Committee:

Daniel A. Enquobahrie, Chair

Michelle A. Williams

Harvey Checkoway

Program Authorized to Offer Degree:

Public Health: Epidemiology

University of Washington

Abstract

Maternal body burden of cadmium: impacts on gestational diabetes and fetal growth

Megan E. Romano

Chair of the Supervisory Committee:

Professor Michelle A. Williams

Department of Epidemiology

Introduction: Cadmium (Cd) exposure has been associated with increased risk of diabetes mellitus, hypertension, and osteoporosis. However, the effect of Cd on gestational diabetes mellitus (GDM) has not been investigated, and the influence of Cd on infant size at birth has not been fully elucidated. Interpretation of studies examining potentially infant sex-specific effects of Cd on fetal growth has been hindered by limited description of sex-specific fetal growth determinants.

Methods: The study population consisted of pregnant women and singletons from the Omega Study, a large prospective cohort study examining risk factors for pregnancy complications based in Seattle and Tacoma, Washington (1996-2008). Data were gathered by interviewer-administered questionnaires, medical record abstraction, and semi-quantitative food frequency questionnaires. Creatinine-corrected Cd in early pregnancy urine samples was quantified by inductively coupled plasma mass spectrometry. Multivariable unconditional logistic regression was used to analyze the association of urinary Cd with GDM. Infant sex-stratified multivariable

linear regression was used to assess potential demographic, obstetric, medical, nutritional, and lifestyle factors influencing birth anthropometric measurements (birthweight, ponderal index, birth length, and head circumference), and to analyze the association of maternal urinary Cd with size at birth.

Results: Controlling for potential confounders, there was a trend of increased risk of GDM with increasing urinary Cd (p-trend=0.015). Multiparity was associated with a greater increase in birthweight among females, whereas among males maternal pre-pregnancy obese or underweight status were associated with greater increases and decreases in birthweight, respectively. For males maternal hypertensive conditions were associated with decreased birthweight and ponderal index. Female infants had reduced birth length with greater tertile of maternal urinary Cd, whereas males had a marginal increase in birth length (females: p-trend=0.11; males; p-trend=0.06; p for interaction=0.03).

Conclusion: Greater Cd is associated with greater risk of GDM. Careful consideration must be given to parity, pre-pregnancy body mass index, and hypertension disorders when assessing factors influencing size at birth due to observed differences in these associations for male and female infants. Finally, our findings suggest a possible infant sex-specific reversal of effects for Cd on birth length; however, replication in other populations and investigation of the underlying mechanisms are warranted.

Table of Contents

List of Figures

List of Tables

Appendix

ACKNOWLEDGEMENTS

I am grateful for the support provided to me throughout my degree program by the Reproductive, Perinatal, and Pediatric Epidemiology (RPPE) Training Program (grant number T32 HD052462 from the National Institute of Child Health and Development (NICHD), NIH). I appreciate the kind support and helpful advice provided to me by Swee May Cripe, Gayle Reiber, and my fellow RPPE trainees during my time at the University of Washington. I would like to thank my reading committee chair, Daniel Enquobahrie and my supervisory committee chair, Michelle Williams, for their generous and thoughtful mentorship throughout the entire dissertation process. I also appreciate the time, expertise, and feedback shared with me by Harvey Checkoway and Christopher Simpson. I would like to recognize the gracious assistance provided to me by the staff at the Center for Perinatal Studies at Swedish Medical Center: Ihunnaya Frederick, Raymond Miller, Chunfang Qiu, and Dejene Abetew. Finally, I would like to thank my parents, Christine and Joseph Romano, and friends for their unfailing support and encouragement throughout my time in graduate school.

DEDICATION

This dissertation is lovingly dedicated to the memory of Philomena and Michael Romano Jr., who imparted their wisdom, emphasized the importance of education and work ethic, and instilled me with the confidence to succeed through their perpetual love.

Introduction

Environmental cadmium (Cd) exposure has been linked to increased risk of cancer (1,2), diabetes mellitus (3), cardiovascular disease (4), osteoporosis (5,6), and impaired kidney function (7–10). However, associations of maternal Cd burden with pregnancy complications (e.g gestational diabetes mellitus) and/or pregnancy outcomes (e.g. fetal growth) have not been investigated or fully described. Further, a clear understanding of sex-specific fetal growth determinants is lacking which limited optimal design, conduct, and interpretation of studies investigating maternal Cd and fetal growth associations, a relation with potentially significant sex-specific differences.

Environmental Cadmium Exposure

Cadmium (Cd) is a natural, toxic metal widely utilized in commercial products, including batteries, pigments, and plastics. It enters the environment as a byproduct of mining, smelting, industrial processes, and burning of coal or household wastes (9). Whereas Cd has long been recognized as an occupational health hazard, less is understood about the effect of environmental Cd exposure among the general population (7,9). Ingestion of food containing Cd and inhalation of tobacco smoke are the primary routes of Cd exposure in the general (7,8,10). A high rate of soil-to-plant transfer makes Cd a common dietary contaminant, particularly of fiber-rich foods. Grains, shellfish, and organ meats have high Cd content, but low levels of Cd are found in most foods (9). Additionally, consumption of eggs, tofu, leafy greens and yams is associated with increased Cd body burden (11). Cd is readily stored in the leaves of tobacco plants; cigarette smokers are thus exposed to high levels of Cd via inhalation (7,9).

After ingestion or inhalation, Cd accumulates in tissues throughout the body with the primary body burden accruing in the liver and kidneys (10,12). No metabolism of Cd is known to occur within the body, though Cd ions can bind to proteins, notably albumin and metallothionein (9,10). Urinary Cd is a good biomarker of exposure and accurately reflects total body burden of Cd (9); however, an extremely small portion of Cd is excreted daily (7,9). The body does not have an efficient mechanism for removing Cd, so Cd has a half-life of 10 to 30 years within the body (13). Calcium, zinc, and iron deficiencies, as well as diets low in protein can augment Cd absorption (14). Women of reproductive age typically have lower iron stores than men of the same age; their body burdens of Cd thus tend to be greater (7,10). Data from the National Health and Nutrition Examination Survey (NHANES) illustrates that women in the United States do have greater body burden of Cd than their male peers (15). During pregnancy iron requirements increase substantially during the second and third trimester, as blood volume expands and iron becomes necessary to support fetal growth (16). The corresponding changes in iron absorption over the course of pregnancy also increase Cd absorption (14,17), which make pregnant women a population of special interest.

Gestational diabetes mellitus and Environmental Cadmium Exposure

Gestational diabetes mellitus (GDM) is glucose intolerance that begins, or is first recognized, during pregnancy. GDM complicates approximately 4% of pregnancies each year in the United States (18), and elevates the lifetime risk of type 2 diabetes mellitus, obesity, and metabolic syndrome for both mother and infant (19,20). Development of GDM is the result of a complex interplay among environmental exposures, lifestyle factors, and genetic predisposition (21). A growing body of evidence suggests an association between body burden of Cd and diabetes, (3,22–24) though some did not observe this association (25,26). Table I in the Appendix

summarizes the epidemiologic literature examining the association between Cd and diabetes. Rodent studies have demonstrated that Cd is diabetogenic and diminishes glucose tolerance (27–29), suggesting that an association between Cd exposure and GDM is biologically plausible. Although the pancreas accumulates less Cd than the kidney and liver, elevations in pancreatic Cd correspond to reductions in serum insulin among rats dosed with Cd at low levels (30). Experimental evidence suggests that Cd may damage the islets of Langerhans (30), which house the pancreatic islet β-cells that monitor blood glucose and secrete insulin into systemic circulation (31). To the best of our knowledge, the relation between Cd and GDM has not been assessed in an epidemiologic study.

Infant Birth Size and Maternal Cadmium Burden

Infant birth size, which reflects fetal growth, has far-reaching implications for neonatal morbidity and mortality, early childhood development, and even health well into adulthood (32).

Optimal fetal growth requires a complex interplay of several factors: genetics, maternal health and nutrition, placental and hormonal influences, and uterine vascular support for the transfer of nutrients to the fetal compartment (21,33). Known risk factors for small size at birth include advanced maternal age, race/ethnicity other than non-Hispanic white, maternal chronic cardiovascular or metabolic medical conditions, both low and high parity, low maternal weight gain, multiple fetal pregnancy, maternal or fetal infection, socioeconomic status, maternal alcohol intake, and maternal smoking (32,34,35). Established risk factors for macrosomia include pre-existing maternal diabetes mellitus and GDM, pregnancy lasting more than 41 weeks, and previous delivery of a macrosomic infant (36). However, little is known about sex-specific determinants of birth size. Male and female growth velocities appear to be somewhat divergent (37,38). Longitudinal assessment of fetal growth by ultrasound suggests that the

female fetus simultaneously grows in both length and weight over the course of gestation. However, the male fetus appears to first grow primarily in length and shifts growth efforts toward accelerated weight gain during the third trimester (37). The notion of sex-specific growth patterns is further supported by the observation that male neonates generally weigh more, are longer, and leaner than female neonates (39–41). These differences in timing and pattern of fetal growth between males and females may explain, at least in part, the different sex-specific fetal growth related outcomes that follow pregnancy risk factors. For example, mild preeclampsia (42,43), severe maternal asthma (44–46), and heavy maternal smoking (47,48) have each been associated with sexually dimorphic size at birth. Recent literature suggests a similar sex-specific effect of Cd on fetal growth (49,50).

Higher maternal Cd burden has been associated with decreases in birth weight (51–60), birth length (54,55,59,61,62), and head circumference (59,62). However, some of the literature does not support these observations (63–65). Studies of the general population from a wide range of countries and using a variety of biomarkers (maternal blood/urine/placenta, cord blood, etc.) suggest that Cd exposure impairs fetal growth (Table II, Appendix). One large prospective cohort study based in Bangladesh observed that Cd exposure correlated with reduced birthweight and head circumference among female neonates only (49). These findings have not yet been replicated in a well-nourished population, such as the general population of the United States. Table II in the Appendix summarizes the epidemiologic literature examining the association between Cd and size at birth.

Conceptual Framework and Overview of the Dissertation Project

A conceptual model for the dissertation project is presented in Figure 1. The specific aims of this research project are: **1)** to determine if greater maternal body burden of Cd increases women's

risk of developing GDM during pregnancy; **2)** to delineate maternal and fetal factors associated with sex-specific differences in fetal growth as measured by neonatal anthropometry (birthweight, birth length, head circumference, and ponderal index); and **3)** to evaluate whether greater maternal body burden of Cd impairs fetal growth as measured by neonatal anthropometry (birthweight, birth length, head circumference, and ponderal index).The research in this dissertation was conducted in the setting of the Omega Study, a large prospective cohort study examining risk factors for complications of pregnancy, based at the Center for Perinatal Studies at Swedish Medical Center and Tacoma General Hospital, Seattle and Tacoma, Washington (1996-2008). Interviewer administered structured questionnaires, medical record abstraction, semi-quantitative food frequency questionnaires, and creatinine-corrected cadmium in early pregnancy urine samples quantified by inductively coupled plasma mass spectrometry (ICP-MS) were used to provide data for the dissertation project. Specific details regarding each research question and study design are provided in the chapters following this introduction. Chapter 1 examines the relation between maternal body burden of Cd and GDM using a case-cohort study (n=621). Chapter 2 uses a cross-sectional study to assess sex-specific determinants of size at birth (n=3033), and Chapter 3 presents a prospective cohort study investigating the effect of maternal body burden of Cd on size at birth (n=472). Finally, we provide a brief conclusion synthesizing Chapters 1-3, discussing the implications or our findings, and suggesting future directions for research.

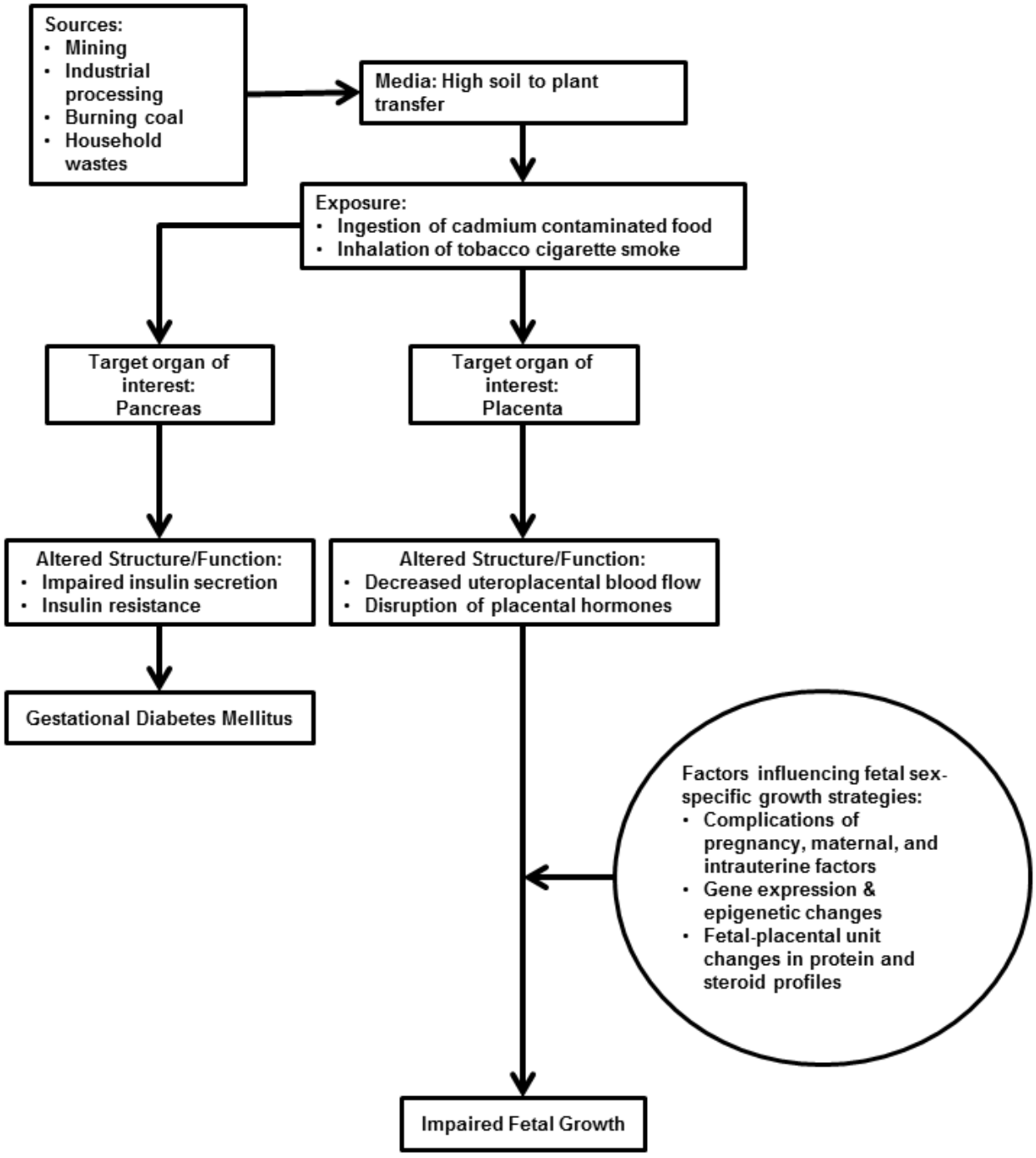


Figure 1: Conceptual model of select adverse outcomes of cadmium exposure

Chapter 1: Maternal body burden of cadmium and risk of gestational diabetes mellitus

ABSTRACT

Background- Environmental cadmium (Cd) exposure has been associated with impaired fasting glucose and type 2 diabetes. However, the association of Cd with risk of gestational diabetes mellitus (GDM) has not been investigated epidemiologically, to the best of our knowledge.

Methods - We conducted a nested case-cohort study among 140 GDM case and 516 randomly selected subcohort members from a large prospective cohort study designed to examine risk factors of pregnancy complications. Urinary Cd in clean-catch spot urine samples (collected at 15 weeks of gestation, on average) was measured using inductively coupled plasma mass spectrometry. Urinary Cd tertiles (<0.29; 0.29-0.42; ≥0.43 μg/g Cr) were based on the Cd distribution in the subcohort. GDM was diagnosed according to the 2003American Diabetes Association guidelines between 24 and 28 weeks gestation. Adjusted odds ratios (ORs) and 95% confidence intervals (CIs) were estimated using multivariable unconditional logistic regression.

Results – The geometric mean of creatinine (Cr)-corrected urinary Cd among GDM cases was 0.39 μg/g Cr (95% CI: 0.37, 0.41) compared with 0.31 μg/g Cr (95% CI: 0.29, 0.33) among non-case women. The OR (95% CI) corresponding to increasing urinary Cd tertiles among all women was: 1.0 (referent); 1.6 (0.9-3.1); 2.1 (1.2, 3.7); p-trend=0.015. In stratified analyses, high urinary cadmium (≥0.29 μg/g Cr) was related to a 2.3-fold increased risk of GDM (95% CI: 1.1, 5.0) compared to low urinary Cd cadmium (<0.29 μg/g Cr) or high urinary cadmium among normal weight women [pre-pregnancy BMI (pp-BMI) >18.5 and <25 kg/m^2], and 1.4-fold increased risk of GDM (95% CI: 0.6, 2.9) among overweight and obese women (pp-BMI ≥ 25 kg/m^2). Overweight and obese women with high urinary Cd had 3.5-fold greater risk of GDM (95% CI: 1.5, 7.8) than normal weight women with low urinary Cd; however, the interaction between maternal urinary Cd and pp-BMI was not statistically significant (p= 0.259).

Conclusion –Greater maternal body burden of Cd increases women's risk of GDM. Although the interaction between Cd and pp-BMI status was not statistically significant, there may be a combined effect of Cd and pp-BMI on GDM risk. Our findings should be replicated in other populations.

INTRODUCTION

Gestational diabetes mellitus (GDM) is a pregnancy-related glucose intolerance that complicates 1-14% of pregnancies each year in the United States (66). GDM increases the lifetime risk of type 2 diabetes mellitus, obesity, and the metabolic syndrome for both mother and infant (19,20,66). Although many demographic (maternal age (67–69), race/ethnicity (69,70)), lifestyle (pre-pregnancy BMI (69,71), smoking (69,72)) and medical risk factors (preeclampsia and chronic hypertension (73), family history of diabetes (69,74,75)) for GDM are known, less is understood about environmental risk factors for GDM.

Cadmium (Cd) is widely utilized in commercial products including batteries, pigments, and plastics. Mining, industrial processing, burning of coal, and household wastes all contribute to the entry of Cd into the environment (9). The general population is primarily exposed to Cd via ingestion of food containing Cd and inhalation of tobacco smoke. Relatively low levels of Cd are found in most foods; however grains, shellfish, and offal have high Cd content (9). In addition, ingestion of eggs, tofu, leafy greens and yams has been associated with increased body burden of Cd (11). A high rate of soil-to-plant transfer makes Cd a common dietary contaminant, particularly of fiber rich foods (9,11). Cd is readily stored in the leaves of tobacco plants. Thus, cigarette smokers are exposed to high levels of Cd via inhalation (7,9). Cd exposure leads to renal damage (12), cardiovascular disease (4,76), osteoporosis (5), and cancer (1,2).

Experimental studies have consistently demonstrated that Cd is diabetogenic (27–30). Rat and mouse models suggest that Cd diminishes glucose tolerance (27–29), potentially by impairing insulin secretion via damage to the islets of Langerhans (77,78). Pancreatic tissue has been shown to accumulate Cd, albeit to a lesser degree than the kidney and liver, and elevations in pancreatic Cd have been correlated with reductions in serum insulin among rats exposed to

low levels of Cd (30,79). Additional laboratory evidence suggests that Cd exposure disrupts lipid homeostasis, as evidenced by abnormal adipocyte differentiation that can lead to insulin resistance (80).

In addition to experimental evidence, a growing body of evidence from population-level studies suggests an association between body burden of Cd and Type 2 diabetes. Several (3,22–24,81,82) but not all (25,26,83,84) studies of non-occupationally Cd-exposed persons have found higher levels of Cd among diabetic individuals or more severe complications of diabetes among those highly exposed to Cd through diet (85,86). Beyond clinically recognized diabetes, increasing body burden of Cd has also been associated with impaired fasting glucose in a dose-dependent fashion (3). Despite biologically plausible mechanisms (**Figure 1**) and demonstrated association between Cd and type 2 diabetes, to the best of our knowledge, the relation between maternal body burden of Cd and GDM risk has not been investigated epidemiologically. The objective of this case-cohort study was to examine whether higher body burden of Cd increases risk of GDM. We also evaluated whether these associations were modified by pre-pregnancy body mass index (BMI), as overweight and obese women are known to be at increased risk of GDM (67,71,87).

METHODS

Study Setting and Study Population

The Omega Study, a large (N=4344) prospective cohort study (1996-2008) based at the Center for Perinatal Studies at Swedish Medical Center in Seattle, was designed to investigate risk factors for pregnancy complications. Participants were recruited from prenatal care clinics affiliated with Swedish Medical Center and Tacoma General Hospital, Tacoma, WA. Women initiating prenatal care at a study clinic prior to 20 weeks gestation were eligible for participation. Women who were less than 18 years of age, did not intend to carry the pregnancy to term, or did not plan to deliver at either of the two institutions were excluded.

In the current case-cohort study (88–91), a sub-cohort of 750 women was randomly drawn from among the 4344 pregnancies in the Omega Study. In addition, all 190 GDM cases among Omega Study participants were identified for the case-cohort study. Among the randomly selected members of the sub-cohort, 44 were GDM cases (Figure 2). Urine samples were available for 922 women [732 (97.6%) non-cases and 190 (100%) cases)]. Women were excluded for the following reasons: 18 subcohort members had missing GDM status, 6 subcohort members and 4 GDM cases had renal disease, 17 women in the subcohort had pre-existing diabetes mellitus, 9 non-case subcohort members delivered prior to 24 weeks gestation, 27 non-cases and 10 cases had multiple fetal births, and 8 women had Cd values suggestive of renal impairment (>2μg/g Cr). WHO guidelines suggest that creatinine concentration may be used to identify spot urine samples that are too dilute (<30 mg/dL) or too concentrated (>300 mg/dL) to provide valid estimates of the concentration of the urinary chemical of interest (92). We excluded 138 subcohort members and 36 GDM cases with dilute urine (<30 mg Cr/dL) and 1 subcohort member with overly concentrated urine (>300 mg Cr/dL). The characteristics of the

women with dilute urine did not differ substantially from the women included in the analytic population, with the exception that GDM cases with low creatinine were less likely to have normal pre-pregnancy BMI (18.5 kg/m^2 ≤ BMI <25) (p=0.02) and slightly more likely to have been underweight prior to pregnancy (BMI<18.5 kg/m^2) than GDM cases with normal creatinine (p=0.05) (Appendix: Table III). Low creatinine is common among individuals with reduced lean body mass (93,94).

After exclusions the analytic population included 621 women (481 non-case subcohort members and 140 GDM cases) (Figure 3). Additional recommendations based on assessment of urine samples from the National Health and Nutrition Examination Survey (NHANES) suggest that for women, the cutoff of <30 mg creatinine/dL urine may be inappropriate, as it is not uncommon for women to have low urinary creatinine (94). Sensitivity analyses were conducted among participants without excluding samples that were deemed dilute (< 30 mg/dL) according to the WHO guidelines (619 non-case subcohort members; 176 GDM cases) (92).

Data Collection

Information on sociodemographic characteristics, reproductive and medical histories, lifestyle factors (such as alcohol and tobacco use), and maternal anthropometry was collected by trained interviewers using a structured questionnaire shortly after enrollment. Maternal medical records were abstracted to ascertain course and outcomes of the pregnancy.

Maternal Cadmium Burden Assessment

At 15 weeks gestation, on average (standard deviation=2.9, range=4-25), a clean-catch spot urine sample was collected in polyethylene containers, promptly separated into 2 mL aliquots, and stored at −80 °C until analyses. Urine heavy metal concentrations, including Cd and total arsenic, were quantified using a validated method of inductively coupled plasma mass-spectrometry

(ICP-MS) following published protocols (95) at Metametrix Clinical Laboratory, a Clinical Laboratory Improvement Amendments (CLIA) certified facility in Duluth, GA. Briefly, urine samples were shaken and 1 mL was acidified with 1% HNO_3 (100 µl) . An internal standard solution containing scandium, rhodium, and germanium (500 µl) was added. Samples were diluted to 5 mL with deionized water. Polyatomic interferences were minimized by utilizing ICP-MS with a dynamic reaction cell (PerkinElmer SCIEX Elan DRC II with ESI SC-4, FAST Autosampler). The accuracy of ICP-MS was checked by conducting proficiency testing using urine reference material (New York Toxic / Trace Elements in Urine Event #1 2012). The limits of detection for urinary Cd and total arsenic were 0.10 and 3.0 µg/g Cr respectively. Urinary creatinine concentration was assessed using a commercially available kit (Genzyme Diagnostics, Catalogue # 221-30/# 221-50) with improved Jaffe Reaction. Laboratory personnel were blinded to case status. We categorized tertiles of urinary Cd according to the distribution in the subcohort.

Gestational Diabetes Mellitus (GDM) Diagnosis

Per American Diabetes Association (ADA) recommendations, a 50g 1-hour oral glucose challenge was used to screen all women in the Omega Study between gestational ages of 24 weeks and 28 weeks. Women who failed the screening test (glucose ≥7.8mmol/L) completed a diagnostic 100g 3-hour oral glucose tolerance test within 2 weeks of the screening test. Women were diagnosed with GDM if two or more 100g 3-hour oral glucose tolerance test levels exceeded ADA criteria: fasting ≥ 5.3 mmol/L; 1-hour ≥10.0 mmol/L; 2-hour ≥8.6 mmol/L; 3-hour ≥ 7.8 mmol/L (96).

Statistical Analysis

We compared the frequency distribution of relevant characteristics of the population between

GDM cases and the subcohort. Tertiles were defined based upon the distribution of urinary creatinine-corrected Cd in the subcohort. Unconditional logistic regression was used to assess the association across tertiles of maternal body burden of Cd and risk of GDM. The case-cohort odds ratio, calculated via the log-link function, is a good estimate of the incidence proportion ratio in cumulative-incidence type case-cohort studies that include thorough case-ascertainment from the full population (89,97–99), such as the present study. Additionally, logistic regression has been successfully used for prior case-cohort studies that did not utilize time-to-event data (100,101). We conducted trend tests using the median values of the distribution within tertiles of maternal urinary exposure as the score variable

Adjustment variables selected based on *a priori* knowledge of their associations with exposure and outcome included maternal age (years), pre-pregnancy BMI (kg/m^2), nulliparity (yes/no), family history of diabetes (yes/no), and maternal race (non-Hispanic white - yes/no). Gestational diabetes in a prior pregnancy (yes/no), current preeclampsia (yes/no), chronic hypertension (yes/no), family history of hypertension (yes/no), marital status (married-yes/no), post high school education (yes/no), smoking during pregnancy(yes/no), and urinary total arsenic were also tested as potential confounding variables. Arsenic exposure has been associated with increased risk of GDM among highly exposed women in the general population (102). Due to historic soil contamination from copper smelting activity in King County, WA, arsenic was likely to be an important co-exposure in our population (103). Total arsenic in urine, reflecting both organic and inorganic species of arsenic, was available from ICP-MS. Speciated arsenic would have been preferable since organic arsenic species are less toxic than inorganic forms and recent ingestion of seafood increases the level of organic arsenic in urine (104,105). We, therefore, adjusted for self-reported fish consumption habits (number of fish meals per week

based upon the food frequency questionnaire) in all models that included total urinary arsenic in order to partially account for expected higher levels of organic arsenic species in the urine of women that regularly consume seafood. Potential confounders were included in the final model if they resulted in a substantial change in risk estimates. Adjusted odds ratios (OR) and 95% confidence intervals (95% CI) are reported.

We repeated the analysis, stratifying by overweight and obese status (pre-pregnancy BMI >25 kg/m^2 versus ≥ 25 kg/m^2), and grouping women according to low urinary Cd (<0.29 μg/g Cr) or high urinary Cd (≥0.29 μg/g Cr). Women who were underweight (pre-pregnancy BMI>18.5 kg/m^2) were excluded from this analysis (n=33: 31 subcohort members, 2 GDM cases). We also examined the joint effect of Cd and pre-pregnancy BMI by adding a term for the interaction between high urinary cadmium and pre-pregnancy overweight and obese status to the multivariable model.

We conducted an exploratory analysis using flexible cubic splines (106) to model risk of GDM in relation to continuous urinary Cd among women with urinary Cd above the limit of detection (>0.10 μg/ g Cr). We fit the models with and without the 7 women with urinary Cd >2 μg/ g Cr. There was no evidence of nonlinearity in the relation of Cd to GDM. (Appendix: Figure I).

All statistical analyses used robust standard error estimates and the alpha level of 0.05 was used to define statistical significance. All analyses were completed using STATA 12 statistical software (107).

RESULTS

Women with GDM were older (mean $_{GDM}$=33.6; mean $_{non\text{-}GDM}$=32.8 years), had greater pre-pregnancy BMI (mean $_{GDM}$=27.0; mean $_{non\text{-}GDM}$= 23.7 kg/ m^2), and reported sedentary behavior more often (GDM 9.2%; non-GDM 4.0%) than women without GDM (Table 1). Compared to women without GDM, fewer GDM cases were non-Hispanic white (GDM 69.8%; non-GDM 83.6%), and a greater proportion was Asian (GDM 18.7%; non-GDM 8.5%). Very few African American women participated in the study (GDM 3.6%; non-GDM 2.3%). Women with GDM had higher rates of preeclampsia (GDM 8.0%; non-GDM 2.5%), chronic hypertension (GDM 9.3%; non-GDM 3.7%), and a positive family history of diabetes (GDM 33.6%; non-GDM 14.6%) or hypertension (GDM 62.9%; non-GDM 44.9%) as compared to women without GDM. There were not substantial differences between women with and without GDM in terms of parity, marital status, education, smoking habits, or gestational age at urine collection (Table 1).

The geometric mean of Cd (0.31 μg Cd/g Cr; 95% CI: 0.29, 0.33) and creatinine (83.6 mg Cr/dL urine; 95% CI 79.5, 87.9) was slightly lower among women without GDM versus women with GDM (0.39 μg Cd/g Cr; 95% CI: 0.37,0.41, 82.6 mg Cr/dL urine; 95% CI 75.3, 90.7) (Table 1). Women in the middle tertile (0.29-0.42 μg/g Cr) for urinary Cd had an elevated, but not statistically significant, risk of GDM (aOR=1.64 95% CI: 0.88–3.05), compared to those in the lowest tertile (<0.29 μg/g Cr) (Table 2). Women in the highest tertile for urinary Cd ≥0.43 μg/g Cr) had a 2-fold higher risk of GDM, compared to those in the lowest tertile (aOR=2.07; 95% CI=1.15-3.73), after adjusting for the confounders. We observed a statistically significant trend of increasing risk of GDM with greater urinary Cd (p-trend=0.015) (Table 2).

In stratified analyses, among women with normal pre-pregnancy BMI (>18.5 and <25 kg/m^2), high urinary Cd (≥0.29 μg/g Cr) was related to a 2.33-fold increased risk of GDM (95% CI: 1.08, 5.02) compared to low urinary Cd (<0.29 μg/g Cr). Among women who were overweight and obese prior to pregnancy (≥25 kg/m^2), women with high urinary Cd had 1.36-fold increased risk of GDM (95% CI: 0.64, 2.91) compared to women with low urinary Cd (Table 3). In our analysis of the joint effect of Cd and overweight and obese status, we observed that compared to women with low urinary Cd (<0.29 μg/g Cr) and normal pre-pregnancy BMI, women with high urinary Cd (≥0.29 μg/g Cr) and normal BMI had 2.15-fold increased risk of GDM, women with low urinary and overweight and obese pre-pregnancy BMI had 2.86 –fold increased risk of GDM, and women with high urinary Cd and overweight and obese BMI had a 3.46-fold increased risk of [aOR (95% CI): $aOR_{low\ \&\ normal\ BMI}$=1.00 (reference), $aOR_{high\ \&\ normal\ BMI}$=2.15 (1.02, 4.53) $aOR_{low\ \&\ overweight/obese}$=2.86 (1.17, 7.61), $aOR_{high\ \&\ overweight/obese}$=3.46 (1.54, 7.78), p for interaction=0.259 (Table 3)].

In the sensitivity analysis that included women with creatinine levels <30 mg/dL, Cd body burden was also positively associated with risk of GDM. Among women with creatinine levels <300 mg/dL, the tertiles of Cd exposure based upon the distribution in the subcohort were: low (<0.30 μg/g Cr), medium (0.30-0.42 μg/g Cr), and high (≥0.43). Women in the middle tertile for urinary Cd (0.30-0.42 μg/g Cr) had an 8% increased risk of GDM (aOR=1.08; 95% CI: 0.63,1.86), compared to those in the lowest tertile (<0.30μg/g Cr). Women in the highest tertile (≥0.43μg/g Cr) had a 1.24-fold increase in risk of GDM, compared to those in the lowest tertile (aOR=1.24; 95% CI: 0.79, 1.95), after adjustment for confounders. The observed trend of increasing risk of GDM with greater urinary Cd was not statistically significant (p-trend=0.355) (Table 4). We stratified according overweight/obese status (≥25 kg/m^2) and grouped women by

dichotomous low (<0.30 μg/g Cr) or high urinary Cd (≥0.30 μg/g Cr) and observed a non-statistically significant increase in risk of GDM among women who were not overweight or obese (18.5<BMI<25) [aOR (95% CI): aOR_{low}=1.00 (reference), aOR_{high}=1.77 (0.96, 3.26)] and no association among overweight and obese women with high urinary Cd (≥25 kg/m^2) [aOR_{low}=1.00 (reference), aOR_{high}=0.92 (0.46, 1.82) (Table 5)]. We observed some evidence of a joint effect of urinary Cd and overweight/obese status. As compared to women with low urinary Cd and normal pre-pregnancy BMI, women with high urinary Cd and normal BMI had 1.68-fold increased risk of GDM, women with low urinary Cd and overweight and obese BMI had 2.99-fold increased risk, and women with high urinary Cd and overweight and obese BMI had a 2.96-fold increased risk of GDM [OR (95% CI): $aOR_{low\ \&\ normal\ BMI}$=1.00 (reference), $aOR_{high\ \&\ normal\ BMI}$=1.68 (0.94, 3.02), $OR_{low\ \&\ overweight/obese}$=2.99 (1.37-6.50), $OR_{high\ \&\ overweight/obese}$=2.96 (1.55, 5.64), p for interaction=0.248 (Table 5)].

DISCUSSION

Our findings suggest that greater body burden of Cd increases risk of GDM in a dose dependent fashion (p-trend=0.015, Table 2) after accounting for other known risk factors. Women with high urinary Cd had twice the risk of GDM as women with low urinary Cd [aOR=2.07; 95 % CI 1.15-3.73 (Table 2)]. Although the interaction between Cd and overweight/obese status was not statistically significant, there was some suggestion of a joint effect of Cd and pre-pregnancy BMI. Overweight/obese women with high body burden of Cd had substantially increased risk of GDM as compared to normal weight women with low body burden of Cd [aOR=3.46; 95% CI 1.54-7.78 (Table 3)].

Although no prior research has specifically addressed the association between Cd and GDM, a majority of cross-sectional studies(3,22–24,81,82) suggest a relation between Cd and type 2 diabetes, but some studies report no association (25,26,83,84) (Appendix: Table I). Schwartz et al. used data from NHANES III (1988-1994) to assess whether increased urinary Cd was associated with impaired fasting glucose (IFG) and type 2 diabetes in the United States. A dose-dependent trend was obvious between tertiles of urinary Cd (0–0.99, 1.00 –1.99, ≥2 μg Cd/g Cr) and IFG [OR (95% CI): 1.48 (1.21–1.82), 2.05 (1.42–2.95) p-trend<0.0001**]**. A similar trend was apparent for type 2 diabetes, [OR (95% CI): 1.24 (1.06 –1.45), 1.45 (1.07–1.97) p-trend<0.0001] (3). Barregard et al. conducted a cross-sectional study based in Sweden with follow-up approximately 5 years later; although not statistically significant they observed a 1.2 – fold increased risk of type 2 diabetes or impaired glucose tolerance (95% CI 0.5, 2.6) at follow-up among women in the highest quartile of urinary Cd (0.56-1.59 μg Cd/g Cr) as compared to lowest (0.10-0.24 μg Cd/g Cr). The authors note that the toxic effect of cadmium may be more apparent in populations with higher prevalence of risk factors for type 2 diabetes, such as obesity

(81). To the best of our knowledge no studies have directly assessed effect modification of the Cd-diabetes association by BMI; however a common theme among the studies that observed no association between cadmium and type 2 diabetes is a population with lower average BMI than the United States. Average pre-pregnancy BMI within our subcohort was 23.9 kg/m^2 [standard deviation (SD)=5.3 kg/m^2] and 27.0 kg/m^2 (SD=7.2 kg/m^2) among GDM cases, whereas average BMI among non-diabetics (mean 23.8 kg/m^2, SD=3.1 kg/m^2) and diabetics (mean=25.3 kg/m^2, SD=3.5 kg/m^2) was lower in the population-based study in Korea (83). Likewise, average BMI was lower among women in the studies based in rural Thailand (mean23.1 kg/m^2, SD=3.8 kg/m^2) (25,26).

Development of either GDM or type 2 diabetes represents the interaction of environmental exposures, lifestyle factors, and genetic predisposition. The pathogenesis of both conditions can be thought of as a continuum of dysglycemia with the development of impaired insulin secretion and insulin resistance and as a common pathogenic link. GDM involves progressive impairment of glucose tolerance due to diminishing insulin receptors and decreasing effect of insulin on targets (21,108). A similar pre-diabetic state of diminished glucose tolerance, prompted by pancreatic islet β-cell failure, precedes the insulin resistance and impaired insulin secretion that are the hallmark of type 2 diabetes (109–111). There is some prior, although not fully consistent, evidence of diabetogenic effects of Cd. A study of smeltery workers and minimally exposed subjects demonstrated that long term Cd exposure is linked with elevations in blood glucose level and lower serum insulin level (112). *In vitro,* the islet of Langerhans in the pancreas of Cd-exposed rats secrete substantially less insulin than similar cells of rats that were not exposed to Cd (77). Additional studies have demonstrated that Cd induces hyperglycemia

(28). The mechanism remains uncertain, but appears to involve damage to the islets of Langerhans and diminished function of glucose transporter type 4 within adipocytes (77).

Arsenic exposure has been associated with type 2 diabetes (113), and recent literature suggests that chronic exposure to arsenic is also a risk factor for GDM (102). Ettinger et al. observed that women with the highest total arsenic blood levels (2.09-24.07 μg/L) had almost 3 times the odds (OR=2.8; 95%CI 1.1-6.9) of impaired glucose tolerance (> 140 mg/dL) compared to the women with the lowest levels (0.23-0.92 μg/L) (102). In an exploratory analysis, there was no clear trend between increasing tertiles of total urinary arsenic and GDM after adjustment for other potential risk factors (data not shown). However, arsenic was an important confounder in our final model for the Cd analyses. There is likely residual confounding by arsenic exposure, as our study utilized an imperfect measure of arsenic, total urinary arsenic. Ettinger et al. also acknowledged the limitation of their use of un-speciated blood arsenic, as inorganic arsenic is the relevant exposure (102).

Micronutrient deficiencies, including calcium, zinc, and iron lead to increased Cd absorption (14). We conducted an exploratory analysis to assess whether dietary intake of these essential micronutrients influenced the observed association between Cd and GDM. However, control for any combination of total daily dietary calcium, iron (total iron, heme, and/or nonheme), and zinc intake or for falling below the Institute of Medicine's recommended dietary allowance for pregnant women (<1000 mg/day calcium, <27 mg/day iron, <11 mg/day zinc) (114) made no substantial impact on the observed estimates (data not shown). More sensitive metrics of calcium, iron, and zinc status, such as blood measures (115), may be necessary to elucidate the complex interrelationship among essential and toxic metals.

Obesity and overweight status in the United States is increasing across all ages, and reproductive aged women are entering into pregnancy with historically high BMI (116). GDM complicates 24.5% of pregnancies among obese women as compared to 2.2% of pregnancies among non-obese women (117). Increasing degree of overweight/obesity is linked with greater risk of developing GDM. Compared to normal weight women (BMI≥20), overweight (BMI>25–29.9), moderately obese (BMI>30-34.9), and morbidly obese women (BMI≥35) had twice (OR=1.97; 95% CI 1.77, 2.19), three times (OR=3.01; 95% CI 2.34, 3.87), and almost 6 times (OR=5.55; 95% CI 4.27, 7.21) the risk of GDM (71). Pre-pregnancy BMI is often indicated as the main modifiable risk factor for GDM. In our study it appeared that Cd and overweight obesity status might jointly affect a woman's risk of GDM (Table 3). If both Cd body burden and BMI could be reduced prior to pregnancy, particularly among overweight/obese women, then GDM risk could be diminished. Data from the National Hospital Discharge Survey in 2007 indicate that GDM augments national medical costs by $636 million. Averting one case of GDM would save $3,305 in immediate maternal health costs and $209 in infant care during the first year of life (118).

Our analyses must be interpreted with caution due to the discrepancy between the primary analyses (Table 2 and Table 3) and the sensitivity analyses (Table 4 and Table 5). The WHO creatinine guidelines have been criticized as being overly restrictive for women, because women tend to have lower creatinine levels than the male occupational cohorts upon which the WHO guidelines were originally based (94). Urinary creatinine levels decrease over the course of normal pregnancy (119), so the WHO recommendations may not be appropriate for pregnant women. Indeed, the creatinine levels within the Omega Subcohort were lower than those observed among all women of reproductive age and reproductive aged non-Hispanic white

women in NHANES III (Table 6). Barr et al. suggest that the WHO guidelines were created at a time when measurement of very small concentrations of toxicants in urine was not technologically feasible, and that the vast improvements in analytical techniques in the intervening years make concerns related to quantification of environmental toxicants in overly dilute urine obsolete (94). However, we are not aware of any published criteria specific to the assessment of creatinine in spot urine samples during pregnancy, and the WHO guidelines are commonly used for research involving spot urine samples, including published NHANES creatinine corrected urinary Cd (15). The ability to put our findings into the context of other published literature steered us toward use of the WHO guidelines for our primary analysis; however, as the debate evolves in the literature new recommendations are likely to emerge.

Other potential limitations of our study deserve mention. Humans are typically exposed to complex mixtures of toxic substances every day, and as with all studies of environmental exposures, there is an inherent difficulty in singling out the effect of Cd alone. Therefore, unknown and unmeasured co-exposures may contribute to the risk of GDM. Generalizability may be reduced since women in the Omega Study are generally non-Hispanic white, married, and affluent, reflective of the underlying population that utilizes Swedish Medical Center and Tacoma General Hospital. However, our study population should represent a highly exposed subpopulation within the general public, as high income is associated with increased body burden of Cd (120). Levels of urinary creatinine corrected Cd in the Omega Study subcohort were generally greater than that of the US population as estimated by the NHANES (Table 7). We had limited variability in smoking status among the study participants, with the majority of our study population being never smokers (71.6%). Because smoking tobacco cigarettes is a major source of Cd exposure in the general population (7,9,121), and GDM risk is increased

among smokers, smoking may confound the Cd-GDM association (69,72). Our results are probably not reflective of the association between Cd and GDM among smokers, and smoking tobacco cigarettes should be considered as a potential confounder in future studies in populations with more variability in smoking habits.

Our study also has several strengths. In this first study to examine maternal Cd body burden and GDM risk, we used a large and well-characterized cohort of pregnant women to complete our research. The prospective nature of the parent Omega study facilitated the exclusion of women with diagnosed pregestational diabetes, and the use of early pregnancy biological samples allowed us to characterize maternal Cd burden during the critical period of early pregnancy when pathophysiologic changes of GDM have been shown to start (122).Urinary metals were assessed by a robust, well-validated, and accurate method (ICP-MS). Structured interviews, medical record abstraction, and a semi-quantitative FFQ provided rich covariate data. In sum, our study and its findings provide new information to address a knowledge gap in the literature.

Collectively, our findings suggest that Cd may be an important environmental risk factor for GDM. Replication of these findings in a larger study and other diverse populations will be important to gain a fuller understanding of the true association between Cd body burden and GDM. Further, to determine if screening for high levels of Cd prior to conception can lead to preventative measures to decrease Cd exposure, potentially by addressing zinc, calcium, and/or iron deficiencies preconceptionally or during early pregnancy is also warranted.

Table 1. Selected characteristics of the study cohort according to gestational diabetes status and tertile of maternal urinary cadmium

	Non-case Subcohort members	Gestational diabetes Cases	Tertile of maternal urinary cadmium (μg/g creatinine)		
	n=481	n=140	Low (<0.29)	Medium (0.29-0.42)	High (≥0.43)
	%	%	%	%	%
Maternal age (years)					
<25	3.5	5.0	6.6	3.0	1.4
25-34	62.2	50.7	64.5	64.0	51.4
≥35	34.3	44.3	28.9	33.0	47.2
Pre-pregnancy body mass index (kg/m^2)					
Under weight (<18.5)	6.2	1.4	4.1	5.0	6.1
Normal weight (18.5 to <25.0)	65.0	50.7	57.1	61.0	67.9
Overweight (25-30.0)	20.8	25.7	24.5	24.0	17.5
Obese (≥30.0)	7.9	22.1	14.3	10.0	8.5
Nulliparous	58.6	55.0	62.4	54.5	56.6
Race/Ethnicity					
Non-Hispanic white	83.6	69.8	83.8	83.0	75.8
African American	2.3	3.6	3.6	2.0	2.4
Asian	8.5	18.7	7.6	9.0	15.2
Other	5.6	7.9	5.1	6.0	6.6
Post high school education	96.2	93.9	95.2	94.8	97.4
Married	84.8	82.9	83.8	87.0	82.1
Preeclampsia [a]	2.5	8.0	4.1	3.5	2.8
Iron deficiency anemia [a]	1.9	4.3	3.1	2.0	2.4
Chronic hypertension	3.7	9.3	4.6	3.0	6.1
Family history of diabetes [b]	14.6	33.6	15.2	19.5	21.2
Family history of hypertension [c]	44.9	62.9	49.7	51.0	44.8

(table continues on next page, notes are located at end of table)

Table 1. Selected characteristics of the study cohort according to gestational diabetes status and tertile or maternal urinary cadmium (continued)

	Non-cases in Subcohort	Gestational Diabetes Cases	Tertile of maternal urinary cadmium (μg/g creatinine)		
	n=481	n=140	Low (<0.29)	Medium (0.29-0.42)	High (≥0.43)
	%	%	%	%	%
Smoking status [d]	29.8	28.2	26.8	32.3	29.5
Never	70.2	71.8	73.3	67.7	70.5
Former	21.6	18.3	19.3	22.4	22.1
Current	8.2	9.9	7.5	9.9	7.4
Ever	29.8	28.2	26.8	32.3	29.5
No leisure time physical activity	4.0	9.2	5.9	4.7	4.2
Dietary micronutrient intake deficiency [e]					
Low calcium (<1000 mg/day)	41.4	52.0	44.8	41.7	43.6
Low iron intake (<27 mg/day)	92.8	96.7	94.5	95.0	91.2
Low zinc intake (<11 mg/day)	47.9	48.0	49.7	45.0	48.6
Mean ± SD					
Dietary micronutrient intake (mg/day) [f]					
Calcium	1224 ± 618	1111 ± 654	1231 ± 595	1224 ± 635	1156 ± 640
Iron	14.4 ± 7.0	12.9 ± 6.2	13.8 ± 6.2	14.0 ± 6.7	14.5 ± 7.5
Zinc	12.6 ± 6.2	12.1 ± 6.0	12.2 ± 5.4	12.7 ± 6.2	12.8 ± 6.9
Average weekly servings of fish [f]	1.3 ± 1.2	1.3 ± 1.2	1.3 ± 1.2	1.4 ± 1.3	1.5 ± 1.5

SD= Standard deviation

a. During study pregnancy
b. Any primary or secondary family member with a diabetic condition
c. Any primary or secondary family member with a hypertensive condition
d. Self-reported smoking status

e. Estimated dietary intake of micronutrients from a semi-quantitative food frequency questionnaire was used designate daily consumption below the Institute of Medicine's daily recommended dietary allowance for pregnant women: calcium (1000 mg), iron (27 mg), zinc (11 mg) (114).
f. Dietary intake estimated from semi-quantitative food frequency questionnaire

Table 2. Odds Ratios for the association between tertiles of urinary cadmium and risk of gestational diabetes among women with urinary creatinine 30-300 mg/dL

Urinary Cadmium (μg/g creatinine)	n	n(%) [a]	aOR[b] (95% CI)
Low (<0.29)	197	32 (16.2)	1.00 (reference)
Middle (0.29-0.42)	200	44 (16.2)	1.64 (0.88, 3.05)
High (≥0.43)	212	52 (24.5)	2.07 (1.15, 3.73)
		p-trend	0.015

a. n(%) with outcome
b. Adjusted for age (years), pre-pregnancy BMI (kg/m^2), nulliparity, preeclampsia, chronic hypertension, family history of diabetes, race/ethnicity, family history of hypertension, total urinary arsenic and fish consumption

Table 3. Interaction of overweight and obesity status and urinary Cd on risk of gestational diabetes among women with urinary creatinine 30-300 mg/dL

Urinary Cd (μg/g creatinine) [Body mass index kg/m^2]	n	n(%)[a]	Stratified aOR[b] (95% CI)	Joint Model aOR[b] (95% CI)
Low (<0.29) [18.5< Body mass index <25]	102	10 (9.8)	1.00 (reference)	1.00 (reference)
High (≥0.29) [18.5< Body mass index <25]	236	49 (20.8)	2.33 (1.08, 5.02)	2.15 (1.02, 4.53)
Low (<0.29) [Body mass index ≥25]	69	17 (24.6)	1.00 (reference)	2.86 (1.17, 7.61)
High (≥0.29) [Body mass index ≥25]	103	33 (32.0)	1.36 (0.64, 2.91)	3.46 (1.54, 7.78)
			p for ϒ [c]	0.259

a. n (%) with outcome
b. Adjusted for age (years), pre-pregnancy BMI (kg/m^2), nulliparity, preeclampsia, chronic hypertension, family history of diabetes, race/ethnicity, family history of hypertension, total urinary arsenic and fish consumption
c. ϒ = interaction between overweight/obese status and cadmium body burden

Table 4. Odds Ratios for the association between tertiles of urinary cadmium and risk of gestational diabetes among women with urinary creatinine <300 mg/dL

Urinary Cadmium (μg/g creatinine)[a]	n	n (%)[b]	aOR[c] (95% CI)
Low (<30)	332	65 (19.6)	1.00 (reference)
Middle (0.30-0.42)	164	35 (21.3)	1.08 (0.63, 1.86)
High (≥0.43)	218	52 (23.9)	1.24 (0.79, 1.95)
		p-trend	0.355

a. Cutoffs for tertiles are based upon the distribution of urinary cadmium among members of the subcohort with creatinine levels <300 μg /dL
b. n (%) with outcome
c. Adjusted for age (years), pre-pregnancy BMI (kg/m^2), nulliparity, preeclampsia, chronic hypertension, family history of diabetes, race/ethnicity, family history of hypertension, gestational week of urine collection, total urinary arsenic and fish consumption

Table 5. Odds Ratios for the association between tertiles of urinary cadmium and risk of gestational diabetes stratified by overweight/obese status (BMI≥25) among women with urinary creatinine <300 mg/dL

Urinary Cd (μg/g creatinine) [Body mass index kg/m^2][a]	n	n(%)[b]	Stratified aOR[c] (95% CI)	Joint Model aOR[c] (95% CI)
Low (<0.30 [18.5< Body mass index <25]	212	67 (31.6)	1.00 (reference)	1.00 (reference)
High (≥0.30) [18.5< Body mass index <25]	100	32 (31.6)	1.77 (0.96, 3.26)	1.68 (0.94, 3.02)
Low (<0.30) [Body mass index ≥25]	458	78 (17.0)	1.00 (reference)	2.99 (1.37, 6.50)
High (≥0.30) [Body mass index ≥25]	100	17 (17.0)	0.92 (0.46, 1.82)	2.96 (1.55, 5.64)
			p for ϒ [d]	0.248

a. Cutoffs for tertiles are based upon the distribution of urinary cadmium among members of the subcohort with creatinine levels <300 μg /dL
b. n (%) with outcome
c. Adjusted for age (years), pre-pregnancy BMI (kg/m^2), nulliparity, preeclampsia, chronic hypertension, family history of diabetes, race/ethnicity, family history of hypertension, gestational week of urine collection, total urinary arsenic and fish consumption
d. ϒ = interaction between overweight/obese status and cadmium body burden

Table 6: Selected percentiles of urinary creatinine levels (mg/dL) in the Omega Study Subcohort and among reproductive aged women in NHANES III

	Ages 20-29 Years			Ages 30-39 Years			Ages 40-49 Years		
Percentile	Omega	White [a]	All [b]	Omega	White [a]	All [b]	Omega	White [a]	All [b]
10th	22.0	31.6	37.2	17.0	25.8	27.4	24.0	18.6	20.5
50th	78.0	120.3	132.8	60.0	103.3	106.9	63.5	78.6	89.6
90th	180.0	233.7	246.6	160.0	221.3	227.7	184.0	182.9	195.1

a. Data from the National Health and Nutrition Examination Survey for non-Hispanic white reproductive aged women (NHANES III) (94)
b. Data from the National Health and Nutrition Examination Survey all reproductive aged women (NHANES III) (94)

Table 7: Selected percentiles and geometric mean of urinary creatinine corrected cadmium levels (µg/g creatinine) in the Omega Study Subcohort and among women in NHANES III

		NHANES [a]		
	Omega Subcohort	Total	Females	non-Hispanic whites
Geometric Mean	0.39	0.21	0.25	0.22
50th percentile	0.38	0.21	0.25	0.22
75th percentile	0.51	0.41	0.49	0.43
90th percentile	0.73	0.68	0.80	0.69
95th percentile	0.90	0.94	1.06	1.00

a. Data from the National Health and Nutrition Examination Survey (NHANES III) (15)

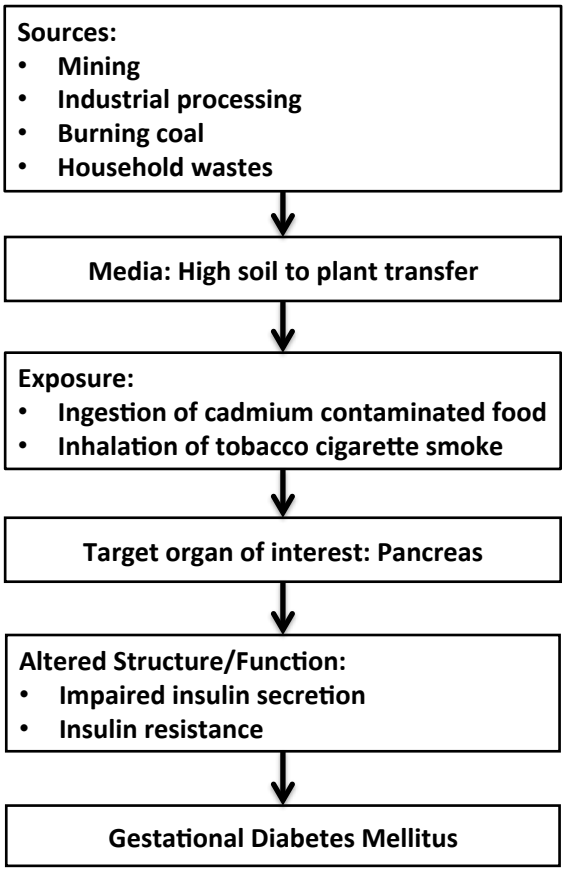


Figure 1: Conceptual model of cadmium exposure and gestational diabetes mellitus

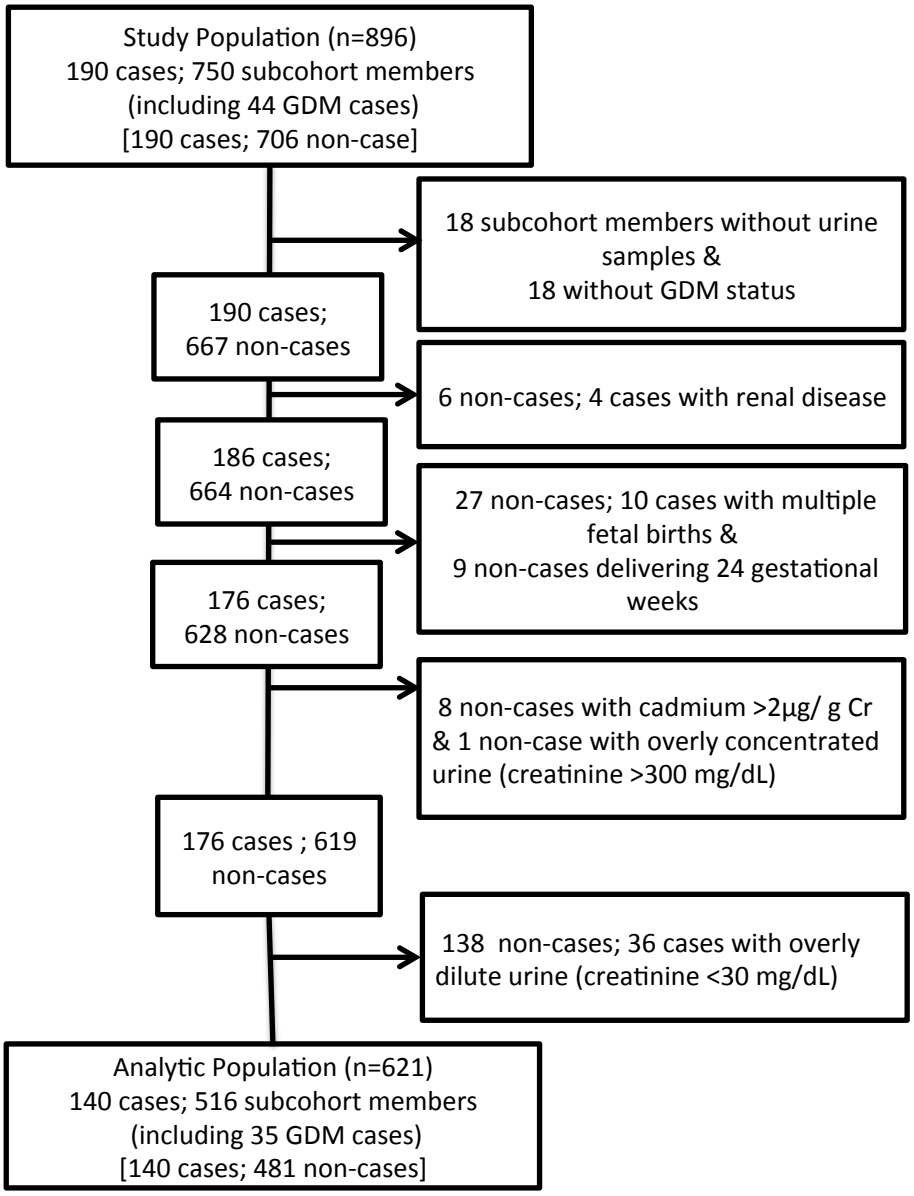


Figure 2: Exclusions based on missing exposure/outcome information, medical history, and pregnancy characteristics

Chapter 2: Sex-specific determinants of birth size among term infants in the Omega Study Population, Seattle/Tacoma, WA, 1996-2008

ABSTRACT

Background - Suboptimal birth size is associated with later life morbidity and mortality. Recent evidence supporting potential differences in response (both in scale and type) of male and female fetuses to poor intrauterine environment suggests that determinants of birth size may vary by infant sex. We investigated this relatively under-explored area of research.

Methods – The current study included 3033 term maternal-singleton pairs from the Omega Study (1996-2008), a large prospective cohort study investigating risk factors of pregnancy complications in Seattle and Tacoma, Washington. Data were collected from interviewer administered questionnaires, food frequency questionnaires, and medical records. Infant sex-stratified multivariable linear regression was used to assess potential risk factors (demographic, obstetric or medical, and nutritional or lifestyle) influencing birth anthropometric measurements (birthweight, ponderal index, birth length, and head circumference).

Results – Multiparity was associated with a larger increase in birthweight among females [β (95% CI) males: 184 g (88, 279 g), females: 256 g (173, 339 g)]. The effect of maternal pre-pregnancy obese or underweight status were associated with birthweight only among males [β(95% CI) obesity, males: 151 g (48, 253 g), females:74 g (-20, 167 g); underweight, males: -117 g (-200, -34 g) females: -40 g (-163, 83 g)]. The presence of a maternal hypertensive condition was associated with birthweight and ponderal index among males, but not females [β (95% CI) birthweight males: -82 g (-145,-20 g), females: -25 g (-83, 33 g); ponderal index males: -0.6 kg/m^3 (-1.0,-0.1 kg/m^3), females: -0.4 kg/m^3, (-0.8, 0.1 kg/m^3)]. Similarly, maternal family history of hypertension was associated with lower birthweight among males [β (95% CI) males: -59 g (-104, -14 g), females: -37 g (-80, 6 g)].

Conclusions – Our findings indicate that associations of parity, pre-pregnancy body mass index, and hypertensive disorders (and family history) with birth size differ for male and female infants. These findings, if replicated, highlight the need for evaluating sex-specific relations in future studies of modifiable and environmental risk factors of fetal growth.

INTRODUCTION

Suboptimal fetal growth has been consistently associated with short and long term morbidity and mortality over the life course (123–128). In the United States, approximately 8.3% of infants are low birthweight (LBW, <2500 g) (32,129), increasing their risk of type 2 diabetes (130) and cardiovascular disease during childhood and adulthood (123,124). On the other hand, macrosomia, defined either as birthweight >4000 g or >4500 g in different settings (131), has a prevalence of 1.1% (>4500 g) to 8.4% (>4000 g) in the US and is associated with long term risk of type 2 diabetes (132), obesity (36), and the metabolic syndrome (133–135). Further, fetal growth restriction (FGR), defined as falling short of inherent growth potential (39,136), can also be asymmetric, which is characterized by low weight for length. Both ponderal index, a measure of leanness in newborns (birthweight (kg)/ [birth length (m)]^3), and head circumference are useful indicators of asymmetric growth restriction (137–139). Infants with low ponderal index and those with small head circumferences have greater lifetime risk of cardiovascular mortality (140,141) and cognitive deficits persisting into adulthood (142–147).

Traditional risk factors for suboptimal (both symmetric and asymmetric) fetal growth can be broadly characterized as demographic, obstetric or medical, nutritional, and environmental or toxic (32,40). Demographic factors resulting in small size at birth include both low (<19 years) and high maternal age (≥ 35 years) (148–151), race/ethnicity other than non-Hispanic white (148,151,152), small maternal stature (149,150), and pre-pregnancy low (<18.5 kg/m^2) or high (≥25 kg/m^2) body mass index (BMI) (36,150). Maternal chronic hypertension (153,154) and nulliparity (149,150), are medical and obstetric factors associated with small size at birth. Nutritional factors and lifestyle factors, including low maternal weight gain (40,155) and micronutrient deficiencies (156–159), maternal alcohol intake and smoking, have also been

associated with reduced birthweight (32,160).Large birth size is associated with older maternal age (>35 years) (36), higher maternal pre-pregnancy BMI (161), male sex of infant (131), GDM or pre-existing maternal diabetes (128,131), and family history of diabetes (162). The determinants of asymmetric growth restriction are less well understood. Nulliparity, taller maternal height, and male infant sex, have been associated with asymmetric fetal growth (39,163,164).

Suboptimal fetal growth can result from *in utero* adaptive programming or restricted organ/tissue growth and development that follow a poor intrauterine environment (123,124). Villar and Belizan's "timing hypothesis" suggests that fetal size and proportionality convey the developmental timing of an insult to fetal growth (165). In general, first trimester insults result in proportionately small infants, whereas second trimester result in short infants, and third trimester insults result in lean (long and thin) infants (165). Accumulating evidence points to sex-specific differences in growth velocity that may differentially impact the effects of *in utero* insult among male and female fetuses (37,38). The observation that male neonates tend to have heavier birthweight, longer birth length, and leaner ponderal index than their female counterparts (39,40) suggests that fetal growth patterns differ between sexes. Additionally, infant sex-specific patterns of fetal growth have been observed in response to preeclampsia (42,43), severe maternal asthma (44–46), environmental heavy metal exposure (49,50), and maternal heavy smoking or environmental tobacco smoke (47,48). However, significant gaps remain in the understanding of sex-specific determinants of fetal growth in the context of traditional/common risk factors. Such investigations will inform the design, conduct, and interpretation of future studies assessing the effect of specific modifiable and environmental risk factors on fetal growth. If sex-specific determinants of fetal growth are identified, then summarizing effects across sexes may obscure

true associations. Therefore, using a prospectively assembled pregnancy cohort, we investigated sex-specific determinants of fetal growth.

METHODS

Study setting and population- The Omega Study is a large prospective cohort study based at the Center for Perinatal Studies at Swedish Medical Center in Seattle, WA, designed to investigate risk factors of pregnancy complications. Study participants (N=4344) were recruited from multiple prenatal care clinics affiliated with Swedish Medical Center and Tacoma General Hospital between 1996 and 2008. Eligible participants were women who speak English, were 18 years or older, initiated prenatal care prior to 20 weeks gestation, intended to carry the pregnancy to term, and planned to deliver at the study hospitals. Approximately 76% of eligible women participated, and 94% of enrolled subjects were successfully followed through delivery. Multiple fetal pregnancies (n=145), pregnancies that ended in preterm birth (<37 gestational weeks, n=352), miscarriage, spontaneous abortion, or stillbirth (n=419), and/or delivery of infants with gross congenital malformations (n=146) were excluded from analyses. For women who had more than one pregnancy (n=203) included in the parent Omega Study, we included the earliest chronological pregnancy. No birth anthropometrics were available for 82 neonates. The final analytic population included 3033 mother-offspring pairs (1514 male infants and 1519 female infants). Written informed consent was provided by all participants, and all procedures and study protocols were approved by the institutional review boards at Swedish Medical Center and Tacoma General Hospital.

Data collection- Study data were gathered by trained interviewers using 1) a structured questionnaire shortly after study enrollment (15 gestational weeks on average), 2) a validated, self-administered food-frequency questionnaire (FFQ) describing nutritional intake over the prior six months (three months before pregnancy through the first three months of pregnancy, on average) (166), and 3) post-delivery abstraction of maternal and infant medical records.

Study variables- Marital status, race/ethnicity, smoking habits (before and during pregnancy), early pregnancy alcohol consumption, and maternal pre-pregnancy weight were self-reported during the structured interview. Maternal age at delivery, height, parity, chronic medical conditions (hypertension, diabetes, renal or thyroid disease), family history of hypertension or diabetes, and infant sex were abstracted from medical records. Pre-pregnancy BMI (kg/m^2) was calculated as self-reported pre-pregnancy weight (in kilograms) divided by the square of height (in meters). Preeclampsia was defined according to the American College of Obstetricians and Gynecologists (ACOG) guidelines from 2000, using data from the medical record (167). Gestational diabetes (GDM) was diagnosed according to the American Diabetes Association 2003 guidelines (96). A combination of self-reported last menstrual period (LMP) and ultrasound data from 20 weeks gestation or earlier were used to estimate gestational week at delivery. When LMP and ultrasound dating were both available and agreed within 14 days, LMP was used to assign gestational age. For the 4% of women for whom the two dates differed by >14 days, the ultrasound-based date was used. The Institute of Medicine (IOM) guidelines were used to classify weight gain during pregnancy based on pre-pregnancy BMI as below optimal, optimal, or above optimal (Appendix: Table IV) (168). Daily caloric intake (kcal/day) and dietary intake of calcium, iron, and zinc (mg/day) were estimated using food composition tables from the University of Minnesota Nutrition Coding Center Nutrient Database (Nutrition Coordinating Center, Minneapolis, MN) (169). Low dietary intake of micronutrients was designated based on the IOM recommended dietary allowance (RDA) for calcium, iron, and zinc during pregnancy (114).

Assessment of birth size – Data on infant anthropometric measurements including birthweight (g), birth length (cm), and head circumference (cm) were abstracted from medical records.

Ponderal index was calculated to quantify the degree of asymmetry in birth size (164) as [birthweight (kg)/birth length $(m)^3$].

Statistical Analysis- We examined distributions, number (%) for categorical variables and mean (sd, standard deviation) for continuous variables, of maternal and infant characteristics stratified by infant sex within the following categories based upon the distribution of birthweight in our study population: below average (less than the infant sex-specific mean birthweight minus one sd), average (between the infant sex-specific mean birthweight minus one standard deviation and the mean plus one standard deviation), above average (greater than the infant sex-specific mean plus one standard deviation). Lowess smoothed (170) percentiles of birthweight, birth length, ponderal index, and head circumferences were plotted against gestational week at birth to visually assess general trends.

Univariable linear regression was performed to assess the independent relations between potential predictors and birth anthropometrics [Appendix (Table V-VII)]. Eight separate multivariable linear regressions were used to estimate coefficients and 95% confidence intervals (95% CI) for the association of the predictors of interest with birthweight, ponderal index, birth length, and head circumference stratifying by infant sex. Advanced maternal age (≥35 years), pre-pregnancy BMI [underweight (BMI <18.5), normal weight (18.5 <BMI <25.0), overweight (25<BMI<30.0), and obese (BMI ≥ 30.0)], and parity were selected as covariates based upon known associations with birth size (32,39). Additional factors tested in the multivariable models were parameterized as follows: continuous (centered at the mean for the study population) height, gestational week at birth, daily caloric intake, dietary micronutrient intake (calcium, iron, zinc), indicator variables for diabetes (none, GDM, pre-pregnancy diabetes), parity (0, 1, ≥2), and Institute of Medicine classifications for weight gain during pregnancy (below optimal,

optimal, above optimal)]; marital status (married/not married), race/ethnicity (Non-Hispanic white: yes/no), smoking during pregnancy (yes/no), alcohol consumption during pregnancy, chronic hypertension, renal disease, thyroid disease, family history of hypertension, family history of diabetes, preeclampsia, and dietary micronutrient intake below RDA (calcium, iron zinc). Because continuous covariates in the multivariable models were centered at the mean value for the study population, the constant term in each model can be interpreted as the mean birthweight, ponderal index, birth length, or head circumference among infants delivered by women without pregnancy complications and with mean values for continuous covariates in the population. A quadratic term for gestational week of delivery, which would account for diminishing fetal weight gain in the final weeks of pregnancy, was also tested. Predictors were included in the final multivariable linear regression models based upon the observed univariable associations, statistical significance of each coefficient ($p<0.05$), and comparison of the adjusted R^2 value for the model with and without each variable. If multiple parameterizations of a variable produced similar adjusted R^2 for the model, we used the most parsimonious parameterization. A two-sided $\alpha=0.05$ was used to assess statistical significance. All analyses utilized robust variance and were conducted using STATA 12 (107).

RESULTS

Selected maternal and offspring characteristics are summarized in Table 1. Women in the study population were predominately married (86.4%), non-Hispanic white (85.6%), nulliparous (64.2 %), and had education beyond high school (96.2%) (Table 1). Mean birthweight was heavier among males versus females (mean± standard deviation (sd), males: 3637±448 g; females: 3490±432 g. On average, males had slightly lower ponderal index than females (mean±sd, males: 26.2±3.2 kg/m^3; females: 26.6± 3.8 kg/m^3), and longer birth length (mean±sd, males: 52.9±2.5 cm; females: 50.9±2.6 cm). Mean head circumference among males was also slightly greater than for females (mean±sd, males: 35.2±1.7 cm; females: 34.6±1.7 cm).

Estimated coefficients (β) and 95% confidence intervals (95% CI) from the multivariable linear regression analyses are presented in Tables 2 and 3. All eight of the final infant sex-stratified multivariable models included control for advanced maternal age, race/ethnicity, parity, and smoking during pregnancy. With the exception of the ponderal index models, all models controlled for gestational age at delivery. The birthweight and ponderal index models further controlled for pre-pregnancy BMI, diabetic conditions, any hypertensive condition, family history of hypertension, pregnancy weight gain by IOM classification, low calcium intake, and low zinc intake. The birth length models also included adjustment for maternal height, diabetic conditions, any hypertensive condition, family history of hypertension, pregnancy weight gain by IOM classification, and average daily dietary calcium intake. In addition to the core variables, the head circumference model adjusted for pre-pregnancy BMI, delivery by Caesarean section, and birthweight (kg). The final models accounted for 21-22% of the variation in birthweight (males: adjusted R^2=0.221; females: adjusted R^2=0.213), 2-4% of the variation in ponderal index (males: adjusted R^2=0.039, females: adjusted R^2=0.018), 12-16 of the variation in birth

length (males: adjusted R^2=0.159; females: adjusted R^2=0.127), and 30% of the variation in head circumference (males: adjusted R^2=0.310, females: adjusted R^2=0.292) . Selected infant sex-specific percentiles of birth anthropometrics are presented in Figures 1-4.

Male infants born to obese mothers had a significantly higher birthweight (β 151 g, 95% CI 48, 253 g) compared with male infants born to normal weight mothers (Table 2), whereas female infants born to obese mothers did not have a significantly higher birthweight (β: 74 g; 95% CI -20, 167 g) compared with their counterparts (Table 3). Similarly, the reduction in birthweight among infants born to underweight mothers was significant among males (β: -117 g, 95% CI -200, -34 g) but not females (β: -40 g, 95% CI -163, 83 g) (Tables 2 & 3).

We observed significant increases in birthweight among male and female infants born to women who had a prior birth compared with women who were nulliparous (β: 132 g, 95% CI 79,185 g for males and β: 151g, 95% CI 102, 200 g for females). However, parity related increases in birth length were observed among females (β: 0.3 cm, 95% CI 0.01, 0.7 cm) but not males (β: 0.2 cm, 95% CI -0.1, 0.5 cm) (Table 2 & Table 3).

Compared to males born to mothers without a hypertensive condition, males born to mothers with history of any maternal hypertension had a lower birthweight and ponderal index (β: -82 g, 95% CI -145,-20 g, for birthweight and β:-0.6 kg/m^3 , 95% CI -1.0,-0.1 kg/m^3 for ponderal index); these associations were not observed among females. Similar relations were observed for maternal family history of hypertension where the associations of birthweight and ponderal index were significant only among males.

Maternal GDM-associated increases in birthweight and birth length were observed among female infants (β: 200 g, 95% CI 88, 312 g for birthweight and β: 0.6 cm, 95% CI 0.1, 1.2

cm for birth length) but not males (β: 48 g, 95% CI -80, 176 g for birthweight and β: 0.2 cm, 95% CI -0.4, 0.8 cm for birth length). Pre-existing diabetes related increase in birthweight was only statistically significant among male infants (β: 403 g, 95% CI 103, 704 g, females: 321 g, -96, 738 g).

For both male and female neonates, each 1 week increase in gestational age at birth among term infants corresponded to an approximately 142 g increase in birthweight, 0.7 cm longer birth length, and roughly 0.12 cm greater head circumference (birthweight: β_{males}: 144 g, 95% CI 125, 162 g, $\beta_{females}$:139 g, 95% CI 120, 158 g; birth length: β_{males}:0.7 cm, 95% CI 0.6, 0.8 cm, $\beta_{females}$:0.7 cm, 95% CI 0.5,0.8 cm; head circumference: β_{males}:0.13 cm, 95% CI 0.06, 0.20 cm, $\beta_{females}$:0.10 cm, 95% CI 0.02, 0.18 cm) (Table 2 & Table 3).

DISCUSSION

In the current study, we found that associations of fetal growth indicators with traditional/common risk factors may differ for male and female infants. These include pre-pregnancy BMI (with birthweight among males), parity (with birth length among females), GDM (with birthweight and birth length among females), pre-existing diabetes (with birthweight among males), and hypertensive conditions (with birthweight and ponderal index among males) with fetal growth indicators.

Our observations are consistent with prior work suggesting that women with lower pre-pregnancy BMI deliver infants with lighter birthweight and lower ponderal index (149,150), whereas obese women are more likely to deliver larger neonates (36,161). However, the extremes of maternal pre-pregnancy BMI appeared to be associated with birth size only among males in our study. Similarly, in a large prospective study based in Spain, males born to underweight mothers (pre-pregnancy BMI <18.5 kg/m^2) had a 46% greater risk than females of weighing less than the 10^{th} percentile for sex-specific birthweight. Likewise among obese mothers, males had and a 48% greater risk of being heavier than the 90^{th} percentile for sex-specific birthweight, compared to females (171). Using longitudinal fetal ultrasound measurements, Lampl et al. described a sex and gestational week specific association between maternal anthropometrics (height, weight, BMI) and fetal growth (weight) rates (172). Among males delivered by the tallest mothers, fetal weight growth was accelerated prior to 18 gestational weeks. Among women with the heaviest weight and also those with the greatest pre-pregnancy BMI the fetal weight growth rate was slower up to 18 gestational weeks and then accelerated. Compared to females, male fetal weight growth rates were influenced more by maternal weight among shorter mothers and by maternal height among mothers with lower

weight. Birthweight also ultimately varied. Among short mothers with low pre-pregnancy weight, males were 60 g heavier in birthweight than females. Among short mothers with high pre-pregnancy weight, males were 150 g heavier than females at birth, whereas among tall mothers with lower pre-pregnancy weight, males were 191 g heavier (172). By extension, the findings of Lampl et al. suggest that the timing of an insult would not only affect the male and female fetus differently, but that maternal height and weight would also play a role in fetal growth response. These collective observations of infant-sex specific effects of maternal anthropometrics suggest that the roll of such factors should be carefully assessed in future research.

Increasing parity was associated with larger size at birth, as illustrated by increases in birthweight among males and females and birth length among females. Compared with primiparous women, multiparous women had infants that were 132 g and 151 g higher in birth weight, for males and females, respectively. These estimates are in agreement with those from a retrospective longitudinal study in England, which observed an average crude increase in infant birthweight of 138 g between a woman's first and second pregnancy (173). The increasing trend in birthweight with increasing parity is consistent with trends in birthweight observed from retrospective longitudinal study of multiparas (174).

Maternal hypertensive disorders have been previously observed to reduce offspring birth size (153,154,175). Investigators in Sweden conducted a prospective cohort study (n=521) which suggested that mean offspring birthweight was decreased by 39 g among mothers with non-proteinuric gestational hypertension and by 200 g among women with mild preeclampsia, compared to normotensive women (175). In our study the presence of any maternal hypertensive disorder was associated with an 82 g decrease in birthweight among males, but not females.

Infant sex-specific birthweight differences in response to mild preeclampsia have been previously observed (42). In contrast to our observations, a small (n=66) prospective cohort study observed that females born at term to mothers with mild preeclampsia had lower birthweight percentile (28^{th} percentile) than females with normotensive mothers (55^{th} percentile; $p<0.001$), whereas birthweight percentile for males did not differ by maternal preeclampsia (42). Inconsistencies in findings between the previous study and the current study may be due to study population differences (our study population consists of only term births which may have excluded severe forms of preeclampsia that lead to preterm birth (176)) and the combining of multiple forms of hypertension in our analysis, which may be uniquely linked to distinct etiologies of fetal growth impairment.

Family history of hypertension was also related to a reduction in birthweight and birth length among male infants in our study. Prior studies have suggested that even among normotensive pregnancies, impaired maternal cardiovascular function is associated with decreasing birthweight (177) and asymmetric growth restriction (178). It has further been demonstrated that subclinical changes to maternal cardiovascular factors can adversely impact uteroplacental perfusion (179). Taken together, this may suggest that women with a family history of hypertension experience a subclinical rise in blood pressure during pregnancy that may adversely impact uteroplacental blood flow and thus limit fetal growth. This is the first report where sex-specific differences in fetal growth markers have been demonstrated in relation to family history of hypertension. Other replication studies and further investigations are needed. Disruption of uteroplacental perfusion due to maternal cardiovascular function may mimic conditions of under nutrition (179). Experimental studies in baboons suggest that female fetuses are more resilient when faced with nutrient deprivation *in utero* due to changes in genetic and

transcriptomic regulation of pathways related to energy regulation (e.g. insulin signaling) (180,181). Taken together, this may suggest that if women with a family history of hypertension experience a subclinical rise in blood pressure during pregnancy resulting in diminished uteroplacental perfusion, the female fetus may be better able to adapt than the male fetus. However, this line of reasoning is speculative and further study is necessary to understand the potential underlying mechanism.

Gestational diabetes and pre-pregnancy diabetes are the most common risk factors of macrosomia (36,161) and high ponderal index (182). Mothers with diabetic conditions, either GDM or pre-existing diabetes, had higher birthweight male and female infants, respectively, in our study, compared with their respective non-diabetic mothers. In contrast, prior research suggests that males born to mothers with GDM have a 67% increased risk of macrosomia (OR=1.67, 95% CI 1.12, 2.49), compared to a 4% increased risk among females (OR=1.04, 95% CI 0.56, 1.96). However, the same research noted a 16% increased risk in large for gestational age (LGA), defined as sex-specific birthweight for gestational age above the 90^{th} percentile of the relevant fetal growth curve, among female neonates born to women with GDM (OR=1.16, 95% CI 0.85, 1.59) and only a 1% increase in risk of LGA among male infants (OR=1.01, 95% CI 0.75, 1.37) (171). We conducted an exploratory multivariable logistic regression, adjusting for the same variables as our birthweight analyses, to assess the effect of maternal diabetic status on macrosomia in our population (>4000g, 276 males and 167 females). Compared to non-diabetic mothers, among mothers with GDM we observed a 3.2-fold and 1.5-fold increased risk of macrosomia among females and males, respectively ($OR_{females}$=3.23, (95 % CI 1.6, 6.5; OR_{males}=1.45, 95% CI 0.70, 3.00). Among mothers with pregestational diabetes, we observed 7-fold and 5.6-fold increased risk of macrosomia among females and males, respectively

($OR_{females}$= 7.00, 95% CI 1.10, 44.45; OR_{males}=5.62, 95% CI 1.40, 22.60). We wish to interpret our observations among diabetic mothers with caution due to the low prevalence of pregestational diabetes in our study population (0.7%). However, if replicated, these findings may advance the idea that timing of insult may elicit different effects for males and females. Pregestational diabetes would expose the developing fetus to maternal glucose intolerance over the full course of pregnancy, whereas with GDM an increasing level of dysglycemia would occur as pregnancy progresses. Differences in treatment of the diabetic condition and control of maternal glucose would have to be carefully considered.

Maternal smoking is typically associated with a 160-190 g reduction in birthweight (47,183). We did not observe significant smoking-related changes in fetal growth markers among males and females in our study. This may be due to misclassification (due to social-desirability impacting reporting of smoking in our study population) or the low prevalence of smoking among women in our study population, both of which can significantly reduce power of our study to detect significant associations. Unfortunately, we did not have available data to distinguish between heavy and light smokers.

Advanced maternal age, race/ethnicity, and gestational age are well-established as determinants of fetal growth (32,39,40,136), and we observed consistent effects of these factors for both sexes. Likewise above optimal maternal weight gain was associated with larger birthweight and birth length for both male infants (β: 116 g birthweight, 95% CI 116, 216; β 0.4 cm birth length, 95% CI 0.1, 0.7) and female infants (β: 183 g birthweight, 95% CI 135, 231; β: 0.8 cm birth length, 95% CI 0.5, 1.1), in accordance with previous literature (184).

The concept of infant sex-specific genetic, proteomic, and hormonal profiles of the fetal-placental unit (185) supports some of our findings of differences in associations of risk factors

with fetal growth markers among males and females. Studies of preeclampsia (42,43) and maternal asthma (44,45) suggest that sex-dependent mechanisms are set in motion subsequent to intrauterine insult (185). Clifton et al. propose that placenta of male infants is more efficient in general, but have less reserve capacity in the face of multiple stressors. In contrast, placenta of female infants responds to an adverse intrauterine environment with adjustments that allow for sustained but symmetrically smaller growth (185). These studies illustrate the need to consider infant sex in study design and analysis of questions related to fetal growth. Failure to account for underlying sex-specific heterogeneity may obscure true associations, leading to inaccurate interpretation of results and limiting the investigator's ability to assess underlying mechanisms. As the Intergrowth Study (186) works toward the creation of universal prescriptive fetal growth standards to advance monitoring and evaluation efforts geared at improving maternal and infant health, a fuller understanding of infant sex-specific dimensions of fetal growth will be essential. Likewise, the US's National Institute of Child Health and Human Development (NICHD) Fetal Growth Study (187) will draw on such knowledge. In particular, the suggestion that maternal anthropometrics may affect male and female growth differently, and that effects may be dissimilar at different time points in gestation has consequences for determining if fetal growth is inadequate (172).

Our research drew on several strengths. The prospective nature of the Omega Study, high follow-up rates (95%), and availability of extensive covariate data within a well-characterized cohort are some of these strengths. There are some limitations that should be considered when interpreting out study results. Complex interrelationships exist among determinants of size at birth, and there may be residual confounding of our estimates by unknown or unmeasured factors. Generalizability may be reduced since women in the Omega Study are generally white,

married, and prosperous, reflective of the underlying population that utilizes Swedish Medical Center and Tacoma General Hospital. Although we had strong *a priori* motivation from previous literature for assessing the many factors under study, we may have encountered a small number of chance findings due to the large number of statistical tests that we performed. Additionally, there was a lack of variation among study subjects for some key factors, including maternal race/ethnicity, education, and smoking. Misclassification of study variables is possible due to the self-reported nature of many of our covariates. However, such misclassification is likely to be non-differential. Finally, we were underpowered for formal statistical assessment of effect modification by infant sex.

In summary, our findings indicate that associations of parity, pre-pregnancy body mass index, and hypertensive disorders (and family history) with birth size differ for male and female infants. These findings, if replicated, highlight the need for evaluating sex-specific relations in future studies of modifiable and environmental risk factors for impaired fetal growth.

Table 1. Maternal and offspring sociodemographic, obstetric and medical, and nutritional characteristics in the Omega Study, Seattle and Tacoma, Washington, 1996-2008

	All	Male infants			Female infants		
		Below Average [a] (<3130 g)	Average (3130-4065 g)	Above average (>4065 g)	Below average [a] (<3016 g)	Average (3016-3902 g)	Above average (>3902 g)
n=	3033	232	1042	240	220	1,067	232
Maternal factors	%	%	%	%	%	%	%
Demographics							
Age (≥35 years)	32.4	34.9	33.6	31.3	25.9	33.2	28.0
Pre-pregnancy BMI (kg/m^2)[b]							
Under weight (<18.5)	4.3	9.9	4.8	1.3	5.9	3.7	0.4
Normal weight (18.5 -24.9)	70.4	68.1	71.1	62.5	73.2	73.2	62.9
Overweight (25-29.9)	16.7	16.4	16.6	23.3	11.8	14.6	25.0
Obese (≥30.0)	8.6	5.6	7.5	12.9	9.1	8.6	11.6
Non-Hispanic white race/ethnicity	85.6	81.0	86.2	83.7	83.6	86.6	86.6
Post High School Education	96.2	96.4	96.7	94.7	95.7	96.3	95.1
Married	86.4	88.4	86.4	86.7	85.5	85.8	87.1
Obstetric factors [c]							
Nulliparous	64.2	65.5	63.9	60.0	74.1	63.2	64.2
Planned pregnancy	80.4	80.2	80.3	83.6	81.8	80.0	78.4
Gestational Diabetes	4.7	2.4	4.2	5.3	3.6	5.0	7.1
Preeclampsia	2.4	3.9	1.1	2.1	2.3	2.8	4.8
Pregnancy-induced hypertension	12.3	16.0	13.1	13.3	11.8	11.3	9.1
Iron deficiency anemia	4.0	4.7	3.1	2.1	6.4	4.4	5.6

(table continues on next page, notes are located at the end of the table)

Table 1. Maternal and offspring sociodemographic, obstetric and medical, and nutritional characteristics in the Omega Study, Seattle and Tacoma, Washington, 1996-2008 (continued)

	All	Male infants			Female infants		
		Below Average (<3130 g)	Average (3130-4065 g)	Above average (>4065 g)	Below average (<3016 g)	Average (3016-3902 g)	Above average (>3902 g)
n=	3033	232	1042	240	220	1,067	232
	%	%	%	%	%	%	%
Medical factors							
Chronic hypertension	3.2	2.4	2.3	3.0	3.5	4.0	3.9
Pre-pregnancy Diabetes	0.7	0.6	0.5	1.3	0.5	0.4	1.7
Any hypertensive disorder [d]	17.8	21.3	16.3	18.6	16.5	17.4	17.0
Renal disease	0.9	1.3	0.7	0.8	1.8	0.9	na
Thyroid disease	8.7	9.9	8.8	10.0	7.3	8.3	8.6
Family history of diabetes [e]	14.4	14.7	14.1	16.2	16.8	14.2	12.5
Family history of hypertension [f]	49.2	57.3	50.1	45.4	53.6	46.4	49.6
Nutritional factors							
Took prenatal vitamins	97.2	98.2	96.6	96.9	97.1	97.6	97.8
No leisure-time physical activity	3.9	3.1	4.3	5.3	3.9	3.1	4.4
IOM weight gain guidelines [g]							
Below optimal	10.1	19.0	8.3	2.5	21.3	10.6	4.8
Optimal	32.2	39.0	31.8	17.6	44.0	34.0	23.4
Above optimal	57.6	42.0	59.8	79.8	34.7	55.4	71.9
Dietary micronutrient intake [h]							
Low calcium intake	42.9	47.8	43.7	43.7	49.5	40.9	42.7
Low iron intake	94.8	96.1	95.2	95.2	92.6	94.5	94.0
Low zinc intake	46.8	51.2	48.3	48.3	53.5	44.7	44.0

(table continues on next page, notes are located at the end of the table)

Table 1. Maternal and offspring sociodemographic, obstetric and medical, and nutritional characteristics in the Omega Study, Seattle and Tacoma, Washington, 1996-2008 (continued)

	All	Male infants			Female infants		
		Below Average (<3130 g)	Average (3130-4065 g)	Above average (>4065 g)	Below average (<3016 g)	Average (3016-3902 g)	Above average (>3902 g)
n=	3033	232	1042	240	220	1,067	232
	%	%	%	%	%	%	%
Lifestyle factors							
Smoked during pregnancy [i]	6.0	7.2	6.1	6.2	5.8	5.4	6.7
Consumed alcohol during pregnancy [j]	25.4	26.3	22.4	31.7	26.4	27.0	23.3
Mean (SD)							
Offspring Characteristics							
Birthweight (g)	3529 (461)	2897 (213)	3585 (213)	4333 (247)	2785 (221)	3445 (236)	4166 (228)
Gestational week at delivery	39.2 (1.2)	38.3 (1.1)	39.2 (1.1)	39.8 (1.1)	38.6 (1.2)	39.3 (1.1)	39.8 (1.0)
Ponderal index (kg/m3)	26.4 (3.5)	24.7 (3.2)	26.1 (3.1)	27.6 (3.1)	24.5 (3.6)	26.6 (3.6)	28.4 (3.8)
Birth length (cm)	51.2 (2.6)	49.1 (2.1)	51.7 (2.2)	54.1 (2.0)	48.6 (2.4)	50.7 (2.3)	52.9 (2.2)
Head circumference (cm)	34.8 (1.7)	33.7 (1.5)	35.1 (1.6)	36.5 (1.4)	33.1 (1.4)	34.5 (1.6)	36.0 (1.3)
Gestational week of 1st prenatal care visit	8.9 (2.1)	8.9 (1.9)	8.9 (1.9)	8.8 (2.1)	9.0 (1.9)	8.8 (2.2)	9.0 (1.9)

SD= Standard deviation

a. Groups are based on the distribution of birthweight in the study population below average (less than the infant sex-specific mean birthweight minus one standard deviation), average (between the infant sex-specific mean birthweight minus one standard deviation and the mean plus one standard deviation), above average (greater than the infant sex-specific mean plus one standard deviation)
b. Body mass index (BMI) based upon World Health Organization classifications (188)
c. Obstetric factors pertaining to the study pregnancy
d. Includes pregnancy-induced hypertension, preeclampsia and chronic hypertension
e. Any maternal primary or secondary relative with hypertension
f. Any maternal primary or secondary relative with a diabetic condition

g. Institute of Medicine (IOM) guidelines for weight gain during pregnancy (168)
h. Low intake designated by consuming less than the IOM daily recommended dietary allowance for pregnant women: calcium (1 g), iron (27 mg), zinc (11 mg) (114)
i. Maternal self-reported smoking of tobacco cigarettes during the study pregnancy
j. Maternal self-reported alcohol consumption during the study pregnancy

Table 2. Estimated coefficients and 95% confidence intervals from multivariable linear regression models examining the relation between predictors of interest and birth anthropometrics of **male infants** in the Omega Study, Seattle and Tacoma, Washington, 1996-2008

Parameter	Birthweight (g) [a] β [95% CI]	Ponderal Index (kg/m^3) [b] β [95% CI]	Birth length (cm) [c] β [95% CI]	Head circumference (cm) [d] β [95% CI]
n	1318	1306	1307	1404
Constant [e]	3514 [3457, 3571]*	25.5 [25.0, 25.9]*	51.6 [51.3, 51.9]*	34.9 [34.7, 35.0]*
Demographics				
Age ≥35 years	-13 [-62, 35]	-0.2 [-0.5, 0.2]	0.02 [-0.25, 0.30]	0.04 [-0.13, 0.21]
Pre-pregnancy BMI (kg/m^2) [f]				
Underweight (<18,5)	-117 [-200, -34]*	-1.0 [-1.7, -0.2]*	---	0.07 [-0.28, 0.42]
Normal (18.5-24.9)	0 [reference]	0 [reference]	---	0 [reference]
Overweight (25-29.9)	47 [-23, 117]	-0.06 [-0.6, 0.4]	---	-0.10 [-0.29, 0.10]
Obese	151 [48, 253]*	1.0 [0.2, 1.7]*	---	0.01 [-0.27, 0.29]
Height (m)	---	---	6.6 [4.6, 8.7]*	---
Race/ethnicity [g]	-62 [-130, 6]	-0.2 [-0.7, 0.3]	0.2 [-0.2, 0.5]	-0.24 [-0.46, -0.03]*
Obstetric & medical factors				
Parity				
Nulliparous	0 [reference]	0 [reference]	0 [reference]	0 [reference]
1	132 [79, 185]*	0.7 [0.3, 1.2]*	0.2 [-0.1, 0.5]	0.16 [-0.01, 0.33]
≥2	184 [88, 279]*	1.0 [0.3, 1.7]*	0.3 [-0.2, 0.8]	0.005 [-0.25, 0.26]
Caesarean-section	---		---	0.43 [0.25, 0.60]*
Diabetic conditions				
None	0 [reference]	0 [reference]	0 [reference]	---
Gestational diabetes	48 [-80, 176]	0.2 [-0.6, 1.0]	0.2 [-0.4, 0.8]	---
Pre-existing diabetes	403 [103, 704]*	1.6 [-0.4, 3.5]	1.0 [-0.7, 2.7]	---
Any hypertension [h]	-82 [-145, -20]*	-0.6 [-1.0, -0.1]*	-0.01 [-0.3, 0.3]	---
Family history of hypertension [i]	-59 [-104, -14]*	0.4 [0.03, 0.7]*	-0.5 [-0.8, -0.2]*	---

(table continues on the next page; notes are located at the end of the table)

Table 2. Estimated coefficients and 95% confidence intervals from multivariable linear regression models examining the relation between predictors of interest and birth anthropometrics of **male infants** in the Omega Study, Seattle and Tacoma, Washington, 1996-2008 (continued)

Parameter	Birthweight (g) [a] β [95% CI]	Ponderal Index (kg/m^3) [b] β [95% CI]	Birth length (cm) [c] β [95% CI]	Head circumference (cm) [d] β [95% CI]
n	1318	1306	1307	1404
Nutritional & lifestyle factors				
IOM weight gain guidelines [j]				
Below optimal	-76 [-162, 9]	-0.4 [-1.1, 0.3]	-0.1 [-0.6, 0.4]	---
Optimal	0 [reference]		0 [reference]	---
Above optimal	166 [116, 216]*	0.6 [0.2, 1.0]*	0.4 [0.1, 0.7]*	---
Dietary micronutrient intake [k]				
Calcium (g/day)	---		0.2 [0.001, 0.4]*	---
Low calcium intake	-31 [-87, 25]	0.1 [-0.3, 0.5]	---	---
Low zinc intake	-9 [-64, 46]	-0.02 [-0.4, 0.4]	---	---
Smoker [l]	-81 [-175, 14]	-0.2 [-1.0, 0.5]	-0.3 [-0.8, 0.3]	-0.11 [-0.40, 0.18]
Neonatal characteristics				
Gestational age (weeks)	144 [125, 162]*		0.7 [0.6, 0.8]*	0.13 [0.06, 0.20]*
Birthweight (kg)	---	---	---	1.78 [1.59, 1.97]*
Adjusted R^2 for model	0.221	0.039	0.159	0.310

β=estimated coefficient; 95% CI=95% Confidence interval; *p<0.05

Each outcome was examined in a separate multivariable linear regression models.

a. Covariates in the birthweight model included advanced maternal age, pre-pregnancy body mass index, race/ethnicity, parity, diabetic conditions, any hypertensive condition, family history of hypertension, pregnancy weight gain, low dietary calcium intake, low dietary zinc intake, smoking during pregnancy, and gestational age.
b. Covariates in the ponderal index model included advanced maternal age, pre-pregnancy body mass index, maternal race/ethnicity, parity, diabetic conditions, any hypertensive condition, family history of hypertension, pregnancy weight gain, low dietary calcium, iron, and zinc intake, and smoking during pregnancy.

c. Covariates in the birth length model included advanced maternal age, maternal height, maternal race/ethnicity, parity, diabetic conditions, any hypertensive condition, family history of hypertension, pregnancy weight gain, daily calcium intake, smoking during pregnancy, and gestational age at delivery.
d. Covariates in the head circumference model included advanced maternal age, pre-pregnancy body mass index, maternal race/ethnicity, parity, delivery via Caesarean section, smoking during pregnancy, birthweight, and gestational age.
e. The constant represents the mean level of the outcome for an average uncomplicated pregnancy in the study population.
f. Body mass index (BMI) based upon World Health Organization classifications (188)
g. Not of non-Hispanic white race/ethnicity
h. Includes pregnancy-induced hypertension, preeclampsia and chronic hypertension
i. Any maternal primary or secondary relative with chronic hypertension,
j. Institute of Medicine (IOM) guidelines for weight gain during pregnancy (168)
k. Low intake designated by consuming less than the IOM daily recommended dietary allowance for pregnant women: calcium (1 g), iron (27 mg), zinc (11 mg) (114)
l. Maternal self-reported smoking of tobacco cigarettes during the study pregnancy (current smoker)

Table 3. Estimated coefficients and 95% confidence intervals from multivariable linear regression models examining the relation between predictors of interest and birth anthropometrics of **female infants** in the Omega Study, Seattle and Tacoma, Washington, 1996-2008

Parameter	Birthweight (g) [a] β [95% CI]	Ponderal Index (kg/m^3) [b] β [95% CI]	Birth length (cm) [c] β [95% CI]	Head circumference (cm) [d] β [95% CI]
n	1337	1314	1316	1389
Constant [e]	3330 [3280, 3380]*	26.3 [25.8, 26.8]*	50.3 [49.9, 50.8]*	34.31 [34.2, 34.4]*
Demographics				
Age ≥35 years	-13 [-60, 34]	-0.2 [-0.7, 0.2]	0.1 [-0.19, 0.38]	-0.04 [-0.20, 0.12]
Pre-pregnancy BMI (kg/m^2) [f]				
Underweight (<18,5)	-40 [-163, 83]	0.4 [-0.5, 1.4]	---	0.01 [-0.40, 0.42]
Normal (18.5-24.9)	0 [reference]	0 [reference]	---	0 [reference]
Overweight (25-29.9)	46 [-17, 108]	0.7 [0.01, 1.4]*	---	-0.01 [-0.22, 0.19]
Obese	74 [-20, 167]	0.6 [-0.1, 1.2]	---	0.09 [-0.14, 0.32]
Height (m)			5.0 [3.0, 6.9]*	
Race/ethnicity [g]	-37 [-104, 30]	0.1 [-0.6, 0.7]	0.01 [-0.4, 0.5]	-0.13 [-0.33, 0.06]
Obstetric & medical factors				
Parity				
Nulliparous				
1	151 [102, 200]*	0.7 [0.2, 1.2]*	0.3 [0.01, 0.7]*	0.16 [0.004, 0.33]*
≥2	256 [173, 339]*	1.2 [0.4, 1.9]*	0.4 [0.01, 0.9]*	0.37 [0.12, 0.63]*
Caesarean-section delivery				0.64 [0.48, 0.80]*
Diabetic conditions				
None	0 [reference]	0 [reference]	0 [reference]	---
Gestational diabetes	200 [88, 312]*	0.5 [-0.4, 1.5]	0.6 [0.1, 1.2]*	---
Pre-existing diabetes	321 [-96, 738]	1.6 [-0.9, 4.1]	0.4 [-1.5, 2.3]	---
Any hypertension [h]	-25 [-83, 33]	-0.4 [-0.8, 0.1]	0.1 [-0.2, 0.4]	---
Family history of hypertension [i]	-37 [-80, 6]	-0.1 [-0.5, 0.3]	-0.1 [-0.4, 0.1]	---

(table continues on the next page; notes are located at the end of the table)

Table 3. Estimated coefficients and 95% confidence intervals from multivariable linear regression models examining the relation between predictors of interest and birth anthropometrics of **female infants** in the Omega Study, Seattle and Tacoma, Washington, 1996-2008 (continued)

Parameter	Birthweight (g)[1] β [95% CI]	Ponderal Index (kg/m^3)[2] β [95% CI]	Birth length (cm)[3] β [95% CI]	Head circumference (cm)[4] β [95% CI]
n	1337	1314	1316	1389
Nutritional & lifestyle factors				
IOM weight gain guidelines [j]				
Below optimal	-119 [-189, -48]*	-0.6 [-1.2, 0.1]	-0.2 [-0.6, 0.2]	---
Optimal				
Above optimal	183 [135, 231]*	0.1 [-0.4, 0.6]	0.8 [0.5, 1.1]*	---
Dietary micronutrient intake [k]				
Calcium (g/day)	---	---	-0.1 [-0.3, 0.1]	---
Low calcium intake	-2 [-54, 49]	0.04 [-0.6, 0.6]	---	---
Low iron intake	---	---	---	---
Low zinc intake	-18 [-69, 33]	-0.4 [-1.0, 0.1]	---	---
Smoker [1]	25 [-66, 117]	0.7 [-0.3, 1.7]	-0.3 [-1.0, 0.3]	0.10 [-0.19, 0.39]
Offspring characteristics				
Gestational age (weeks)	139 [120, 158]*	---	0.7 [0.5, 0.8]*	0.10 [0.02, 0.18]*
Birthweight (kg)				1.82 [1.61, 2.02]*
Adjusted R^2 for model	0.213	0.018	0.127	0.292

β=estimated coefficient; 95% CI=95% Confidence interval; *p<0.05

Each outcome was examined in a separate multivariable linear regression models.

a. Covariates in the birthweight model included advanced maternal age, pre-pregnancy body mass index, race/ethnicity, parity, diabetic conditions, any hypertensive condition, family history of hypertension, pregnancy weight gain, low dietary calcium intake, low dietary zinc intake, smoking during pregnancy, and gestational age.

b. Covariates in the ponderal index model included advanced maternal age, pre-pregnancy body mass index, maternal race/ethnicity, parity, diabetic conditions, any hypertensive condition, family history of hypertension, pregnancy weight gain, low dietary calcium, iron, and zinc intake, and smoking during pregnancy.

c. Covariates in the birth length model included advanced maternal age, maternal height, maternal race/ethnicity, parity, diabetic conditions, any hypertensive condition, family history of hypertension, pregnancy weight gain, daily calcium intake, smoking during pregnancy, and gestational age at delivery.
d. Covariates in the head circumference model included advanced maternal age, pre-pregnancy body mass index, maternal race/ethnicity, parity, delivery via Caesarean section, smoking during pregnancy, birthweight, and gestational age.
e. The constant represents the mean level of the outcome for an average uncomplicated pregnancy in the study population.
f. Body mass index (BMI) based upon World Health Organization classifications (188)
g. Not of non-Hispanic white race/ethnicity
h. Includes pregnancy induced hypertension, preeclampsia and chronic hypertension
i. Any maternal primary or secondary relative with chronic hypertension
j. Institute of Medicine (IOM) guidelines for weight gain during pregnancy (168)
k. Low intake defined as consuming less than the IOM daily recommended dietary allowance for pregnant women: calcium (1 g), iron (27 mg), zinc (11 mg) (114)
l. Maternal self-reported smoking of tobacco cigarettes during the study pregnancy (current smoker)

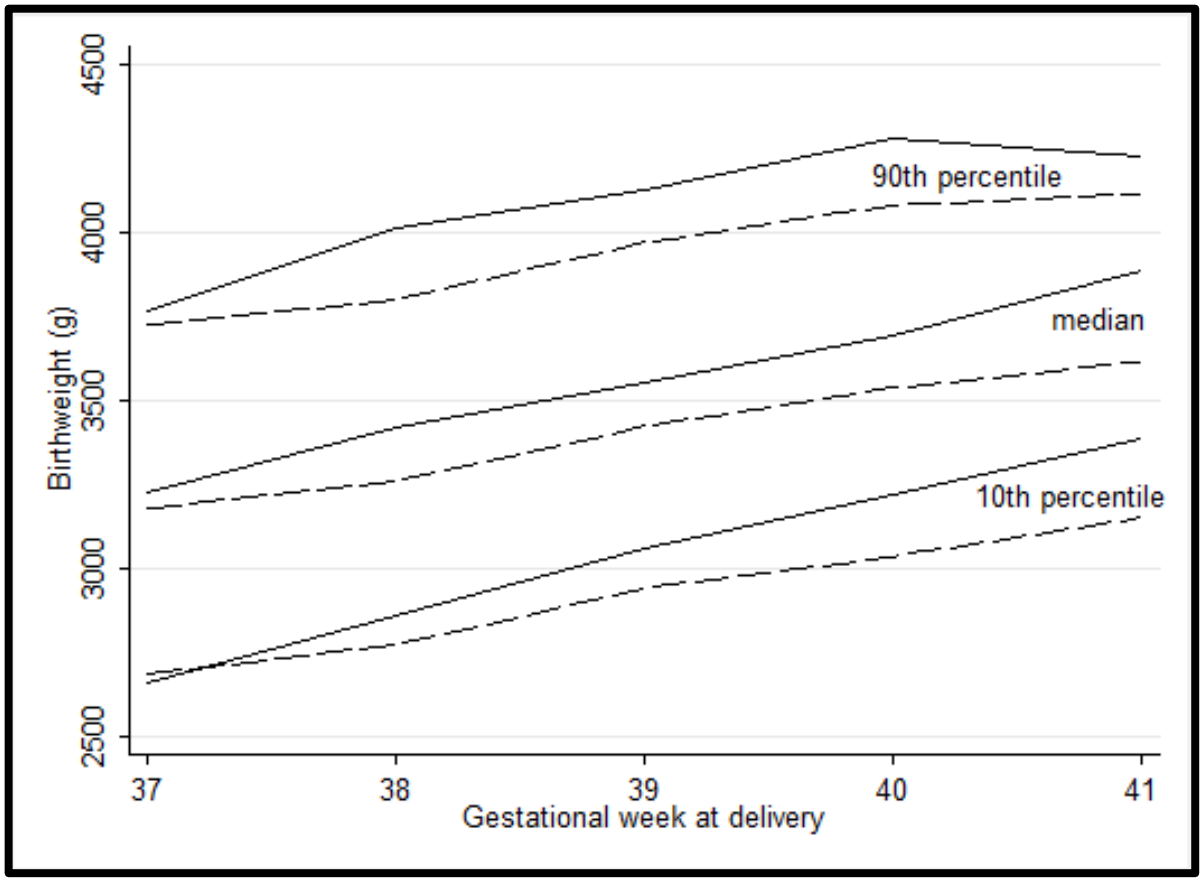


Figure 1: Selected percentiles of birthweight (g) by gestational week for term births (37 gestational weeks) in the Omega Study cohort (1996-2008), stratified by infant sex. Solid lines represent percentiles for male infants and dashed lines represent percentiles for female infants

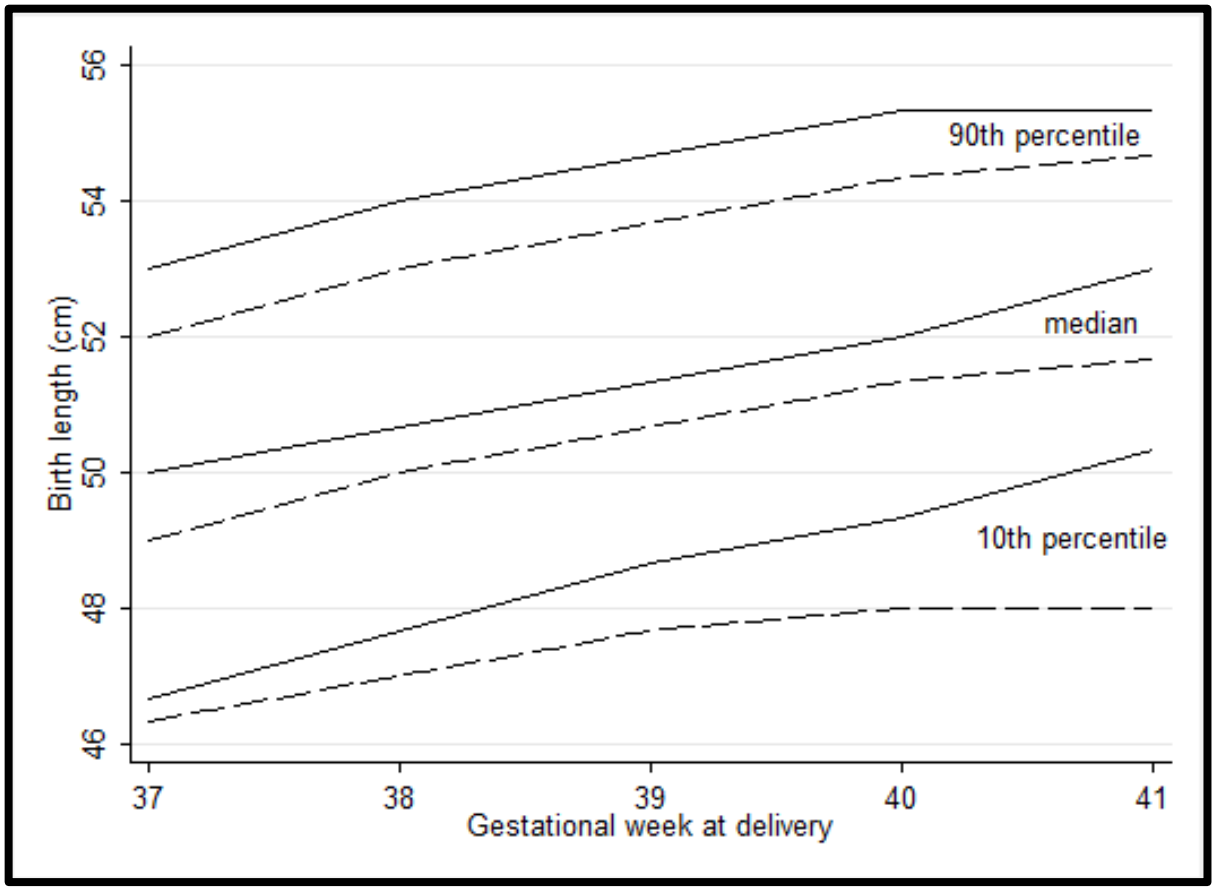


Figure 2: Selected percentiles of birth length (cm) by gestational week for term births (≥37 gestational weeks) in the Omega Study cohort (1996-2008), stratified by infant sex. Solid lines represent percentiles for male infants and dashed lines represent percentiles for female infants

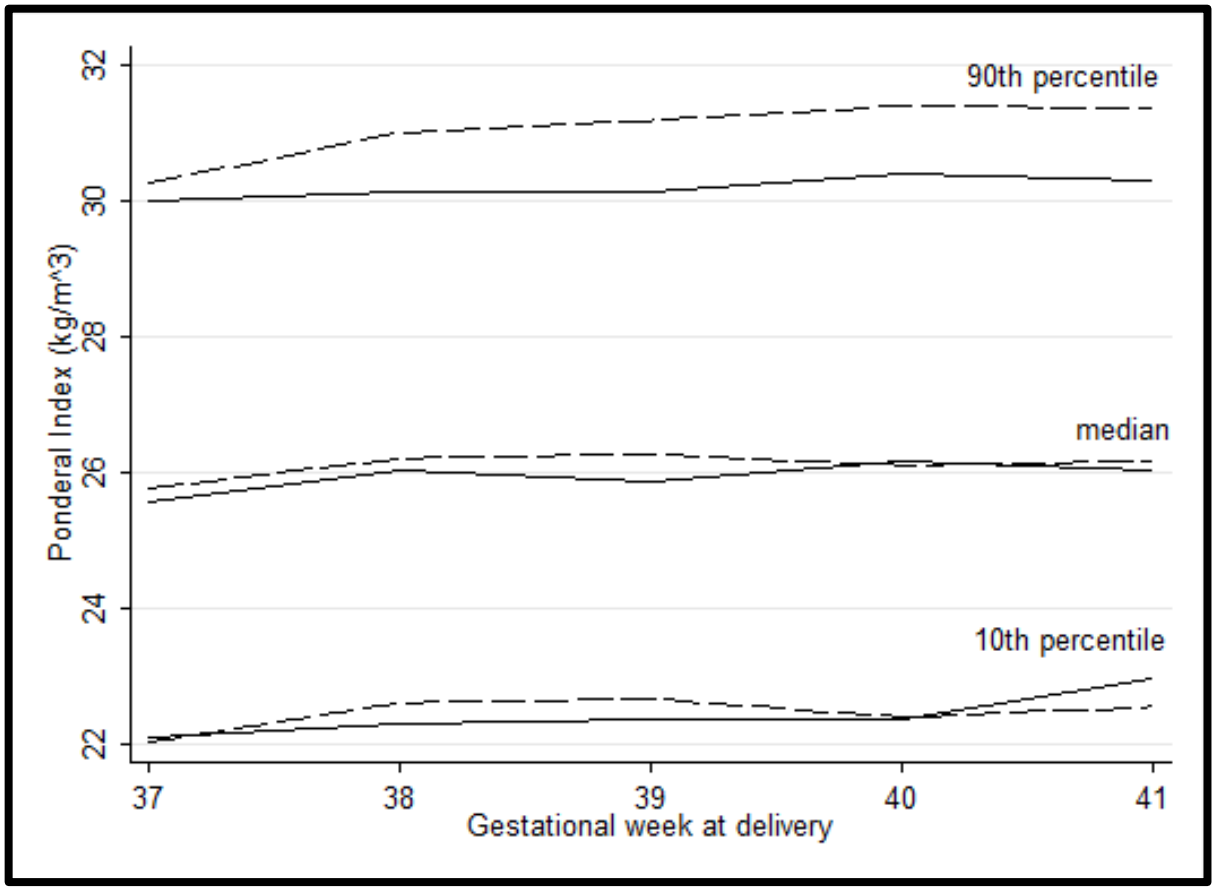


Figure 3: Selected percentiles of ponderal index (kg/m^3) by gestational week for term births (≥37 gestational weeks) in the Omega Study cohort (1996-2008), stratified by infant sex. Solid lines represent percentiles for male infants and dashed lines represent percentiles for female infants

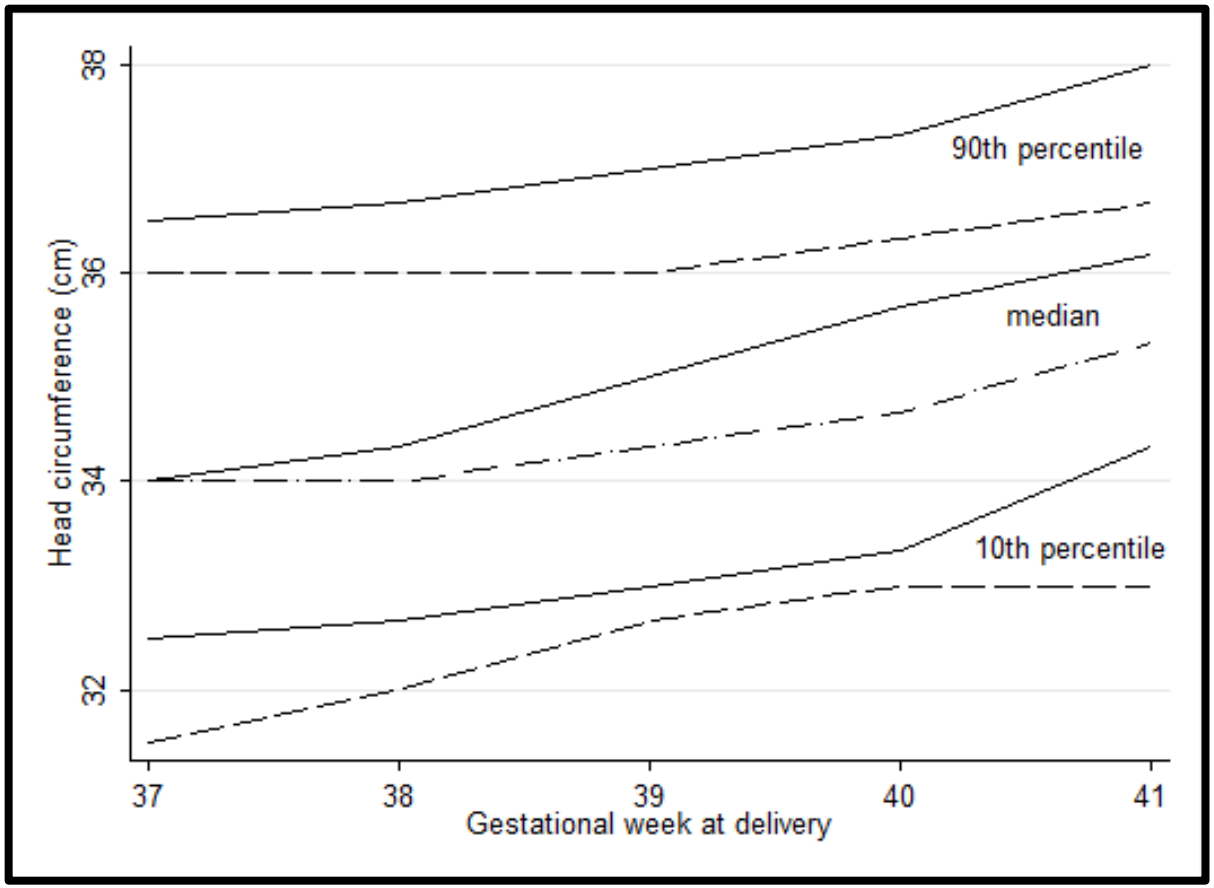


Figure 4: Selected percentiles of head circumference (cm) by gestational week for term births (≥37 gestational weeks) in the Omega Study cohort (1996-2008), stratified by infant sex. Solid lines represent percentiles for male infants and dashed lines represent percentiles for female infants

Chapter 3: Maternal body burden of cadmium and offspring size at birth

ABSTRACT

Background: Increasing evidence suggests an inverse association between cadmium and size at birth, and recent evidence suggests that the effect of cadmium may be greatest among female neonates.

Methods: We utilized 750 randomly selected maternal-infant pairs from the Omega Study (1996-2008), a large (N=4344) prospective cohort study designed to examine risk factors for pregnancy complications. Creatinine-corrected cadmium (Cd) in maternal clean-catch spot urine samples was quantified by inductively coupled plasma mass spectrometry. Continuous natural log-transformed Cd (lnCd) and categorical Cd (tertile categories: low [<0.29 μg/g Cr], middle [0.29-0.42 μg/g Cr], high [≥0.43 μg/g Cr]) were used in infant sex-stratified multivariable linear regression models.

Results: Females had reduced birth length with greater tertile of maternal urinary Cd, whereas males had a marginal increase in birth length [β(95% CI) females: low=reference, middle= -0.52 cm (-1.31,0.26), high=-0.71 cm (-1.59,0.17), p-trend=0.11; males: low=reference, middle=0.19 cm (-0.58,0.97), high=0.79 cm (–0.06, 1.63), p-trend=0.06; p for interaction=0.03]. The interaction between lnCd and infant gender was statistically significant for ponderal index [p=0.005; β(95% CI): female=0.22 kg/m^3 (-0.52,0.96); male= -0.95 kg/m^3 (1.56,-0.35)] and birth length [p =0.002; β(95% CI): female= -0.73 cm (-1.15, -0.30), male= 0.49 cm (-0.01, 0.99)]. Statistically insignificant inverse relations between lnCd and birthweight, ponderal index, and head circumference were observed among all infants.

Conclusion – Our findings suggest potential sex-specific reversal of effects for Cd on birth length and contribute to the growing body of evidence detailing the adverse effect of Cd exposure on fetal growth. Replication in other populations and investigation of potential underlying mechanisms is warranted.

INTRODUCTION

Cadmium (Cd) is widely used in production of batteries, pigments, plastics, and a variety of other commercial products (9). Cd toxicity has long been recognized as an occupational health hazard (9) and experimental studies in several animal models suggest embryotoxic effects of Cd (189). However, less is known about the impact of environmental Cd exposure among the general population, particularly in early life. Ingestion of food containing Cd and inhalation of tobacco smoke are the primary routes of Cd exposure in the general population (8–10,190). Cd exposure leads to renal damage (12), cardiovascular disease (4,76), and osteoporosis (5). Following ingestion or inhalation, Cd is absorbed and not readily excreted (9,10,190). Though Cd is primarily stored in the liver and kidneys (9,10,12,190), the placenta also accumulates Cd (51). Calcium, zinc, and iron deficiencies, as well as diets low in protein can augment Cd absorption (14,191). Pregnant women and the developing fetus are particularly susceptible to Cd because of the typically lower iron stores in reproductive age women (7,10), substantial pregnancy-related increase in iron requirements (16), and resulting increased Cd absorption during pregnancy (17,51,192).

Accumulation of Cd in the placenta may contribute to decreased uteroplacental blood flow (193) or disrupt the synthesis and metabolism of placental hormones, leading to fetal growth restriction (194,195). Small birth size has been linked with diabetes, cardiovascular disease, hypertension, and stroke, during both childhood and adulthood (123,124). Several previous studies have observed inverse associations between gestational exposure to Cd (characterized using measures in cord blood, placenta, or maternal blood and urine) and birthweight (49,51–59), birth length (54,61,62), and head circumference (49,62), although a few have observed no association with fetal growth (63–65). The majority of the these studies have

included fewer than 80 maternal-infant pairs (51,53,55–58,61,62), and control for confounding has been inconsistent. In particular, few of these studies have accounted for arsenic (As) exposure (55,58,196), which has been suggested to reduce birthweight (58,197–199). Only two studies have examined the association between Cd and fetal growth restriction (FGR), defined as birthweight below the 10^{th} percentile for gestational age (60,63). In a modestly sized (n=101) cross-sectional study, placental Cd was 1.08 ng/g greater among FGR infants, compared to average for gestational age infants ($p<0.05$)(63), whereas greater maternal blood Cd was associated with a 41% increase in risk of FGR in a larger prospective cohort study based in France (OR=1.41, 95% confidence interval 1.00-1.99)(60). Assessment of continuous measures of asymmetric growth restriction have been inconsistent with one study observing no association between Cd cord blood and ponderal index (59), and another observing a direct relation between placental Cd and newborn body mass index (64).

The two largest studies to date each included roughly 1600 maternal-infant pairs (59,196), and both observed statistically significant inverse associations between Cd and birthweight. Al-Saleh et al. observed a 2-fold increased risk of birthweight less than the 10^{th} percentile for gestational week among infants with higher cord blood (95% confidence interval: 1.1, 3.6) (59). Kippler et al. observed a 45 g decrease in birthweight for each 1 μg/L increase in maternal urinary Cd among female neonates only (95% confidence interval: -82.5, -7.3 g) (196). However, both of these studies were conducted in areas with fairly high general population exposure to Cd, and it is unknown if Cd exposure levels in the United States lead to reduced size at birth. Likewise, most studies have not investigated sex-specific effects of Cd on fetal growth (Appendix: Table II).

The goal of the present study was to evaluate whether greater maternal body burden of

Cd impairs fetal growth as measured by infant anthropometry (birthweight, birth length, head circumference, and ponderal index), and to evaluate whether these associations are different for male versus female neonates.

METHODS

Study Setting and Study Population- The Omega Study, a large prospective cohort study at the Center for Perinatal Studies at Swedish Medical Center in Seattle, WA, was designed to investigate risk factors for pregnancy complications. Subjects were recruited from multiple prenatal care clinics affiliated with Swedish Medical Center/Tacoma General Hospital (1996-2008). English speaking women initiating prenatal care at a study clinic prior to 20 weeks gestation were eligible for participation. Women who were less than 18 years of age, planning to deliver at a non-study hospital, or not intending to carry the pregnancy to term were excluded. The population for the present study was comprised of a subcohort of 750 women randomly drawn from among participants (N=4,344) in the Omega Study. Women with a history of renal disease (n=7), pre-existing diabetes mellitus (n=17), chronic hypertension (n=33), multiple fetal births (n=27), and known complications of pregnancy [preeclampsia (n=27), gestational diabetes (n=44), preterm delivery (n=95), infant with congenital anomaly (n=22)] were excluded from analyses. The urinary level of 2 μg Cd/g creatinine has been suggested as a real-world threshold for preliminary stages of Cd induced renal effects (12), so women with urinary Cd above this level were also excluded (n=7). World Health Organization (WHO) guidelines suggest that creatinine concentration may be used to flag spot urine samples that are too dilute (<30 mg/dL) or too concentrated (>300 mg/dL) to provide valid estimates of the concentration of the urinary chemical of interest (92). We excluded 165 women who had creatinine levels outside of the acceptable range. Aside from their low urinary creatinine level, the characteristics of these women were similar to those remaining in the study (Appendix Table III). The exclusions described above are not mutually exclusive; some women were excluded for multiple reasons. The final analytic population included 472 maternal-infant pairs (n_{male}=246, n_{female}=226).

Data Collection - Information on sociodemographic characteristics, reproductive and medical histories, lifestyle factors such as alcohol and tobacco use, and maternal anthropometry was collected by trained interviewers using a structured questionnaire shortly after study enrollment. Maternal medical records were abstracted to ascertain pregnancy outcomes, covariate data related to pregnancy complications, and relevant characteristics during pregnancy. Participants also completed a self-administered, validated, and semi-quantitative food-frequency questionnaire (FFQ) describing nutritional intake over the prior six months (166) (three months before pregnancy through the first three months of pregnancy, on average).

Exposure Assessment - At 15 weeks gestation, on average (range 4-25 gestational weeks), clean-catch spot urine samples were collected in polyethylene containers, promptly separated into 2 mL aliquots, and stored at −80 °C until analysis. Among the 750 randomly selected, 732 women provided urine samples (97.6%).

Urine concentrations of Cd and total As were quantified using a validated method of inductively coupled plasma mass-spectrometry (ICP-MS) following published protocols (95) at Metametrix Clinical Laboratory, a Clinical Laboratory Improvement Amendments (CLIA) certified facility in Duluth, GA. Briefly, urine samples were shaken and 1 mL was acidified with 1% HNO_3 (100 µl). An internal standard solution containing scandium, rhodium, and germanium (500 µl) was added. Samples were diluted to 5 mL with deionized water. Polyatomic interference was minimized through the use of ICP-MS with a dynamic reaction cell (PerkinElmer SCIEX Elan DRC II with ESI SC-4, FAST Autosampler). The accuracy of ICP-MS was checked by conducting proficiency testing using urine reference material (New York Toxic / Trace Elements in Urine Event #1 2012). The limits of detection for urinary Cd and total As were 0.12 and 3.0 µg/g Cr respectively. Urinary creatinine concentration was assessed using a commercially

available kit (Genzyme Diagnostics, Catalogue # 221-30/# 221-50) with improved Jaffe Reaction.

The distribution of creatinine-corrected, urinary Cd concentration was right-skewed. We used a natural log-transformation of creatinine-corrected, urinary Cd concentrations (lnCd) to diminish the influence of extreme values on the regression coefficients. The majority of the women in the analytic population (84%) had urinary Cd greater than the limit of detection (LOD=0.12 μg/g Cr). Appropriate to our observed distribution of Cd, we substituted LOD/(√2) for women with urinary Cd values below the LOD (200,201). Maternal urinary Cd was also categorized into low (<0.29 μg/g Cr), middle (0.29-0.42 μg/g Cr), high (≥0.43 μg/g Cr) tertiles.

Outcome measurements- Information on infant birthweight (g), birth length (cm), and head circumference (cm) was abstracted from medical records. Ponderal index, a measure of leanness in newborns used to quantify asymmetric fetal growth restriction, was calculated as: [birthweight (kg)]/ [birth length (m)]^3.

Statistical Analysis- We examined the frequency distribution of maternal sociodemographic characteristics, medical and reproductive histories, according to tertile of maternal urinary Cd and infant sex. Multivariate linear regression was used to model the relation between continuous lnCd or tertile of maternal Cd and each continuous outcome [birthweight (g), ponderal index (kg/m^3), birth length (cm), and head circumference (cm)]. The models adjusted for the following confounders based on *a priori* knowledge of their associations with exposure and outcome: continuous maternal age (years), parity (nulliparous/multiparous), continuous maternal pre-pregnancy BMI, maternal race (non-Hispanic white: yes/no), gestational age (continuous weeks plus quadratic term). However, we did not adjust for gestational age in the ponderal index analysis, as ponderal index remains fairly steady at term (164). For the birth length analysis,

maternal height (m) was substituted for maternal pre-pregnancy BMI, as it tends to be strongly associated with birth length (34,55,202,203).

Environmental exposure to inorganic As can decrease birthweight (58,197–199), but inorganic As exposure is not associated with birth length and head circumference (55,199). We conducted an exploratory analysis to assess the impact of confounding by As exposure on the Cd-birthweight and Cd-ponderal index associations. Total As in urine, which reflects both organic and inorganic species of As, was available from ICP-MS. Ideally, we would quantify speciated As, as the organic As species are less toxic than inorganic forms, and recent ingestion of seafood increases the level of organic As in urine (104,105). In order to partially account for expected higher levels of organic As species in the urine of women that regularly consume seafood, we further adjusted for self-reported fish consumption habits (fish and seafood servings per year from food frequency questionnaire) in models containing total As. Because only 6.3% of the women in the full Omega study population are current smokers, we did not *a priori* adjust for smoking status in the main analyses. However, we tested the effect of smoking during pregnancy (yes/no) and included it the final birthweight and ponderal index models. We also conducted a series of sensitivity analyses in which we restricted to never smokers. Exploratory multivariable logistic and multinomial regressions were utilized to assess the effect of cadmium on low birthweight (birthweight<2500 g) and three levels of each birth anthropometric measurement (<10^{th} percentile, 10^{th}-90^{th} percentile, and >90^{th} percentile). Finally, we conducted exploratory analyses assessing the impact of nutritional factors including, weight gain during pregnancy, calcium, and zinc intake. The Institute of Medicine (IOM) guidelines were used to classify weight gain during pregnancy based on pre-pregnancy BMI as below optimal, optimal, or above optimal (Appendix: Table IV) (168). Daily caloric intake (kcal/day) and dietary intake

of calcium, iron, and zinc (mg/day) were estimated using food composition tables from the University of Minnesota Nutrition Coding Center Nutrient Database (Nutrition Coordinating Center, Minneapolis, MN) (169). Low dietary intake of micronutrients was designated based on the IOM recommended dietary allowance (RDA) for calcium, iron, and zinc during pregnancy (114).

An interaction term was added to each of the final models to formally assess the joint impact of urinary Cd (either lnCd or tertile) and infant gender on outcomes of interest (204). We conducted trend tests using the median values of the distribution within tertiles of maternal urinary exposure as the score variable. Analyses were also repeated stratified by infant gender.

As there are no specific recommendations for creatinine-based exclusions among pregnant women, we performed a sensitivity analysis in which we relaxed the urinary creatinine exclusion criteria. New recommendations based upon assessment of urine samples from NHANES suggest that for women the cutoff of <30 mg creatinine/dL urine may be inappropriate, because it is common for women to have low urinary creatinine (94). In our sensitivity analysis, we still excluded women with urine samples that were too concentrated (> 300 mg/dL) according to both the guidelines of the WHO and the NHANES based recommendations, but included all women with dilute urine (creatinine <30 mg/dL) (92,94).

All statistical analyses used robust standard error estimates and the alpha level of 0.05 to define statistical significance. All analyses were completed using STATA 12 statistical software (107).

RESULTS

Table 1 presents selected characteristics (mothers and their offspring) of the study participants. Women in the study population were predominately married, Non-Hispanic white, nulliparous, and had education beyond high school (Table 1). The geometric mean of urinary Cd was similar for women who delivered female (0.306 μg/g Cr; 95% CI 0.276, 0.339 μg/g Cr) and male infants (0.307 μg/g Cr; 95% CI 0.279, 0.337 μg/g Cr). Regardless of infant gender, women in the low tertile for urinary Cd tended to be younger ($mean_{female}$=31.7, $mean_{male}$=32.3 years) than women in the higher two tertiles. Among women who delivered female infants only, those in the highest tertile of urinary Cd had a smaller proportion of nulliparous women (47.5%; p=0.02) and lower average maternal pre-pregnancy BMI (mean=21.8 kg/m^2; p=0.001) than the lower two tertiles. Average birth anthropometrics were not significantly different across tertiles of maternal urinary Cd among women who delivered male or female infants (Table 1).

The interaction between lnCd and infant gender on ponderal index was statistically significant (p=0.005). An inverse association between lnCd and ponderal index was observed among male infants (β= -0.95 kg/m^3; 95% CI: -1.56, -0.35 kg/m^3) but not female infants (β=0.22 kg/m^3; 95% CI: -0.52, 0.096 kg/m^3). Additionally the interaction between lnCd and infant gender on birth length was statistically significant (p=0.002). An inverse association between lnCd and birth length was observed among female infants (β= -0.73 cm; 95% CI: -1.15, -0.30 cm), whereas a direct relation was observed among male infants (β= 0.49 cm; 95% CI: -0.01, 0.99 cm). The interaction between tertile of maternal urinary Cd and sex of neonate for the birth length analysis was statistically significant (p=0.03). Among female neonates, we observed reduced birth length with increasing tertile of maternal urinary Cd [β(95% CI): low=reference; middle= -0.52 cm (-1.31,0.26 cm); high=-0.71 (-1.59,0.17 cm); p-trend=0.11]. A marginal

increase in birth length across increasing tertiles of maternal urinary Cd was observed among male neonates [β(95% CI): low=reference; middle=0.19 cm (-0.58,0.97 cm); high=0.79 cm (–0.06, 1.63 cm); p-trend=0.06] (Table 2).

Overall, among all infants, no statistically significant associations were observed between maternal Cd (either continuous lnCd or tertiles of Cd) and birthweight, ponderal index, birth length or head circumference (Table 3). The directions, magnitude, and statistical significance of observed associations between maternal Cd and fetal growth markers were generally the same for the sensitivity analyses including women with dilute urine (<30 mg Cr/dL) (Table 4), and those restricted to never smokers (Table 5). The addition of nutritional factors to the ponderal index and head circumference models did not substantially change the observed estimates, consistent with our previous observations (Chapter 2). However, with the addition of maternal weight gain, low calcium intake and low zinc intake to the birthweight model, we observed decreased birthweight among males in the high tertile of maternal urinary Cd [β(95% CI): low=reference; middle= -62.7 g (-206.0, 80.5 g); high=-22.7 g (-160.9, 115.6 g); p-trend=0.754]. The Cd birth length associations became stronger when we further controlled for maternal weight gain and dietary calcium intake [β(95% CI): males, low=reference; middle= 0.38 cm (-0.42, 1.18 cm); high=1.07 cm (0.24, 1.89 cm); p-trend=0.01; females, low=reference; middle=-0.55 cm (-1.33, 0.24 cm); high=-0.81 cm (-1.81, 0.19 cm); p-trend=0.105] (Table 6). Maternal dietary intake of calcium was previously indicated as a significant factor influencing birth length among male infants in our study population (Chapter 2). The multinomial logistic regressions did not provide any evidence that Cd differentially affected infants with smaller or larger size at birth (data not shown).

DISCUSSION

In the current study, we found that maternal Cd is associated with sex-specific differences in birth length and ponderal index. Maternal Cd was inversely associated with birth length among females (β= -0.73 cm; 95% CI: -1.15, -0.30 cm) and directly associated with birth length among males (β=0.49 cm; 95% CI: -0.01, 0.99) (p for interaction=0.002). Maternal Cd was also directly associated with ponderal index among females (β=0.22 kg/m^3; 95% CI: -0.52 ,0.96 kg/m^3) and inversely associated with ponderal index among males (β= -0.95 kg/m^3 (95% CI:-1.56,-0.35 kg/m^3) (p for interaction=0.005); however, it is likely that the observed effect modification of birth length by infant sex was driving the observed relations between maternal Cd and ponderal index. In addition, we observed modest, statistically insignificant, decreases in birthweight, head circumference, and ponderal index among all neonates in response to increasing lnCd.

Our findings are broadly in line with prior research of Cd and birthweight, but previous work assessing Cd's impact on head circumference and ponderal index has been inconsistent. We observed a statistically insignificant decrease in birthweight corresponding to greater maternal lnCd among all neonates, in agreement with prior research (Appendix: Table II). Several previous studies have observed a similar link between greater Cd exposure and reduced weight at birth (51–59,62), though a few have not (61,63–65). Among all infants, we also observed a small and statistically insignificant decrease in head circumference. The literature regarding Cd and head circumference has been fairly equivocal with one study observing decreasing head circumference with increasing Cd (62), one observing increasing head circumference with increasing Cd (57), and three suggesting no association (51,55,59,65). Likewise, only two prior studies have assessed Cd and either newborn BMI or ponderal index. In

a cross-sectional study (n=262) spanning 6 regions across the Russian and Norwegian arctic and subarctic regions, Odland et al. observed a statistically insignificant increase of 4.4 kg/m^2 in neonatal BMI with each μg/g increase in placental Cd (95% CI (-3.95, 12.75 kg/m^2)(64). Al-Saleh et al. conducted a large cross-sectional study (n=1578) among Saudi Arabian women, in which they assessed lead, cadmium and mercury in cord blood, placenta, and maternal blood. They reported that there was no association between ponderal index less than the 10th percentile and Cd (data not shown) (59). Neither of these studies stratified by infant sex (59,64).

Kippler et al. conducted a large prospective study in which sex-specific impacts of Cd exposure on size at birth were suggested. Specifically, they observed reductions in birthweight and head circumference among girls only and no clear impact of Cd on fetal growth impairment among males (49). Although we did not observe any statistically significant impact of Cd on birthweight, our findings are in general agreement with their birthweight findings. In our exploratory analysis, we observed a 2.7-fold increased risk of low birth weight (LBW, birthweight<2500 g) among infants in the middle tertile for maternal urinary Cd (95% CI: 0.26, 29.0), and a 2.4 fold increased risk of LBW among infants in the high tertile (95% CI: 0.39, 15.1) compared to infants in the low tertile and controlling for gestational age, maternal age, pre-pregnancy BMI, race/ethnicity, parity, gestational week of urine collection, total maternal urinary arsenic, and maternal fish consumption.

Our findings also potentially suggest a sex-specific impact of gestational Cd exposure on ponderal index, though this may be driven by the observed differences in Cd's effect on birth length among male and female infants. We observed a statistically significant interaction between continuous maternal lnCd and infant sex on ponderal index. Girls experienced modest increases in ponderal index, whereas boys experienced decreased ponderal index with greater

maternal lnCd. For the Cd-birth length association, we observed unanticipated and statistically significant interactions between both lnCd (p=0.002) and maternal tertile of Cd (p=0.03) with infant sex, suggesting an inverse dose-response relation between maternal Cd exposure and birth length among female neonates and a positive dose response association among male infants (Table 3). Although small and not statistically significant, Kippler et al. previously observed an inverse relation between increasing maternal urinary Cd (μg/L) and birth length among girls (β= –0.043 cm; 95% CI–0.21, 0.12 cm) and a direct relation among boys (β= 0.071; 95% CI –0.18, 0.32) (49). To the best of our knowledge, ours is the first study to identify potential sex-specific differences in ponderal index or birth length related to maternal Cd exposure.

Several biological mechanisms have been proposed to explain Cd-induced fetal growth impairment. Experimental evidence in multiple animal models supports Cd's placental toxicity and ability to inhibit transfer of nutrients to the fetal compartment through reduced uteroplacental blood flow (193,194). Additionally, *in vitro* study of perfused human placenta indicate that Cd is related to reduced volume of the fetal vasculature, necrosis of the placenta, and inhibited transfer of zinc to the fetal unit (205). Disruption of the synthesis, metabolism, or release of hormones by the placenta also occurs as Cd accumulates in placental tissue. Placental metabolism of glucocorticoids diminishes in the presence of Cd (206), failing to shield the fetus from the growth-inhibiting properties of glucocorticoids. Likewise, Cd is associated with reductions in placental leptin synthesis (195), which has been linked to intrauterine growth restriction (207). However, given the recent observation of different relations between Cd and fetal growth among girls versus boys (49), the previously described mechanisms incompletely define the effect of Cd on fetal growth. Likewise, part of the discrepancy in results across prior studies is likely due to failure to account for sex-specific effects of Cd on fetal growth.

Sex-specific Cd-birthweight associations may be accomplished by Cd-induced changes to epigenetic regulation of genes governing growth. A recent study found that in the presence of Cd-exposure, hypomethylation of DNA isolated from cord blood was observed predominantly among genes associated with organ development, morphology, and bone development among females versus global hypermethylation among males (50). Prior research has observed Cd-induced DNA hypomethylation among adult women (208) and altered epigenetic regulation of the fetal hepatic glucocorticoid receptor promotor among female fetal rats (209). Additional evidence suggests that glucocorticoids may themselves have sex-specific impacts on the fetal-placental unit, with the male placenta better resisting the growth inhibiting impact of cortisol (210). Decreased fetal growth among females has been associated with reductions in 11β-hydroxated dehydrogenase type 2 (11β-HSD 2) and increased cortisol (210). However, the evidence supporting the role of Cd in reducing 11β-HSD 2 has been inconclusive (206,211).

It is well established that male and female intrauterine growth velocities differ, with females growing symmetrically throughout gestation and males growing first in length and then in weight (37). Recently it has been suggested that there are sex-specific responses of the fetal-placental unit to insults in the intrauterine environment. Based upon observations of sex-specific growth responses to maternal preeclampsia (42,43) and asthma (44,45), Clifton et al. have proposed that the male fetus adopts strategies to maintain typical fetal growth in response to an initial *in utero* insult. The efforts to maintain the initial growth trajectory in a poor intrauterine environment places the male fetus at greater risk of growth restriction should a second acute insult occur. In contrast, the female fetus responds to a suboptimal intrauterine environment by growth adaptive strategies that result in reduced but symmetrical growth. The female strategy provides an advantage to the female fetus if additional insults occur (185).

Regardless of support in prior literature for sex-specific growth strategies, the apparent reversal of the Cd-birth length association between sexes is puzzling, but may be explained somewhat by sex-specific differences in Cd induced bone damage. Experimental studies have suggested that the male skeleton is somewhat resistant to Cd induced bone damage, as evidenced by sex-specific differences in the impact of Cd exposure on bone mineral density, bone mineral content, and bone metabolism (212–214). Cd-exposed young female rats experienced adverse effects on bone mineral density, bone mass accumulation during skeletal growth, and bone metabolism (213), whereas, there were no observable effects for young male rats (212). Likewise, a shorter duration of low-moderate Cd exposure was necessary to induce adverse effects on bone mineral content among female rats (212,213). Epidemiologic studies have demonstrated that Cd diminishes bone mineral density and increases fractures and osteoporosis risk for both adult men and women; however, the effects tends to be more pronounced among women (5,6,215). Although these findings do not explain the positive association observed between Cd and birth length among male infants in our study, they demonstrate that less Cd induced bone damage occurs among males than among females. Additional work in this area is necessary to clarify the observed associations.

Our study drew on several important strengths, including its prospective design and the availability of extensive information for a broad array of potential confounders within a well-characterized cohort. We were able to measure Cd exposure in maternal urine, which is a well-recognized marker of Cd body burden (7,9). Both Cd and total As were measured using a robust, well validated, and sensitive method (ICP-MS) to quantify Cd and total As in maternal urine. Finally, ours is the first study, to the best of our knowledge, to assess sex-specific differences in ponderal index.

There are also limitations that should be considered when interpreting our study results. Humans are exposed to complex mixtures of toxic substances every day, and as with all studies of environmental exposures there is an inherent difficulty in singling out the effect of Cd alone. Although we were able to control for total As, unmeasured co-exposures may contribute to the risk of impaired fetal growth. Likewise, we did not assess iron, zinc, and calcium status among the women in our population. Cd absorption increases in the presence of iron, zinc, and calcium deficiencies, so our inability to assess interactions among toxic and nutrient metals may limit our findings. External validity may be reduced since women in the Omega Study are generally white, married, and prosperous, reflective of the underlying population that utilizes Swedish Medical Center and Tacoma General Hospital. However, these women should represent a highly exposed subpopulation within the general public, as high income has been associated with increased body burden of Cd (120).

Our study provides further evidence that future assessments of Cd's impact on fetal growth should account for potential sex-specific differences. We observed a potentially important sex difference in the impact of Cd exposure on ponderal index, suggesting that Cd may cause symmetric growth restriction among female neonates and asymmetric growth restriction among males. Likewise, we observed divergent effects of Cd on birth length among boys and girls. Future research is warranted to replicate these findings in diverse populations and to further clarify the observed relations. Future studies should also account for iron, zinc, and calcium deficiencies. This study reiterates the deleterious impact of gestational exposure to Cd on fetal growth and adds to the growing body of evidence suggesting that the male and female fetus may employ differing growth strategies in response to similar intrauterine insults.

Table 1. Demographic, medical and obstetric history, and pregnancy, delivery, and infant characteristics of the study population according to tertile of maternal urinary cadmium and stratified by sex of neonate

	Female Neonates Tertiles of Maternal Urinary Cadmium (μg/g Cr)				**Male Neonates** Tertiles of Maternal Urinary Cadmium (μg/g Cr)		
	<0.29 n=68	0.29-0.42 n=62	≥0.43 n=61		<0.29 n=67	0.29-0.42 n=72	≥0.43 n=64
Mean±SD							
Infant Characteristics							
Birthweight (g)	3475 ±452	3522 ±403	3400 ±475		3642 ±459	3570 ±472	3686 ±365
Birth length (cm)	51.2 ±2.6	50.8 ±2.2	50.5 ±2.4		51.6 ±2.6	51.8 ±2.2	52.3 ±2.3
Head circumference (cm)	34.6 ±1.7	34.7 ±1.7	34.3 ±1.6		35.1 ±1.7	35.2 ±1.6	35.4 ±1.7
Ponderal index (kg/m^3)	25.9 ±3.5	26.9 ±3.5	26.6 ±4.3		26.5 ±3.0	25.8 ±3.6	25.8 ±3.0
Maternal Characteristics							
Maternal age years	31.7 ±4.7	33.4 ±4.2	34.5 ±4.7	*	32.3 ±4.4	32.8 ±3.8	33.6 ±4.1
Pre-pregnancy BMI (kg/m^2) [a]	24.5 ±5.1	23.2 ±3.6	21.8 ±3.1	*	23.9 ±6.9	23.4 ±3.9	23.5 ±4.0
Gestational week of urine collection	15.3 ±2.8	15.4 ±2.7	15.1 ±2.9		15.4 ±2.9	15.2 ±3.0	15.1 ±2.7
%							
Non- Hispanic white race/ethnicity	86.8	91.9	83.6		86.6	77.8	79.7
Post high school education	98.5	98.3	96.4		95.2	95.6	96.8
Married	85.3	90.3	82.0		83.6	87.5	93.8
Nulliparous	72.1	59.7	47.5	*	55.2	52.8	60.9
Family history of diabetes [b]	8.8	16.1	23.0		9.0	19.4	10.9
Family history of hypertension [c]	44.1	37.1	36.1		46.3	51.4	39.1
Current smoker [d]	6.1	6.7	3.6		11.3	8.8	8.2
Consumed alcohol during pregnancy [e]	27.9	29.0	37.7		29.0	29.0	29.0

(table continues on next page, notes are located at end of table)

Table 1. Demographic, medical and obstetric history, and pregnancy, delivery, and infant characteristics of the study population according to tertile of maternal urinary cadmium and stratified by sex of neonate (continued)

	Female Neonates Tertiles of Maternal Urinary Cadmium (μg/g Cr)				**Male Neonates** Tertiles of Maternal Urinary Cadmium (μg/g Cr)		
	<0.29 n=68	0.29-0.42 n=62	≥0.43 n=61		<0.29 n=67	0.29-0.42 n=72	≥0.43 n=64
No leisure time physical activity	7.6	3.3	1.8		3.2	2.9	4.9
IOM weight gain guidelines [f]							
Below optimal	13.2	12.9	8.5		3.0	4.2	4.8
Optimal	30.9	35.5	44.1		35.8	28.2	27.0
Above optimal	55.9	51.6	47.5		61.2	67.6	68.3
Dietary micronutrient intake [g]							
Calcium (g/day)							
Low calcium intake	36.9	38.6	52.0		46.0	46.2	37.3
Low iron intake	95.4	94.7	84.0		96.83	95.4	94.9
Low zinc intake	38.5	47.4	48.0		49.21	55.4	39.0
Geometric mean (95% confidence interval)							
Maternal Urinary Measures							
Cadmium μg/g Cr	0.14 (0.13, 0.16)	0.35 (0.34, 0.36)	0.63 (0.58, 0.69)		0.14 (0.13, 0.16)	0.35 (0.34, 0.36)	0.59 (0.55, 0.63)
Total Arsenic μg/g Cr	15.9 (11.8, 21.4)	18.8 (15.1, 23.5)	26.0 (19.8, 34.3)	*	18.2 (14.2, 23.2)	17.7 (13.9, 22.5)	21.7 (17.9, 26.1)
Creatinine mg/dL	81 (69, 94	87 (78, 97)	75 (65, 88)		79 (69, 92)	91 (80, 103)	79 (70, 90)

Cr=creatinine, SD=standard deviation

*p<0.005

a. Body mass index (BMI)

b. Any maternal primary or secondary relative with a diabetic condition

c. Any maternal primary or secondary relative with hypertension
d. Maternal self-reported smoking of tobacco cigarettes during the study pregnancy
e. Maternal self-reported alcohol consumption during the study pregnancy
f. Institute of Medicine (IOM) guidelines for weight gain during pregnancy (168)
g. Low intake designated by consuming less than the IOM daily recommended dietary allowance for pregnant women: calcium (1 g), iron (27 mg), zinc (11 mg) (114)

Table 2. Multivariate linear regression analyses of the association between maternal urinary Cd and infant anthropometric measures, stratified by infant sex

	Birthweight (g)		Ponderal Index (kg/m3)		Birth Length (cm)		Head Circumference (cm)	
Urinary Cadmium (μg/g creatinine)	n	β[a] (95% CI)	n	β[b] (95% CI)	n	β[c] (95% CI)	n	β[d] (95% CI)
Female infants								
Continuous ln(Cd)	167	-73.4(-168.4, 21.7)	162	0.22(-0.52, 0.96)	186	**-0.73 (-1.15, -0.30)**	183	-0.32 (-0.66, 0.16)
Low (<0.29)	64	0 (reference)	61	0 (reference)	65	0 (reference)	61	0 (reference)
Medium (0.29-0.42)	56	-15.9(-162.8, 131.0)	55	0.64(-0.67, 1.94)	61	-0.52(-1.31, 0.26)	62	0.02(-0.52, 0.56)
High (≥0.43)	47	-67.0(-242.2, 108.2)	46	0.02(-1.50, 1.55)	60	-0.71(-1.59, 0.17)	60	-0.12(-0.71, 0.46)
		p-trend=0.457		p-trend=0.457		p-trend=0.114		p-trend=0.664
Male infants								
Continuous ln(Cd)	179	-10.5(-100.9, 79.8)	179	**-0.95(-1.56, -0.35)**	201	0.49(-0.01, 0.99)	201	0.14 (-0.24, 0.53)
Low (<0.29)	61	0 (reference)	60	0 (reference)	66	0 (reference)	66	0 (reference)
Medium (0.29-0.42)	62	-24.1(-169.9, 121.7)	63	-0.86(-2.06, 0.34)	72	0.19(-0.58, 0.97)	72	0.15(-0.40, 0.70)
High (≥0.43)	56	42.8(-100.6, 186.1)	56	-0.85(-2.03, 0.33)	63	0.79(-0.06, 1.63)	63	0.33(-0.24, 0.91)
		p-trend=0.535		p-trend=0.535		p-trend=0.063		p-trend=0.254
p for lnCd*sex interaction		0.39		**0.005**		**0.002**		0.062
p for Cd tertile*sex interaction		0.64		0.15		**0.03**		0.50

a. Controlling for maternal age, pre-pregnancy BMI, race/ethnicity, nulliparity, gestational age at delivery, smoking during pregnancy, gestational week of urine sample collection, and maternal total urinary arsenic adjusted for fish consumption
b. Controlling for maternal age, pre-pregnancy BMI, race/ethnicity, nulliparity, smoking during pregnancy, gestational week of urine sample collection, and maternal total urinary arsenic adjusted for fish consumption
c. Controlling for maternal age, maternal height, race/ethnicity, nulliparity, gestational age at delivery, and gestational week of urine sample collection
d. Controlling for maternal age, pre-pregnancy BMI, race/ethnicity, nulliparity, gestational age at delivery, and gestational week of urine sample collection

Table 3. Multivariate linear regression analyses of the association between maternal urinary Cd and infant anthropometric measures among all neonates

	Birthweight (g)		Ponderal Index (kg/m^3)		Birth Length (cm)		Head Circumference (cm)	
Urinary Cadmium (μg/g creatinine)	n	β[a] (95% CI)	n	β[b] (95% CI)	n	β[c] (95% CI)	n	B[d] (95% CI)
Continuous ln(Cd)	358	-32.8(-95.1, 29.5)	352	-0.29(-0.76, 0.19)	352	0.02(-0.33, 0.36)	349	-0.12(-0.39, 0.15)
Low (<0.29)	127	0 (reference)	123	0 (reference)	123	0 (reference)	119	0 (reference)
Medium (0.29-0.42)	122	-18.9(-118.9, 81.1)	121	0.06(-0.81, 0.94)	121	-0.14(-0.73, 00.4)	122	0.16(-0.2, 0.57)
High (≥0.43)	109	11.0(-94.9, 117.0)	108	-0.07(-1.05, 0.91)	108	0.14(-0.49, 0.78)	108	0.07(-0.4, 0.49)
		p-trend=0.830		p-trend=0.879		p-trend=0.647		p-trend=0.708

a. Controlling for maternal age, pre-pregnancy BMI, race/ethnicity, nulliparity, gestational age at delivery, smoking during pregnancy, gestational week of urine sample collection, maternal total urinary arsenic, and fish consumption
b. Controlling for maternal age, pre-pregnancy BMI, race/ethnicity, nulliparity, smoking during pregnancy, gestational week of urine sample collection, maternal total urinary arsenic, and fish consumption
c. Controlling for maternal age, maternal height, race/ethnicity, nulliparity, gestational age at delivery, and gestational week of urine sample collection
d. Controlling for maternal age, pre-pregnancy BMI, race/ethnicity, nulliparity, gestational age at delivery, and gestational week of urine sample collection

Table 4. Multivariate linear regression analyses of the association between maternal urinary Cd and infant anthropometric measure, stratified by infant sex, including women with dilute urine (<30 mg creatinine/dL)

	Birthweight (g)		Ponderal Index (kg/m3)		Birth Length (cm)		Head Circumference (cm)	
Urinary Cadmium (µg/g creatinine)	**n**	**β[a] (95% CI)**	**n**	**β[b] (95% CI)**	**n**	**β[c] (95% CI)**	**n**	**β[d] (95% CI)**
Female infants								
Continuous ln(Cd)	215	-44.6(-114.1, 24.9)	215	0.3(-0.2, 0.7)	240	-0.45 (-0.80, -0.11)	234	-0.16(-0.42, 0.10)
Low (<0.29)	77	0 (reference)	76	0 (reference)	80	0 (reference)	74	0 (reference)
Medium (0.29-0.42)	79	15.0(-114.2, 144.3)	76	1.1(0.0, 2.2)	83	-0.40(-1.14, 0.33)	84	-0.23(-0.71, 0.26)
High (≥0.43)	64	-64.7(-212.4, 83.0)	63	0.2(-0.9, 1.2)	77	-0.67(-1.42, 0.08)	76	-0.19(-0.72, 0.33)
		p-trend=0.440		p-trend=0.440		**p-trend=0.045**		p-trend=0.823
Male infants								
Continuous ln(Cd)	230	18.6(-52.0, 89.2)	230	-0.7(-1.2, -0.2)	258	0.43(0.06, 0.81)	258	0.19(-0.08, 0.46)
Low (<0.29)	75	0 (reference)	76	0 (reference)	84	0 (reference)	84	0 (reference)
Medium (0.29-0.42)	79	4.0(-136.2, 144.3)	78	-1.2(-2.3, -0.2)	90	0.49(-0.27, 1.24)	90	0.03(-1.63, 1.69)
High (≥0.43)	76	48.4(-89.0, 185.8)	76	-1.4(-2.4, -0.3)	84	0.99(0.26, 1.71)	84	0.04(-2.29, 2.38)
		p-trend=0.635		p-trend=0.635		**p-trend=0.032**		p-trend=0.293
p for lnCd*sex interaction		0.353		**0.005**		**0.001**		0.091
p for Cd tertile*sex interaction		0.454		0.119		**0.024**		0.476

a. Controlling for maternal age, pre-pregnancy BMI, race/ethnicity, nulliparity, gestational age at delivery, smoking during pregnancy, gestational week of urine sample collection, and maternal total urinary arsenic adjusted for fish consumption
b. Controlling for maternal age, pre-pregnancy BMI, race/ethnicity, nulliparity, smoking during pregnancy, gestational week of urine sample collection, maternal total urinary arsenic, and fish consumption
c. Controlling for maternal age, maternal height, race/ethnicity, nulliparity, gestational age at delivery, and gestational week of urine sample collection
d. Controlling for maternal age, pre-pregnancy BMI, race/ethnicity, nulliparity, gestational age at delivery, and gestational week of urine sample collection

Table 5: Multivariate linear regression analyses of the association between maternal urinary Cd and infant anthropometric measures among mothers who are never smokers, stratified by infant sex

	Birthweight (g)		Ponderal Index (kg/m3)		Birth Length (cm)		Head Circumference (cm)	
Urinary Cadmium (µg/g creatinine)	n	β[a] (95% CI)	n	β[b] (95% CI)	n	β[c] (95% CI)	n	β[d] (95% CI)
Female infants								
Continuous ln(Cd)	120	-67.5(-177.7, 42.6)	**118**	0.50(-0.43, 1.43)	**128**	-0.73(-1.17, -0.28)	**125**	-0.37(-0.81, 0.06)
Low (<0.29)	48	0 (reference)	47	0 (reference)	49	0 (reference)	46	0 (reference)
Medium (0.29-0.42)	39	-98.6(-273.3, 76.1)	39	0.66(-0.83, 2.15)	41	-0.71(-1.72, 0.30)	41	-0.06(-0.71, 0.59)
High (>0.42)	33	-63.9(-281.2, 153.4)	32	0.42(-1.08, 1.91)	38	-0.56(-1.52, 0.40)	38	-0.37(-1.14, 0.40)
		p-trend=0.513	118	p-trend=0.544		p-trend=0.219		p-trend=0.340
Male infants								
Continuous ln(Cd)	129	-14.1(-125.0, 96.8)	128	-0.93(-1.64, -0.21)	136	0.49(-0.14, 1.12)	135	0.02(-0.46, 0.50)
Medium (0.29-0.42)	42	0 (reference)	41	0 (reference)	41	0 (reference)	41	0 (reference)
High (>0.42)	46	-27.5(-195.9, 140.9)	46	-1.33(-2.76, 0.11)	50	0.63(-0.39, 1.64)	49	0.05(-0.66, 0.76)
Low (<0.29)	41	30.0(-152.8, 212.8)	41	-1.19(-2.64, 0.26)	45	0.91(-0.23, 2.05)	45	0.03(-0.72, 0.78)
		p-trend=0.727		p-trend=0.121		p-trend=0.119		p-trend=0.943
p for lnCd*sex interaction		0.511		**0.008**		**0.001**		0.254
p for Cd tertile*sex interaction		0.454		0.119		**0.024**		0.476

a. Controlling for maternal age, pre-pregnancy BMI, race/ethnicity, nulliparity, gestational age at delivery, smoking during pregnancy, gestational week of urine sample collection, and maternal total urinary arsenic adjusted for fish consumption
b. Controlling for maternal age, pre-pregnancy BMI, race/ethnicity, nulliparity, smoking during pregnancy, gestational week of urine sample collection, maternal total urinary arsenic, and fish consumption
c. Controlling for maternal age, maternal height, race/ethnicity, nulliparity, gestational age at delivery, and gestational week of urine sample collection
d. Controlling for maternal age, pre-pregnancy BMI, race/ethnicity, nulliparity, gestational age at delivery, and gestational week of urine sample collection

Table 6. Exploratory multivariate linear regression analyses including control for nutritional factors in the examination of the association between maternal urinary Cd and infant anthropometric measures, stratified by infant sex

	Birthweight (g)		Birth Length (cm)	
Urinary Cadmium (μg/g creatinine)	n	β[a] (95% CI)	n	β[b] (95% CI)
Female infants	167		166	
Low (<0.29)	64	0 (reference)	62	0 (reference)
Medium (0.29-0.42)	56	-16.6(-155.3, 122.0)	56	-0.55(-1.33, 0.24)
High (≥0.43)	47	-66.6(-235.5, 102.3)	48	-0.81(-1.81, 0.19)
		p-trend=0.444		p-trend=0.105
Male infants	179		184	
Low (<0.29)	61	0 (reference)	62	0 (reference)
Medium (0.29-0.42)	62	-62.7(-206.0, 80.5)	64	0.38(-0.42, 1.18)
High (≥0.43)	56	-22.7(-160.9, 115.6)	58	1.07(0.24, 1.89)
		p-trend=0.754		p-trend=0.011

a. Controlling for maternal age, pre-pregnancy body mass index, race/ethnicity, nulliparity, gestational age at delivery, smoking during pregnancy, gestational week of urine sample collection, maternal total urinary arsenic, fish consumption, weight gain during pregnancy according to categories established by the Institute of Medicine (IOM) guidelines (below optimal, optimal, above optimal) (168), low maternal dietary intake of calcium, and low maternal dietary intake of zinc ± designated as consuming less than the IOM daily recommended dietary allowance (RDA) for pregnant women: calcium (<1 g), zinc (<11 mg) (114)

b. Controlling for maternal age, maternal height, race/ethnicity, nulliparity, gestational age at delivery, gestational week of urine sample collection, IOM weight gain, and maternal average daily calcium intake from semi-quantitative food frequency questionnaire

Conclusions

The described research aimed to determine if greater maternal body burden of Cd increases women's risk of developing GDM during pregnancy; to delineate maternal and fetal factors associated with sex-specific differences in fetal growth as measured by neonatal anthropometry (birthweight, birth length, head circumference, and ponderal index); and to evaluate whether greater maternal body burden of Cd impairs fetal growth as measured by neonatal anthropometry (birthweight, birth length, head circumference, and ponderal index). We accomplished these aims, using the Omega prospective cohort study as a parent study. We investigated the relationship between maternal body burden of Cd and GDM in a nested case-cohort study (n=621), identified infant sex-specific factors influencing size at birth using a cross-sectional study (n=3033), and assessed the effect of maternal body burden of Cd on birth size in a prospective cohort study (n=472). The primary strength of these studies was the use of prospectively collected data from a large and well-characterized cohort of pregnant women.

Within a highly exposed subgroup of the general population (103,120),We observed evidence of a direct relation between body burden of Cd and GDM (p-trend=0.015). Although the interaction between Cd and pre-pregnancy BMI status was not statistically significant, there was some suggestion of a joint effect of Cd and pre-pregnancy BMI on GDM risk. Although prior studies have observed an association between Cd and type 2 diabetes (22,3,23,24), we know of no other studies reporting on the association between Cd and GDM

Using a large and well-characterized cohort of maternal-singleton pairs, we described associations of parity, pre-pregnancy BMI, hypertensive disorders, and family history of hypertension with birth size that differ for male and female infants. Prior work in this area has been fragmented, focusing on a single factor of interest or individual metrics of fetal growth.

These findings, if replicated, highlight the need for evaluating sex-specific relations in future studies of modifiable and environmental risk factors of fetal growth. Indeed, the knowledge gained in these analyses informed the sensitivity analyses for our study of Cd and size at birth, and it is our hope that this research will increase interest in this rather under-studied area of inquiry.

To the best of our knowledge, we are the first to investigate whether Cd exposure in the US general population leads to reduced size at birth. Additionally, few other studies (49,50) have examined sex-specific effects of Cd on size at birth. We observed an intriguing sex-specific reversal of the effect of Cd on birth length, with an inverse relation among female infants and a direct relation among male infants. These results were robust across multiple sensitivity analyses. However, a potential mechanism by which Cd would increase bone formation among males is not readily evident, and it is possible that some additional environmental or hormonal co-exposure is responsible. Taken together, Chapters 2 and 3 reinforce the need to further investigate genetic, placental, and hormonal mechanisms of fetal growth impairment that may differ for male and female fetuses (185).

We have examined previously unexplored questions in this research, but our findings also have limitations. Each of these studies warrants replication in diverse and larger populations. We had limited ability to assess interactions between micronutrients and toxic metals. In particular, dietary iron is a poor proxy for iron status (115). Multiple indicators of iron status, including serum iron, total iron-binding capacity, serum ferritin, transferrin saturation, and hemoglobin, are frequently utilized in the literature to assess iron status(216–221). Iron and zinc deficiencies are known to augment Cd absorption (14). Body burden of zinc is particularly challenging to measure (222,223), but plasma Zn would be a more meaningful measurement, as it represents

roughly 0.2% of total body Zn and is generally a very useful biomarker of Zn status (224). Because there is currently no treatment for Cd toxicity (9), studies of the effect of calcium, iron, and zinc supplementation both pre-conception and during pregnancy could have substantial clinical utility for preventing Cd-induced GDM and fetal growth impairment.

In summary, our study lends additional support to research indicating a diabetogenic effect of Cd, reinforces the need to thoughtfully assess factors that may affect the male and female fetus in different ways, and provides additional support for infant sex-specific effects of Cd on fetal growth. Due to the ubiquity of Cd, a more thorough understanding of the disease burden associated with Cd exposure is desirable. Exploring the utility of preventative efforts, such as vitamin supplementation, that have proven health benefits beyond preventing or diminishing Cd toxicity would be prudent.

Table I: Summary of major findings of epidemiologic studies of cadmium and diabetes

First Author (Year)	Study Design, population (n)	Relevant Outcome(s)	Association measure: Cadmium related Findings	Biological matrix [lab method] median(range), [geometric mean±sd], or arithmetic mean±sd
Afridi (22) (2008)	Cross-sectional, Hyderabad, Pakistan, men only, non-smokers (T2D =196; non T2D=238)	Comparison of Cd levels between groups	Students t-test: T2D men had greater levels of Cd in their blood and urine than non-T2D men, regardless of smoking status (p<0.001 for all).	hair, blood, urine [AAS] T2D / Non-T2D hair (μg/g) 2.5±0.3 / 1.4±0.3 blood (μg/L) 5.7±1.3 / 4.2±1.3 urine (μg/L) 4.7±0.8 / 2.3±0.4
Afridi (82) (2013)	Age-matched case-control, Ireland & Pakistan, men and women ages 30-50, (T2D=145; non T2D=171)	Comparison of Cd levels between groups	Student's t-test: For both Ireland and Pakistan, hair Cd for both male and female Irish T2Ds were significantly higher compared to male and female controls (p<0.001).	hair [ICP-MS] for Ireland; AAS for Pakistan] hair (μg/g) Ireland Pakistan Control Male 0.68 ± 0.15 1.63 ± 0.25 Female 0.62 ± 0.07 1.54 ± 0.17 T2D Male 2.26 ± 0.32 3.19 ± 0.3 Female 1.76 ± 0.06 2.85 ± 0.33
Barregard (81) (2013)	Cross-sectional with 5.4 year follow-up, Gothenburg, Sweden, 64-year old women (816 at baseline & n=244 for follow-up)	IGT T2D β-cell function	Logistic regression: Compared to Q1 of blood Cd, Q4 had1.2 (95% CI:0.6-2.4) or 2.2 (95% CI 0.9-6.1) times the odds of T2D or IGT at baseline and follow-up respectively. Compared to Q1 of urinary Cd, Q4 had 1.2 (0.5-2.6) times the odds of T2D or IGT at follow-up. Controlled for pack-years, waist circumference, and serum adiponectin.	blood, urine [ICP-MS] baseline blood (μg/L) urine (μg/g Cr) NGT 0.3 (0.2-1.7) 0.4 (0.2-0.9) IGT 0.4 (0.1, 1.3) 0.4 (0.2-1.0) T2D 0.4 (0.1-2.0) 0.3 (0.1-1.0)
Chen (86) (2006)	Cross-sectional, Shanghai, China, all with T2D (229)	Urinary indicators of renal dysfunction	Logistic regression: Subjects with greater urinary Cd (≥1 μg/g Cr) had 3.34 (95%CI 1.17-9.53) times the odds of renal dysfunction (β_2-microglobulinuria) compared to those with lower urinary Cd, adjusted for smoking & MT-Ab.	blood, urine [AAS] 0.61(0.03-4.54) μg/L blood; 0.38 (0.05-4.17) μg/g Cr urine
Haswell-Elkins (24) (2007)	Cross-sectional, Torres Strait, Australia* (124)	Comparison of Cd levels between groups Predictors of Cd level	Backward stepwise linear regression: DM (fasting blood glucose ≥7.8 mmol/L or 2h glucose >11.0 mmol/L) was associated with greater levels of Cd (β=0.10; p=0.05), controlling for age sex, smoking status, and community.	urine [ICP-MS] 0.83 μg/g Cr urine
Haswell-Elkins (85) (2008)	Cross-sectional, Torres Strait, Australia* (182)	Albuminuria	Stepwise multiple linear regression: Cd levels were not significantly different between T2D and non-T2D. Among T2D, log 10 Cd levels were related to albuminuria (β=0.99, p<0.001), controlling for hypertension.	urine [ICP-MS] 0.93 μg/g Cr urine (table continues on next page – notes at end)

(table continues on next page; key to abbreviations is located at end of table)

Table I. Summary of major findings of epidemiologic studies of cadmium and diabetes (continued)

First Author (Year)	Study Design, population (n)	Relevant Outcome(s)	Association measure: Cadmium related Findings	Bio Matrix [lab method] median(range), [geometric mean±sd], or arithmetic mean±sd
Ikeda (84) (2013)	Case-control; Japan, never-smoking women (39 DM; 3 control groups, each with 117 controls)	Comparison of Cd levels between groups	Logistic regression: No association was observed between Cd and DM, controlling for age and other non-specific covariates.	Urine [AAS] [1.8 µg/g] Cr for DM [1.8 µg/g] Cr for groups 1 & 3 [1.9 µg/g] Cr for groups 2
Kolachi (23) (2010)	Cross-sectional, Pakistan, pregnant women, T2D (76) and referent mothers (68)	Comparison of Cd levels between groups	Student's t-test: Women with T2D had higher Cd levels than referent women in blood ($p<0.001$), hair ($p<0.01$), and urine ($p<0.005$).	blood, hair, urine [AAS] referent / T2D blood (µg/L) 3.1±0.5 / 4.7±0.9 hair (µg/g) 1.6±0.4 / 2.4±0.5 urine (µg/L) 1.8±0.2 / 2.9±0.5
Moon (83) (2013)	Cross-sectional, KNHANES IV-V, Korea (2009-2010) (3184)	Prevalent DM (fasting plasma glucose ≥126 mg/dL, use of antiDM Tx, self-reported DM)	Logistic regression: Q4 had 0.898 (0.633-1.275) times the odds of DM compared to Q1, controlling for age, sex, region, smoking, alcohol, exercise.	blood [AAS] DM [1.10 ±1.72 µg/L] Not DM [1.16 ± 1.72 µg/L]
Schwartz (3) (2003)	Cross-sectional, NHANESIII (1988-94), USA, age ≥40 (8722)	T2D; Impaired Fasting Glucose (IFG=110 ≤ fasting plasma glucose ≤126 mg/dl)	Logistic regression: As Cd increased, the odds of IFG increased in a dose dependent fashion [OR_{medium} =1.48, 95%CI 1.21–1.82; OR_{high} 2.05, 95%CI 1.42–2.95 as compared to low]. The same relation was observed between Cd and T2D, [OR_{medium} =1.24, 95%CI 1.06 –1.45; OR_{high} =1.45, 95% CI 1.07–1.97) as compared to low]. Controlled for age, sex, race/ethnicity, and BMI.	urine [AAS] Ever Smokers: 0.92 µg/g Cr urine Never Smokers 0.63 µg/g Cr urine low/referent: ≤0.99 µg/g Cr medium: 1.0-1.99 µg/g Cr high: ≥2 µg/g Cr
Swaddiwudhipong (25) (2010)	Cross-sectional, Rural Mae Sot District, Tak Province, Thailand* (5273)	DM (2 fasting plasma glucose measurements ≥126 mg/dl or current use of antiDM Tx)	Logistic regression: No association was observed between Cd and DM, controlling for age, alcohol consumption, smoking, BMI, and hypertension. Prevalence of DM increased across tertiles of Cd ($p=0.068$); and this increase was significant among women ($p=0.024$).	urine [AAS] [2.2±2.3] µg/g Cr urine tertiles: low <1.36 µg/g Cr, medium 1.36-2.89 µg/g Cr, high >2.89 µg/g Cr
Swaddiwudhipong (26) (2012)	5 year longitudinal study, Rural Mae Sot District, Tak Province, Thailand*, all subjects had urinary Cd ≥5 µg/g Cr at baseline (n=436)	DM prevalence (2 fasting plasma glucose measurements ≥126 mg/dl or current use of antidiabetic treatment)	GEE regression analysis: no association between urinary Cd and DM was observed. A 2% increase in prevalence of DM from baseline was observed ($p=0.006$). Among those who reduced Cd exposure, DM prevalence rose 1% ($p=0.375$), while prevalence rose 4.2% among those with continuing Cd exposure ($p=0.021$).	urine [AAS] Baseline: [9.5±1.6] µg/g Cr urine Follow-up: 8.8±1.6 µg/g Cr urine

*Indicates increased likelihood of Cd exposure based upon known Cd contamination in geographical location [AAS=atomic absorption spectrometry, antiDM Tx= antidiabetic medication or treatment, Cd=cadmium, Cr= creatinine, DM=diabetes mellitus, ICP-MS=inductively coupled mass spectrometry, IGT=Impaired glucose tolerance, IQR=interquartile range, MT-Ab=metallothionein antibody, NGT=normal glucose tolerance, Q1=lowest quartile, Q4=highest quartile, T2D=type 2 diabetes]

Table II. Summary of major findings of epidemiologic studies of cadmium and fetal growth

First Author (Year)	Study Design, population (n) [Outcome(s)]	Biological Matrix (timing of collection) [lab method]	Association Measure: Cadmium related Findings	Cadmium median (range) or arithmetic mean±sd
Al-Saleh (59) (2013)	Cross-sectional, Al-Kharj hospital, Saudi Arabia, (1578) [BW, HC, BL, PI, SGA]	CB, MB, & placenta (delivery) [AAS]	Multivariate unconditional logistic regression: Likelihood of <10th percentile for each measure was increased with greater cord Cd [OR (95% CI)]: BL [1.7(1.0–2.8); p=0.03], BW [2.0 (1.1–3.6); p=0.02], HC [1.5 (0.8–2.6); p=0.230] and SGA [1.8 (951.0–3.1); p=0.05], adjusted for maternal age, parity, maternal 3rd trimester BMI, infant sex, and additional outcome specific factors. Cd was not associated with PI.	0.704 (0.245-15.325) µg/L CB 0.983 (0.233-3.157) µg/L MB 0.035(LOD-4.363) µg/g placenta (dry weight)
Frery (52) (1993)	Cross-sectional, Paris, France (102) [Placental features, BW]	Placenta, maternal and newborn hair (delivery) [AAS]	Willcoxon test: Placental calcifications of the parenchyma corresponded with greater Cd concentrations in placental tissue and lower newborn hair Cd (p<0.05). Among women with parenchymal calcifications, on average newborns with hair cadmium in the highest quartile were 473 g lighter than those in the first quartile (p<0.01).	Without calcifications: 8.5 (5th percentile 3.6, 95th percentile 21.4) ng/g placenta With calcifications: 11.8 (5th percentile 4.6, 95th percentile 22.7) ng/g placenta (wet weight)
Galicia-Garcia (56) (1997)	Cross-sectional, Mexico City, Mexico (49) [BW]	Maternal, cord, & newborn blood (delivery) [AAS]	Multiple linear regression: Marginal inverse association between Cd in CB and BW only [β=-321.8 g (p=0.06)], controlled for non-specific "questionnaire items."	1.2 (0.8-2.9) µg/L maternal blood 1.2 (0.6-2.0) µg/L CB 1.1 (0.8-2.1) µg/L newborn blood
Kippler (51) (2010)	Cross-sectional, Matlab, Bangledesh (44) [BW, CC, HC , BL]	Urine (gw 14), CB, placenta (delivery) [ICP-MS]	Spearman rank correlation: Negative correlations between placental Cd and BW (r=-0.31;p=0.04), CC (r=-0.37;p=0.02), and GA (r=-0.39;p<0.01), but no correlation with BL or HC. Non-significant inverse association between placental Cd and both BL and CC. Multiple linear regression: Inverse association between ln placental Cd (µg/kg) and BW (β=-173, 95%CI -432,-86). Inverse association between ln placental Cd and CC (β=--0.86, 95% CI -2.1-0.37). Controlled for CB Zn, and GA	0.110 (0.040-0.492) µg/g placenta (dry weight) 0.00016 (0.000074–0.00032) µg/g CB 0.79 µg/L (0.29-10) urine, adjusted for specific gravity 1.012 g/mL
Kippler (49) (2012)	Prospective cohort study, Matlab, Bangledesh (1616) [BW, CC, HC , BL]	Urine (gw 8), CB, placenta (delivery) [ICP-MS]	Multiple linear regression: Among girls, HC (β = -0.26; 95% CI: –0.43, –0.088), CC (β = -0.24; 95% CI: –0.44, –0.030) and BW (β =-45; 95% CI: –82.5, -7.3 g) Controlled for for maternal age, BMI, SES, hemoglobin at 14 gw, urinary As at 8 gw, betel use, and infant season of birth & GA	Median (IQR), 0.63 µg/L (0.38-1.0)

(table continues on next page; key to abbreviations is located at end of table)

Table II. Summary of major findings of epidemiologic studies of cadmium and fetal growth (continued)

First Author (Year)	Study Design, population (n) [Outcome(s)]	Biological Matrix (timing of collection) [lab method]	Association Measure: Cadmium related Findings	Cadmium median (range) or arithmetic mean±sd
Klapec (63) (2008)	Cross-sectional, Osijek, Croatia, AGA (n=44); IUGR (n=57) (101) [BW]	Placenta (delivery) [AAS]	Univariate linear regression: Placental Cd was not significantly associated with BW in either group.	IUGR: 0.00911 (0.00263-0.02700) µg/g placenta; AGA: 0.00803(0.00210-0.01920) µg/g placenta (wet weight)
Llanos (58) (2009)	Cross-sectional, Santiago, Chile, normal BW (n=20) and LBW (n=20) (40) [LBW]	Placenta (delivery) [AAS]	Mann-Whitney U-test: Average placental Cd was higher in the LBW group than in the normal BW group ($p < 0.01$).	For LBW: 0.050±0.006 µg/g placenta, and for normal BW: 0.023±0.002 µg/g placenta (dry weight)
Menai (60) (2012)	Prospective cohort study, Poitiers & Nancy, France (901) [BW; FGR]	MB (24-28 gw) [AAS]	Multiple linear regression: Cd associated with decreased BW among women who smoked during pregnancy ($b = -113.7$; $p = 0.001$). Logistic regression: Cd OR = 1.41; 95% CI: 1.00–1.99) & smoking both impacted FGR (OR 1.89; 95% CI: 1.00–3.58	Median (IQR) 0.8 (0.1–4.6)
Nishijo (57) (2002)	Cross-sectional, Toyama, Japan* (57) [BW, BL, HC, CC, GA]	Urine and colostrum milk (5-8 days postpartum) [AAS]	Multiple linear regression: BW (β=-0.015), and BL (β=-0.015) are inversely related to maternal urinary Cd; CC (β=0.089) and HC (β=0.094) are positively associated with maternal urinary Cd ($p>0.05$ for all). Controlled for maternal age and GA.	Among women with urinary Cd < 2 µg/g Cr: 2.8 ±1.7 nmol/L breast milk Among women with urinary Cd ≥ 2 µg/g Cr: 4.6±2.5 nmol/L breast milk
Nishijo (62) (2004)	Prospective cohort study, Toyama, Japan* (55) [BW, BL, HC, CC]	MB (30-32 gw) [AAS]	Spearman rank correlation: BW ($r=-0.233$;$p>0.05$), BL ($r=-0.337$;$p<0.05$), HC ($r=-0.145$; $p>0.05$), and CC($r=-0.168$; $p>0.05$), are negatively correlated to MB Cd. Spearman rank correlation: BW ($r=-0.233$;$p>0.05$), BL ($r=-0.337$;$p<0.05$), HC ($r=-0.145$; $p>0.05$), and CC($r=-0.168$; $p>0.05$), are negatively correlated to MB Cd.	9.29±6.29 nmol/L blood 9.29±6.29 nmol/L blood
Odland (64) (2004)	Cross-sectional, 6 sites across Russian & Norwegian arctic and subarctic areas (262) [BW, NBMI]	MB & neonatal blood, placenta (delivery) [AAS]	Multiple linear regression: Positive association between placental Cd both BW (1081 g/unit; 95%CI -1887 to 4048, $p>0.05$) and NBMI(4.40 kg/m^2 per unit; 95%CI -3.95 to 12.75, $p>0.05$). Controlled for country and GA.	2.0 (0.5-35.2) nmol/L maternal blood 0.5 (0.5-4.8) nmol/L neonatal blood 0.032 (0.011-0.201) µg/g placenta

(table continues on next page; key to abbreviations is located at end of table)

Table II. Summary of major findings of epidemiologic studies of cadmium and fetal growth (continued)

First Author (Year)	Study Design, population (n) [Outcome(s)]	Biological Matrix (timing of collection) [lab method]	Association Measure: Cadmium related Findings	Cadmium median (range) or arithmetic mean±sd
Osman (65) (2000)	Cross-sectional, Solna, Sweden (106) [Interactions among (Pb, Cd, Se, Zn), [BW]	MB (gw 36) & CB (delivery)[AAS], placenta (delivery) [ICP-MS]	Multiple linear regression: Pb was inversely associated with BW and HC , but not Cd. Controlled for maternal age, parity, smoking, GA, infant gender, BW, BL, HC and Apgar score.	1.4 (0.12–18) nmol/L whole blood 46 (10–170) nmol/kg placenta (wet weight)
Salpietro (53) (2002)	Cross-sectional, Messina, Italy (45) [BW]	Maternal & cord serum (delivery) [AAS]	Spearman's rank correlation: Inverse correlation of maternal (r=-0.546; p=0.0003) and CB (r=-0.509; p=0.0007) to BW.	119±75 ng/L maternal serum 133±99 ng/L cord serum
Shirai (55) (2010)	Cross-sectional, Tokyo, Japan (78) [BW, BL, HC]	Maternal urine (9-40 gw) [ICP-MS]]	Stepwise multiple linear regression: Negative association between urinary Cd and BW (B=-135; p=0.021: controlling for GA and maternal BMI) was observed. Tested GA, sex, birth order, BMI, age, maternal/paternal smoking, urinary metals (As, Be, Cu, Mo, Se, Sb, Sn, Pb, Zn) with maternal height replacing maternal BMI in the BL analysis.	0.766 µg/g Cr (geometric mean) urine 91.1 ± 53.7µg/g Cr urine
Tian (54) (2009)	Prospective cohort study, Da-Ye County, China* (109) [BW, BL]	MB (<7 days prior to delivery) & CB, placenta (delivery) [ICP-MS]	Multiple linear regression: Inverse association of CB Cd with both BW (β=-1.31; p<0.05) and BL (β=-1.04; p<0.01). Controlled for CB Pb, maternal age, height, weight, gravidity, GA, delivery method	1.80(0.43-25.25) µg/L MB 0.60 (0.02-1.78) µg/L CB 0.15 (0.03-3.9) µg/g placenta (dry weight)
Zhang (61) (2004)	Prospective cohort study, Da-Ye County, China* (44) [PT, BW, BL]	MB (<72 h prior to delivery) & CB, placenta (delivery) [ICP-MS]	Multiple linear regression: Inverse association of Cd in CB (not MB or placental Cd) with BL (β=-2.24; p=0.025); no association with BW. Controlled for maternal age, height, weight, GA.	1.72 (0.80-25.20) µg/L MB 0.40 (0.020-1.48) µg/L CB 0.145(0.082-3.97) µg/g placenta (dry weight)

*Indicates increased likelihood of Cd exposure based upon known Cd contamination in geographical location
AGA=birth weight appropriate for gestational age, AAS=atomic absorption spectrometry, As=arsenic, Be=beryllium, BL=birth length, BMI=body mass index, BW=birth weight, CB=cord blood, CC=chest circumference, Cd=cadmium, Cr=creatinine, Cu=copper, FGR= birthweight lower than the 10^{th} percentile, adjusted for gestational age & neonatal sex, GA=gestational age, gw= gestational week, HC =head circumference, ICP-MS=inductively coupled mass spectrometry, IUGR=intrauterine growth restriction; LBW= birthweight < 2500 g, Mo=molybdenum, na=not applicable, NBMI= newborn body mass index, Pb=lead, Se=selenium, SGA=small for gestational age (BW <10^{th} percentile per GA and infant gender), Sb=antimony, Sn=tin, Zn=zinc

Table III. Characteristics of women in the study population with low urinary creatinine (<30 mg/dL)

	Subcohort (n=138)	GDM Cases (n=36)
Maternal age (years)	**%**	**%**
<25	1.4	na
25-34	60.9	52.8
35+	37.7	47.2
Mean (SD)	33.2 (3.8)	3416.3 (619.3)
Pre-pregnancy BMI (kg/m^2)		
Under weight (<18.5)	6.5	5.6
Normal weight (18.5 to <25.0)	73.9	44.4
Overweight (25.0 to <30.0)	14.5	33.3
Obese (≥30.0)	5.1	16.7
Mean (SD)	22.4 (3.9)	25.5 (6.2)
Nulliparous	65.2	63.9
Race/Ethnicity		
Non-Hispanic White	92.7	80.6
African American	0.7	na
Asian	2.9	16.7
Other	3.6	2.8
Post high school education	99.3	94.3
Married	87.7	91.7
Preeclampsia during current pregnancy	2.2	2.9
Anemic during current pregnancy	3.0	2.9
Chronic hypertension	2.9	2.8
Family history of diabetes	11.6	27.8
Family history of hypertension	47.8	50.0
Smoking status		
Never	73.7	80.0
Former	24.1	11.4
Current	2.3	8.6
No leisure time physical activity	na	2.9
Urinary cadmium < LOD (0.10 μg/g Cr)	76.8	77.8
Urinary arsenic <LOD (3 μg/g Cr)	76.8	77.8
Mean gestation week of urine collection	15.3 (2.7)	15.3 (2.7)
Creatinine in urine mg/dL		
Geometric mean (95% Confidence Interval)	20.1(19.3, 20.8)	18.9 (18.9, 22.0.)

BMI=Body mass index, Cr=creatinine, LOD= Limit of detection, SD= Standard deviation

Table IV. Institute of Medicine guidelines for weight gain during pregnancy(168) based upon World Health Organization classifications of pre-pregnancy body mass index (188)

Pre-pregnancy body mass index (kg/m^2)	Total weight gain (lbs.)
Underweight (<18.5)	28-40
Normal weight (18.5-24.9)	25-35
Overweight (25.0-29.9)	15-25
Obese (≥30)	11-20

Table V. Estimated coefficients and 95% confidence intervals from univariable linear regression models examining the relation between predictors of interest and birth anthropometrics in the Omega Study, Seattle and Tacoma, Washington, 1996-2008

	Birthweight (g)		Ponderal Index (kg/m^3)	
	Males	**Females**	**Males**	**Females**
Covariate	β [95% CI]	β [95% CI]	β [95% CI]	β [95% CI]
Maternal age (≥35 years)	-8 [-58, 41]	8 [-38, 54]	-0.05 [-0.39, 0.29]	-0.05[-0.46, 0.35]
Pre-pregnancy body mass index (kg/m^2)	**15 [10, 2047]**	**13 [8, 18]**	**0.1 [0.03, 0.1]**	**0.1 [0.03, 0.1]**
Maternal height (m)	**1443 [1088, 1797]**	**1131 [799, 1463]**	-0.8 [-3.1, 1.5]	0.2 [-2.3, 2.8]
Unmarried	1.4 [-67.1, 70.0]	8 [-56, 71]	0.4 [-0.2, 0.9]	0.4 [-0.2, 0.9]
Not non-Hispanic white race/ethnicity	**-91 [-161, -21]**	-31 [-102, 40]	-0.1 [-0.6, 0.3]	0.1 [-0.5, 0.6]
Nulliparous	**-73 [-121, -24]**	**-71 [-116, -27]**	**-0.9 [-1.2, -0.5]**	**0.8 [-1.2, -0.4]**
Chronic hypertension	-141 [-301, 19]	17 [-86, 120]	-0.1 [-0.9, 0.7]	-0.3 [-1.1, 0.5]
Preeclampsia	-115 [-369, 139]	57 [-101, 214]	-0.5 [-1.6, 0.6]	0.6 [-0.5, 1.7]
Gestational Diabetes	-24 [-153, 106]	102 [-1, 205]	0.2 [-0.6, 0.9]	0.4 [-0.4, 1.3]
Any hypertensive disorder [a]	-144 [-289, 1]	32 [-64, 127]	-0.3 [-1.0, 0.4]	-.005 [-0.70, 0.69]
Renal disease	-150 [-428, 129]	-174 [-433, 84]	-0.2 [-1.4, 0.9]	**-1.8 [-3.6, -0.06]**
Thyroid disease	-18 [-100, 64]	-3 [-87, 82]	0.3 [-0.3, 0.9]	0.2 [-0.4, 0.8]
Pre-existing diabetes	143 [-164, 450]	183 [-166, 531]	1.6 [-0.1, 3.3]	1.6 [-0.2, 3.4]
Family history of hypertension [b]	**-66 [-113, -19]**	-36 [-80, 9]	-0.1 [-0.5, 0.3]	-0.1 [-0.5, 0.3]
Family history of diabetes [c]	-17 [-88, 53]	-21 [-89, 47]	-0.2 [-0.7, 0.2]	0.2 [-0.3, 0.8]
Total Weight gain (lbs)	**10 [8,12]**	**8 [6,10]**	**0.01 [0.0002, 0.03]**	0.01 [-0.006, 0.03]
Calories (cal/day)	33 [-6, 71]	8 [-28, 44]	-0.04 [-0.30, 0.22]	**0.4 [0.02, 0.7]**
Dietary calcium intake (g/day)	**76 [34, 118]**	17 [-21, 56]	0.03 [-0.25, 0.31]	0.2 [-0.1, 0.6]
Dietary iron intake (mg/day)	3.6 [-0.2, 7.5]	0.3 [-3.4, 4.0]	0.008 [-0.017, 0.033]	0.03 [-0.001,0.06]
Dietary zinc intake (mg/day)	**6.6 [2.2, 10.9]**	0.9 [-3.4, 5.1]	0.02 [-0.01, 0.04]	0.03 [-0.003, 0.06]
No leisure time physical activity	9 [-105, 123]	0.7 [-132, 134]	-0.37 [-1.11, 0.38]	0.21 [-0.86, 1.28]
Smoked during pregnancy [d]	-39 [-140, 63]	83 [-24, 190]	-0.03 [-0.74, 0.67]	**0.98 [0.03, 1.92]**
Alcohol during pregnancy [e]	36 [-19, 92]	-17.0 [-66, 32]	-0.13 [-0.51, 0.26]	0.04 [-0.38, 0.47]
Gestational week of delivery	**144 [126, 163]**	**124 [106, 143]**	0.02 [-0.11, 0.16]	0.01 [-0.16, 0.19]

(table continues on next page; notes are located at end of table)

Table V. Estimated coefficients and 95% confidence intervals from univariable linear regression models examining the relation between predictors of interest and birth anthropometrics in the Omega Study, Seattle and Tacoma, Washington, 1996-2008 (continued)

	Birth Length (cm)		Head Circumference (cm)	
	Males	**Females**	**Males**	**Females**
Covariate	β [95% CI]	β [95% CI]	β [95% CI]	β [95% CI]
Maternal age (≥35 years)	0.01 [-0.26, 0.27]	0.1 [-0.1, 0.4]	0.07 [-0.12, 0.25]	0.07 [-0.12, 0.25]
Pre-pregnancy body mass index (kg/m^2)	**0.03 [0.003, 0.05]**	0.02 [-0.01, 0.05]	**0.03 [0.01, 0.05]**	**0.05 [0.03, 0.07]**
Maternal height (m)	**7.1 [5.2, 9.1]**	**5.8 [3.9, 7.6]**	**2.8 [1.5,4.2]**	**2.3 [1.1, 3.5]**
Unmarried	-0.2 [-0.6, 0.2]	-0.1 [-0.5, 0.3]	-0.20 [-0.44, 0.05]	-0.06 [-0.30, 0.19]
Not non-Hispanic white race/ethnicity	-0.3 [-0.7, 0.01]	-0.2 [-0.6, 0.2]	**-0.41 [-0.66, -0.16]**	-0.17 [-0.41, 0.07]
Nulliparous	0.2 [-0.1, 0.4]	0.1 [-0.2, 0.3]	**-0.2 [-0.4, -0.03]**	**-0.3 [-0.4, -0.1]**
Chronic hypertension	-0.7 [-1.5, 0.004]	0.3 [-0.3, 0.9]	-0.35 [-0.89, 0.20]	0.46 [0.03, 0.90]
Preeclampsia	-0.3 [-1.8, 1.1]	-0.1 [-0.9, 0.6]	-0.20 [-0.96, 0.55]	0.17 [-0.38, 0.72]
Gestational Diabetes	-0.6 [-1.3, 0.1]	0.1 [-0.4, 0.6]	-0.32 [-0.80, 0.17]	0.34 [-0.03, 0.70]
Any hypertensive disorder [a]	-0.3 [-0.9, 0.3]	0.2 [-0.3, 0.7]	-0.03 [-0.47, 0.41]	-0.09 [-0.42, 0.24]
Renal disease	-0.4 [-1.9, 1.1]	-0.2 [-1.6, 1.1]	-0.12 [-1.07, 0.84]	0.05 [-0.92, 1.01]
Thyroid disease	-0.6 [-2.1, 0.7]	0.3 [-0.9, 1.4]	-0.62 [-1.26, 0.02]	0.12 [-0.47, 0.71]
Pre-existing diabetes	-0.2 [-0.7, 0.2]	-0.1 [-0.6, 0.3]	0.10 [-0.20, 0.41]	0.15 [-0.13, 0.44]
Family history of hypertension [b]	**-0.5 [-0.7, -0.3]**	-0.1 [-0.4, 0.1]	0.00 [-0.17, 0.18]	-0.07 [-0.24, 0.11]
Family history of diabetes [c]	0.06 [-0.3, 0.4]	-0.2 [-0.6, 0.1]	0.05 [-0.21, 0.31]	-0.14 [-0.38, 0.10]
Total Weight gain (lbs)	**0.04 [0.03, 0.05]**	**0.03 [0.02, 0.04]**	**0.02 [0.01, 0.03]**	**0.02 [0.01, 0.02]**
Calories (cal/day)	**0.2 [0.004, 0.4]**	-0.2 [-0.4, 0.1]	0.05 [-0.09, 0.18]	-0.03 [-0.17, 0.12]
Dietary calcium intake (g/day)	**0.3 [0.1, 0.6]**	-0.01 [-0.25, 0.23]	0.1 [-0.01, 0.3]	-0.01 [-0.16, 0.14]
Dietary iron intake (mg/day)	0.01 [-0.01, 0.04]	-0.02[-0.04,0.003]	0.0003 [-0.015, 0.015]	-0.009 [-.024, 0.005]
Dietary zinc intake (mg/day)	**0.02 [0.0001, 0.05]**	-0.01[-0.03, 0.01]	0.01 [-0.01, 0.02]	-0.01 [-0.02, 0.01]
No leisure time physical activity	0.3 [-0.3, 0.8]	-0.1 [-0.9, 0.7]	-0.23 [-0.71, 0.26]	0.05 [-0.35, 0.44]
Smoked during pregnancy [d]	-0.2 [-0.7, 0.4]	-0.2 [-0.8, 0.4]	-0.2 [-0.5, 0.1]	0.3 [-0.1, 0.6]
Alcohol during pregnancy [e]	0.3 [-0.04, 0.5]	-0.1 [-0.4, 0.2]	0.13 [-0.08, 0.33]	0.01 [-0.18, 0.19]
Gestational week of delivery	**0.7 [0.6, 0.8]**	**0.6 [0.5, 0.7]**	**0.37 [0.30, 0.44]**	**0.28 [0.21, 0.36]**

Each covariate was entered into a separate univariable linear regression model with each outcome (birthweight, ponderal index, birth length, and head circumference) to assess the independent effect of that covariate on each individual outcome.

β=estimated coefficient; 95% CI=95% Confidence interval; Values with $p<0.05$ presented in bold.

a. Includes preeclampsia and chronic hypertension

b. Any maternal primary or secondary relative with a known hypertensive condition
c. Any maternal primary or secondary relative with a known diabetic condition
d. Any maternal self-reported smoking of tobacco cigarettes
e. Any maternal self-reported consumption of alcohol during pregnancy

Table VI. Estimated coefficients and 95% confidence intervals from linear regression models examining the relation between maternal pre-pregnancy body mass index and birth anthropometrics in the Omega Study, Seattle and Tacoma, Washington, 1996-2008

	Birthweight (g)		Ponderal Index (kg/m^3)	
	Males	**Females**	**Males**	**Females**
Pre-pregnancy BMI (kg/m^2) [a]	β [95% CI]	β [95% CI]	β [95% CI]	β [95% CI]
Underweight (<18,5)	**-222 [-315, -128]**	**-163 [-278, -48]**	**0 .4 [-0.5, 1.3]**	**-1.1 [-1.8, -0.4]**
Normal (18.5-24.9)	0 [reference]	0 [reference]	0 [reference]	0 [reference]
Overweight (25-29.9)	**71 [3, 140]**	**111 [48, 175]**	**0.8 [0.2,1.4]**	0.2 [-0.3, 0.6]
Obese (≥30)	**7 [84, 269]**	**86 [2, 169]**	**0.7 [0.2,1.3]**	**1.0 [0.4, 1.6]**

	Birth Length (cm)		Head Circumference (cm)	
	Males	**Females**	**Males**	**Females**
Pre-pregnancy BMI (kg/m^2) [a]	β [95% CI]	β [95% CI]	β [95% CI]	β [95% CI]
Underweight (<18,5)	**-0.3 [-0.9, 0.3]**	**1.0 [-1.6, -0.3]**	**-0.44 [-0.83, -0.05]**	**-0.28 [-0.77, 0.20]**
Normal (18.5-24.9)	0 [reference]	0 [reference]	0 [reference]	0 [reference]
Overweight (25-29.9)	0.2 [-0.1, 0.6]	0.1 [-0.2, 0.5]	0.07 [-0.15, 0.31]	**0.26 [0.02, 0.50]**
Obese (≥30)	0.2 [-0.3, 0.7]	-0.05 [-0.5, 0.4]	**0.44 [0.12, 0.75]**	**0.39 [0.10, 0.68]**

Four separate linear regressions were performed to assess the independent effect of body mass index (BMI) on each outcome of interest (birthweight, ponderal index, birth length, and head circumference).
β=estimated coefficient; 95% CI=95% Confidence interval; Values with $p<0.05$ presented in bold.

a. Each model included only indicator variables for pre-pregnancy BMI classified by World Health Organization classifications (188), using normal weight women as the reference group.

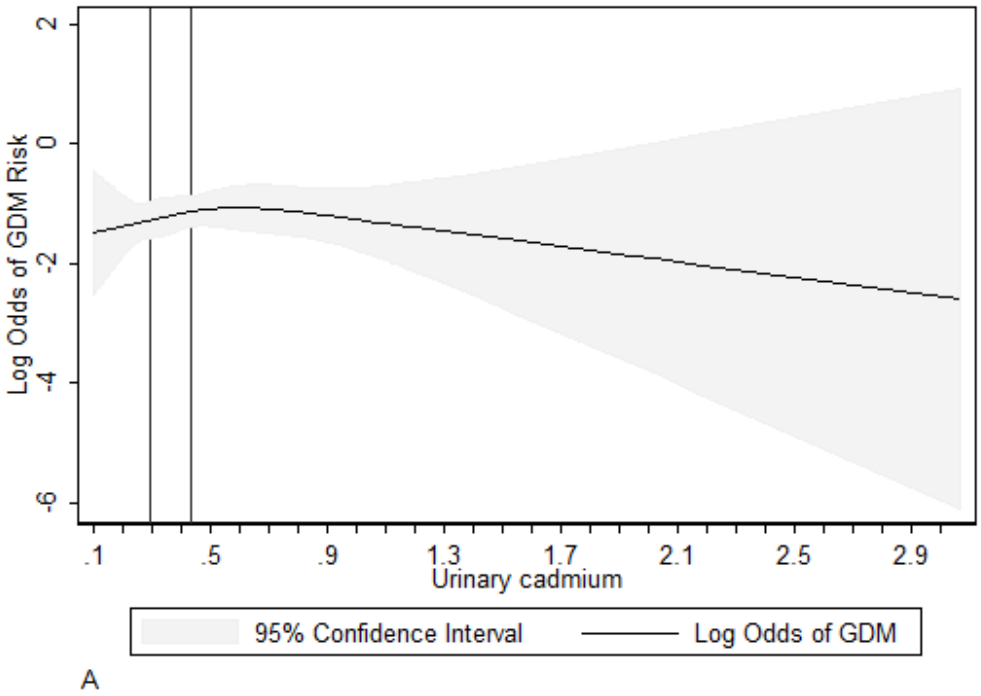
Table VII. Estimated coefficients and 95% confidence intervals from linear regression models examining the relation between maternal weight gain during pregnancy and birth anthropometrics in the Omega Study, Seattle and Tacoma, Washington, 1996-2008

	Birthweight (g)		**Ponderal Index (kg/m^3)**	
	Males	**Females**	**Males**	**Females**
IOM weight gain guidelines [a]	β [95% CI]	β [95% CI]	β [95% CI]	β [95% CI]
Below optimal	**-160 [-246, -73]**	**-133 [-207, -58]**	-0.4 [-1.0, 0.2]	-0.5 [-1.1, 0.1]
Optimal	0 [reference]	0 [reference]	0 [reference]	0 [reference]
Above optimal	**189 [139, 238]**	**184 [136, 231]**	**0.5 [0.2, 0.9]**	0.1 [-0.3, 0.6]
	Birth Length (cm)		**Head Circumference (cm)**	
	Males	**Males**	**Males**	**Females**
IOM weight gain guidelines [a]	β [95% CI]	β [95% CI]	β [95% CI]	β [95% CI]
Below optimal	**-0.5 [-1.0, -0.1]**	-0.3 [-0.8, 0.1]	**-0.4 [-0.8, -0.03]**	-0.3 [-0.6, 0.03]
Optimal	0 [reference]	0 [reference]	0 [reference]	0 [reference]
Above optimal	**0.6 [0.3, 0.8]**	**0.8 [0.6, 1.1]**	**0.3 [0.1, 0.5]**	**0.4 [0.2, 0.6]**

Four separate linear regressions were performed to assess the independent effect of body mass index (BMI) on each outcome of interest (birthweight, ponderal index, birth length, and head circumference)., using normal weight women as the reference group.

β=estimated coefficient; 95% CI=95% Confidence interval; Values with $p<0.05$ presented in bold.

a. Each model included only indicator variables for weight gain categories based on the Institute of Medicine (IOM) guidelines for weight gain during pregnancy(168). Women with optimal weight gain during pregnancy serve as the reference group.



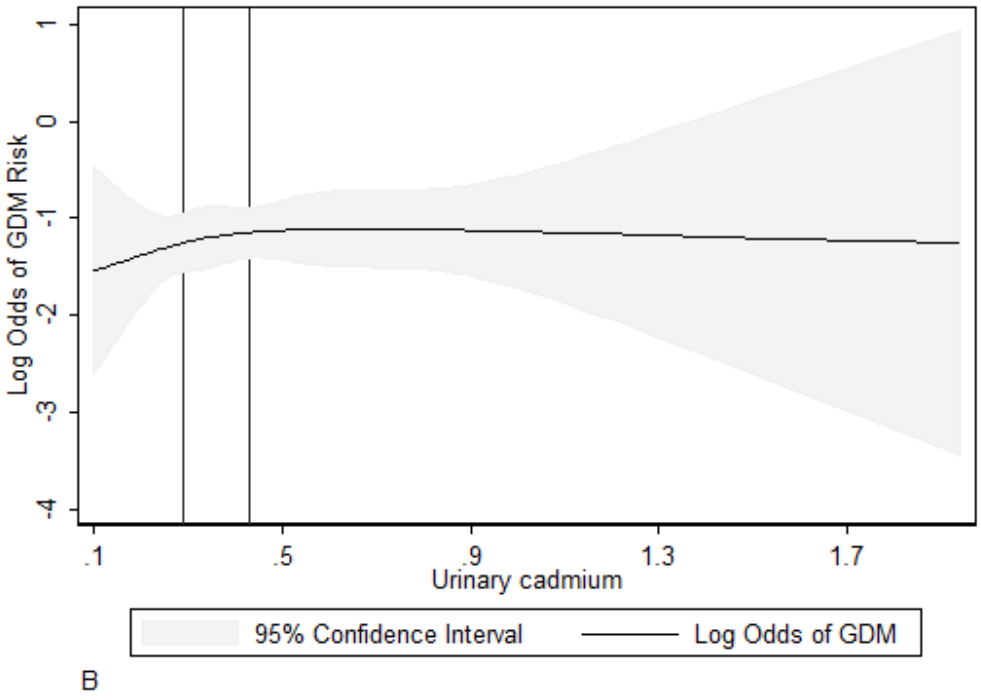


Figure I: Relation between maternal urinary cadmium and risk of gestational diabetes (GDM) (solid line) with 95% Confidence Intervals (gray area). The vertical bars along the urinary cadmium axis indicate the bounds of the tertiles based on distribution of urinary cadmium in the subcohort. (A) Includes 7 women with urinary cadmium >2µg/g Cr (B) Excludes 7 women with urinary cadmium >2µg/g Cr

References

1. Akesson A, Julin B, Wolk A. Long-term dietary cadmium intake and postmenopausal endometrial cancer incidence: a population-based prospective cohort study. *Cancer Res.* 2008;68(15):6435–6441.

2. Julin B, Wolk A, Bergkvist L, et al. Dietary cadmium exposure and risk of postmenopausal breast cancer: a population-based prospective cohort study. *Cancer Res.* 2012;72(6):1459–1466.

3. Schwartz GG, Il'yasova D, Ivanova A. Urinary cadmium, impaired fasting glucose, and diabetes in the NHANES III. *Diabetes Care*. 2003;26(2):468–470.

4. Tellez-Plaza M, Guallar E, Howard BV, et al. Cadmium exposure and incident cardiovascular disease. *Epidemiol. Camb. Mass*. 2013;24(3):421–429.

5. Alfvén T EC. Low-level cadmium exposure and osteoporosis. *J. Bone Miner. Res. Off. J. Am. Soc. Bone Miner. Res.* 2000;15(8):1579–86.

6. Engström A, Michaëlsson K, Vahter M, et al. Associations between dietary cadmium exposure and bone mineral density and risk of osteoporosis and fractures among women. *Bone*. 2012;50(6):1372–1378.

7. Järup L, Akesson A. Current status of cadmium as an environmental health problem. *Toxicol. Appl. Pharmacol.* 2009;238(3):201–208.

8. Satarug S, Garrett SH, Sens MA, et al. Cadmium, environmental exposure, and health outcomes. *Environ. Health Perspect.* 2010;118(2):182–190.

9. ATSDR, U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES, Public Health Service. Toxicological Profile for Cadmium. U.S. Dept of Health and Human Services, Agency for Toxic Substances and Disease Registry; 2012. (http://www.atsdr.cdc.gov/ToxProfiles/tp.asp?id=48&tid=15). (Accessed March 28, 2013)

10. Casarett L. Casarett and Doull's toxicology : the basic science of poisons. 7th ed. New York: McGraw-Hill; 2008.

11. Adams SV, Newcomb PA, Shafer MM, et al. Sources of cadmium exposure among healthy premenopausal women. *Sci. Total Environ.* 2011;409(9):1632–1637.

12. Kido T, Nordberg GF, Roels HA. Cadmium-induced renal effects. In: Broe ME, Porter GA, Bennett WM, et al., eds. *Clinical Nephrotoxins*. Dordrecht: Springer Netherlands; 2004:507–530.

13. Vesey DA. Transport pathways for cadmium in the intestine and kidney proximal tubule: focus on the interaction with essential metals. *Toxicol. Lett.* 2010;198(1):13–19.

14. Kippler M, Goessler W, Nermell B, et al. Factors influencing intestinal cadmium uptake in pregnant Bangladeshi women--a prospective cohort study. *Environ. Res.* 2009;109(7):914–921.

15. Centers for Disease and Control Prevention (USA). Fourth Report on Human Exposure to Environmental Chemicals, Updated Tables 2013. *Available CDC Natl. Cent. Environ. Heal. Atlanta Ga 30341*. 2013;(http://www.cdc.gov/exposurereport/). (Accessed June 1, 2013)

16. Bothwell TH. Iron requirements in pregnancy and strategies to meet them. *Am. J. Clin. Nutr.* 2000;72(1 Suppl):257S–264S.

17. Vahter M, Berglund M, Akesson A, et al. Metals and women's health. *Environ. Res.* 2002;88(3):145–155.

18. American Diabetes Association. Diagnosis and classification of diabetes mellitus. *Diabetes Care*. 2007;31 Suppl 1:S55–60.

19. Chu S, Callagan W, Kim S, et al. Maternal obesity and risk of gestational diabetes mellitus. *Diabetes Care*. 2007;30:2070–2076.

20. Metzger BE. Long-term outcomes in mothers diagnosed with gestational diabetes mellitus and their offspring. *Clin. Obstet. Gynecol.* 2007;50(4):972–979.

21. Blackburn ST. Maternal, Fetal, & Neonatal Physiology: A Clinical Perspective. 3rd ed. St. Louis, Mo: Saunders Elsevier; 2007.

22. Afridi HI, Kazi TG, Kazi N, et al. Evaluation of status of toxic metals in biological samples of diabetes mellitus patients. *Diabetes Res. Clin. Pract.* 2008;80(2):280–288.

23. Kolachi NF, Kazi TG, Afridi HI, et al. Status of Toxic Metals in Biological Samples of Diabetic Mothers and Their Neonates. *Biol. Trace Elem. Res.* 2011;143(1):196–212.

24. Haswell-Elkins M, Imray P, Satarug S, et al. Urinary excretion of cadmium among Torres Strait Islanders (Australia) at risk of elevated dietary exposure through traditional foods. *J. Expo. Sci. Environ. Epidemiol.* 2007;17(4):372–377.

25. Swaddiwudhipong W, Mahasakpan P, Limpatanachote P, et al. Correlations of urinary cadmium with hypertension and diabetes in persons living in cadmium-contaminated villages in northwestern Thailand: A population study. *Environ. Res.* 2010;110(6):612–616.

26. Swaddiwudhipong W, Limpatanachote P, Mahasakpan P, et al. Progress in cadmium-related health effects in persons with high environmental exposure in northwestern Thailand: a five-year follow-up. *Environ. Res.* 2012;112:194–198.

27. Merali Z, Singhal RL. Diabetogenic effects of chronic oral cadmium adminstration to neonatal rats. *Br. J. Pharmacol.* 1980;69(1):151–157.

28. Bell RR, Early JL, Nonavinakere VK, et al. Effect of cadmium on blood glucose level in the rat. *Toxicol. Lett.* 1990;54(2-3):199–205.

29. Han JC, Park SY, Hah BG, et al. Cadmium induces impaired glucose tolerance in rat by down-regulating GLUT4 expression in adipocytes. *Arch. Biochem. Biophys.* 2003;413(2):213–220.

30. Edwards JR, Prozialeck WC. Cadmium, diabetes and chronic kidney disease. *Toxicol. Appl. Pharmacol.* 2009;238(3):289–293.

31. Rutter GA. Nutrient-secretion coupling in the pancreatic islet beta-cell: recent advances. *Mol. Aspects Med.* 2001;22(6):247–284.

32. Valero D. Risk factors for low birth weight: a review. *Eur J Obstet Gynecol Reprod Biol*. 2004;116(1):3–15.

33. Miller H. Fetal growth in humans. Chicago: Year Book Medical Publishers; 1979.

34. Anderson GD, Blidner IN, McClemont S, et al. Determinants of size at birth in a Canadian population. *Am. J. Obstet. Gynecol.* 1984;150(3):236–244.

35. Campbell MK, Cartier S, Xie B, et al. Determinants of Small for Gestational Age Birth at Term. *Paediatr. Perinat. Epidemiol.* 2012;26(6):525–533.

36. Ornoy A. Prenatal origin of obesity and their complications: Gestational diabetes, maternal overweight and the paradoxical effects of fetal growth restriction and macrosomia. *Reprod. Toxicol. Elmsford N*. 2011;32(2):205–212.

37. Lampl M, Jeanty P. Timing is everything: a reconsideration of fetal growth velocity patterns identifies the importance of individual and sex differences. *Am. J. Hum. Biol. Off. J. Hum. Biol. Counc.* 2003;15(5):667–680.

38. Parker AJ, Davies P, Mayho AM, et al. The ultrasound estimation of sex-related variations of intrauterine growth. *Am. J. Obstet. Gynecol.* 1984;149(6):665–669.

39. Kramer MS, Olivier M, McLean FH, et al. Determinants of fetal growth and body proportionality. *Pediatrics*. 1990;86(1):18–26.

40. Kramer MS. Determinants of low birth weight: methodological assessment and meta-analysis. *Bull. World Health Organ.* 1987;65(5):663–737.

41. Wells JCK. Sexual dimorphism of body composition. *Best Pract. Res. Clin. Endocrinol. Metab.* 2007;21(3):415–430.

42. Stark MJ, Clifton VL, Wright IMR. Neonates born to mothers with preeclampsia exhibit sex-specific alterations in microvascular function. *Pediatr. Res.* 2009;65(3):292–295.

43. Stark MJ, Dierkx L, Clifton VL, et al. Alterations in the maternal peripheral microvascular response in pregnancies complicated by preeclampsia and the impact of fetal sex. *J. Soc. Gynecol. Investig.* 2006;13(8):573–578.

44. Murphy VE, Gibson PG, Giles WB, et al. Maternal asthma is associated with reduced female fetal growth. *Am. J. Respir. Crit. Care Med.* 2003;168(11):1317–1323.

45. Murphy VE, Gibson P, Talbot PI, et al. Severe asthma exacerbations during pregnancy. *Obstet. Gynecol.* 2005;106(5 Pt 1):1046–1054.

46. Clark JM, Hulme E, Devendrakumar V, et al. Effect of maternal asthma on birthweight and neonatal outcome in a British inner-city population. *Paediatr. Perinat. Epidemiol.* 2007;21(2):154–162.

47. Zarén B, Lindmark G, Bakketeig L. Maternal smoking affects fetal growth more in the male fetus. *Paediatr. Perinat. Epidemiol.* 2000;14(2):118–126.

48. Varvarigou AA, Asimakopoulou A, Beratis NG. Impact of maternal smoking on birth size: effect of parity and sex dimorphism. *Neonatology*. 2009;95(1):61–67.

49. Kippler M, Tofail F, Gardner R, et al. Maternal Cadmium Exposure during Pregnancy and Size at Birth: A Prospective Cohort Study. *Environ. Health Perspect.* 2012;120(2):284–289.

50. Kippler M, Engström K, Mlakar SJ, et al. Sex-specific effects of early life cadmium exposure on DNA methylation and implications for birth weight. *Epigenetics Off. J. Dna Methylation Soc.* 2013;8(5).

51. Kippler M, Hoque AMW, Raqib R, et al. Accumulation of cadmium in human placenta interacts with the transport of micronutrients to the fetus. *Toxicol. Lett.* 2010;192(2):162–168.

52. Fréry N, Nessmann C, Girard F, et al. Environmental exposure to cadmium and human birthweight. *Toxicology*. 1993;79(2):109–118.

53. Salpietro CD, Gangemi S, Minciullo PL, et al. Cadmium concentration in maternal and cord blood and infant birth weight: a study on healthy non-smoking women. *J. Perinat. Med.* 2002;30(5):395–399.

54. Tian L-L, Zhao Y-C, Wang X-C, et al. Effects of gestational cadmium exposure on pregnancy outcome and development in the offspring at age 4.5 years. *Biol. Trace Elem. Res.* 2009;132(1-3):51–59.

55. Shirai S, Suzuki Y, Yoshinaga J, et al. Maternal exposure to low-level heavy metals during pregnancy and birth size. *J. Environ. Sci. Health Part A Tox. Hazard. Subst. Environ. Eng.* 2010;45(11):1468–1474.

56. Galicia-García V, Rojas-López M, Rojas R, et al. Cadmium levels in maternal, cord and newborn blood in Mexico City. *Toxicol. Lett.* 1997;91(1):57–61.

57. Nishijo M, Nakagawa H, Honda R, et al. Effects of maternal exposure to cadmium on pregnancy outcome and breast milk. *Occup. Environ. Med.* 2002;59(6):394–397.

58. Llanos MN, Ronco AM. Fetal growth restriction is related to placental levels of cadmium, lead and arsenic but not with antioxidant activities. *Reprod. Toxicol. Elmsford N*. 2009;27(1):88–92.

59. Al-Saleh I, Shinwari N, Mashhour A, et al. Birth outcome measures and maternal exposure to heavy metals (lead, cadmium and mercury) in Saudi Arabian population. *Int. J. Hyg. Environ. Health*. 2013;

60. Menai M, Heude B, Slama R, et al. Association between maternal blood cadmium during pregnancy and birth weight and the risk of fetal growth restriction: the EDEN mother-child cohort study. *Reprod. Toxicol. Elmsford N*. 2012;34(4):622–627.

61. Zhang Y-L, Zhao Y-C, Wang J-X, et al. Effect of environmental exposure to cadmium on pregnancy outcome and fetal growth: a study on healthy pregnant women in China. *J. Environ. Sci. Health Part A Tox. Hazard. Subst. Environ. Eng.* 2004;39(9):2507–2515.

62. Nishijo M, Tawara K, Honda R, et al. Relationship between newborn size and mother's blood cadmium levels, Toyama, Japan. *Arch. Environ. Health*. 2004;59(1):22–25.

63. Klapec T, Cavar S, Kasac Z, et al. Selenium in placenta predicts birth weight in normal but not intrauterine growth restriction pregnancy. *J. Trace Elem. Med. Biol. Organ Soc. Miner. Trace Elem. Gms*. 2008;22(1):54–58.

64. Odland JO, Nieboer E, Romanova N, et al. Elements in placenta and pregnancy outcome in arctic and subarctic areas. *Int. J. Circumpolar Health*. 2004;63(2):169–187.

65. Osman K, Akesson A, Berglund M, et al. Toxic and essential elements in placentas of Swedish women. *Clin. Biochem.* 2000;33(2):131–138.

66. American Diabetes Association. Gestational diabetes mellitus. *Diabetes Care*. 2004;27:S88–90.

67. Cypryk K, Szymczak W, Czupryniak L, et al. Gestational diabetes mellitus - an analysis of risk factors. *Endokrynol. Pol.* 2008;59(5):393–397.

68. Xiong X, Saunders LD, Wang FL, et al. Gestational diabetes mellitus: prevalence, risk factors, maternal and infant outcomes. *Int. J. Gynaecol. Obstet. Off. Organ Int. Fed. Gynaecol. Obstet.* 2001;75(3):221–228.

69. Solomon CG, Willett WC, Carey VJ, et al. A prospective study of pregravid determinants of gestational diabetes mellitus. *Jama J. Am. Med. Assoc.* 1997;278(13):1078–1083.

70. Dooley SL, Metzger BE, Cho NH. Gestational diabetes mellitus. Influence of race on disease prevalence and perinatal outcome in a U.S. population. *Diabetes*. 1991;40 Suppl 2:25–29.

71. Torloni MR, Betrán AP, Horta BL, et al. Prepregnancy BMI and the risk of gestational diabetes: a systematic review of the literature with meta-analysis. *Obes. Rev. Off. J. Int. Assoc. Study Obes.* 2009;10(2):194–203.

72. England LJ, Levine RJ, Qian C, et al. Glucose tolerance and risk of gestational diabetes mellitus in nulliparous women who smoke during pregnancy. *Am. J. Epidemiol.* 2004;160(12):1205–1213.

73. ACOG Practice Bulletin. Clinical management guidelines for obstetrician-gynecologists. Number 30, September 2001 (replaces Technical Bulletin Number 200, December 1994). Gestational diabetes. *Obstet. Gynecol.* 2001;98(3):525–538.

74. Williams MA, Qiu C, Dempsey JC, et al. Familial aggregation of type 2 diabetes and chronic hypertension in women with gestational diabetes mellitus. *J. Reprod. Med.* 2003;48(12):955–962.

75. Annis AM, Caulder MS, Cook ML, et al. Family history, diabetes, and other demographic and risk factors among participants of the National Health and Nutrition Examination Survey 1999-2002. *Prev. Chronic. Dis.* 2005;2(2):A19.

76. Tellez-Plaza M, Navas-Acien A, Crainiceanu CM, et al. Cadmium exposure and hypertension in the 1999-2004 National Health and Nutrition Examination Survey (NHANES). *Environ. Health Perspect.* 2008;116(1):51–56.

77. Chen YW, Yang CY, Huang CF, et al. Heavy metals, islet function and diabetes development. *Islets*. 2009;1(3):169–176.

78. El Muayed M, Raja MR, Zhang X, et al. Accumulation of cadmium in insulin-producing β cells. *Islets*. 2012;4(6):405–416.

79. Ficková M, Eybl V, Kotyzová D, et al. Long lasting cadmium intake is associated with reduction of insulin receptors in rat adipocytes. *Biometals Int. J. Role Met. Ions Biol. Biochem. Med.* 2003;16(4):561–566.

80. Kawakami T, Sugimoto H, Furuichi R, et al. Cadmium reduces adipocyte size and expression levels of adiponectin and Peg1/Mest in adipose tissue. *Toxicology*. 2010;267(1-3):20–26.

81. Barregard L, Bergström G, Fagerberg B. Cadmium exposure in relation to insulin production, insulin sensitivity and type 2 diabetes: a cross-sectional and prospective study in women. *Environ. Res.* 2013;121:104–109.

82. Afridi HI, Kazi TG, Brabazon D, et al. Comparative metal distribution in scalp hair of Pakistani and Irish referents and diabetes mellitus patients. *Clin. Chim. Acta Int. J. Clin. Chem.* 2013;415:207–214.

83. Moon S-S. Association of lead, mercury and cadmium with diabetes in the Korean population: the Korea National Health and Nutrition Examination Survey (KNHANES) 2009-2010. *Diabet. Med. J. Br. Diabet. Assoc.* 2013;30(4):e143–148.

84. Ikeda M, Moriguchi J, Sakuragi S, et al. Association of past diseases with levels of cadmium and tubular dysfunction markers in urine of adult women in non-polluted areas in Japan. *Int. Arch. Occup. Environ. Health*. 2013;86(3):343–355.

85. Haswell-Elkins M, Satarug S, O'Rourke P, et al. Striking association between urinary cadmium level and albuminuria among Torres Strait Islander people with diabetes. *Environ. Res.* 2008;106(3):379–383.

86. Chen L, Lei L, Jin T, et al. Plasma metallothionein antibody, urinary cadmium, and renal dysfunction in a Chinese type 2 diabetic population. *Diabetes Care*. 2006;29(12):2682–2687.

87. Harrison CL, Lombard CB, Teede HJ. Understanding health behaviours in a cohort of pregnant women at risk of gestational diabetes mellitus: an observational study. *Bjog Int. J. Obstet. Gynaecol.* 2012;119(6):731–738.

88. Barlow WE, Ichikawa L, Rosner D, et al. Analysis of case-cohort designs. *J. Clin. Epidemiol.* 1999;52(12):1165–1172.

89. Prentice R. A Case-Cohort Design for Epidemiologic Cohort Studies and Disease Prevention Trials. *Biometrika*. 1986;73(1):1.

90. Morgenstern H, Thomas D. Principles of study design in environmental epidemiology. *Environ. Health Perspect.* 1993;101 Suppl 4:23–38.

91. Wacholder S. Practical considerations in choosing between the case-cohort and nested case-control designs. *Epidemiol. Camb. Mass*. 1991;2(2):155–158.

92. WHO. Biological Monitoring of Chemical Exposure in the Workplace. Geneva: World Health Organization; 1996.

93. Boeniger M. Interpretation of urine results used to assess chemical exposure with emphasis on creatinine adjustments: a review. *Am. Ind. Hyg. Assoc. J.* 1993;54(10):615–615–27.

94. Barr DB, Wilder LC, Caudill SP, et al. Urinary creatinine concentrations in the U.S. population: implications for urinary biologic monitoring measurements. *Environ. Health Perspect.* 2005;113(2):192–200.

95. Heitland P. Fast, simple and reliable routine determination of 23 elements in urine by ICP-MS. *J. Anal. At. Spectrom.* 2004;19(12):1552–1558.

96. Expert Committee on the Diagnosis and, Classification of Diabetes Mellitus. Report of the Expert Committee on the Diagnosis and Classification of Diabetes Mellitus. *Dia Care*. 2003;26(suppl 1):s5–s20.

97. Kass PH, Gold EB. Modern Epidemiologic Study Designs. In: Ahrens W, Pigeot I, eds. *Handbook of Epidemiology*. Berlin, Heidelberg: Springer Berlin Heidelberg; 2005:321–344.

98. Kupper LL, McMichael AJ, Spirtas R. A Hybrid Epidemiologic Study Design Useful in Estimating Relative Risk. *J. Am. Stat. Assoc.* 1975;70(351):524–528.

99. Sato T. Risk ratio estimation in case-cohort studies. *Environ. Health Perspect.* 1994;102 Suppl 8:53–56.

100. Chevrier C, Limon G, Monfort C, et al. Urinary biomarkers of prenatal atrazine exposure and adverse birth outcomes in the PELAGIE birth cohort. *Environ. Health Perspect.* 2011;119(7):1034–1041.

101. Overvad K, Wang DY, Olsen J, et al. Copper in human mammary carcinogenesis: a case-cohort study. *Am. J. Epidemiol.* 1993;137(4):409–414.

102. Ettinger AS, Zota AR, Amarasiriwardena CJ, et al. Maternal arsenic exposure and impaired glucose tolerance during pregnancy. *Environ. Health Perspect.* 2009;117(7):1059–1064.

103. King County Environmental Health Services. Findings from Soil Sampling of Vashon-Maury Island Soil Study. *King Cty. Wash.* 2010;(http://www.kingcounty.gov/healthservices/health/ehs/toxic/soilsamples.aspx). (Accessed December 8, 2011)

104. Orloff K, Mistry K, Metcalf S. Biomonitoring for environmental exposures to arsenic. *J. Toxicol. Environ. Health B Crit. Rev.* 2009;12(7):509–524.

105. ATSDR. Toxicological Profile for Arsenic. U.S. Dept of Health and Human Services, Agency for Toxic Substances and Disease Registry; 2007. (http://www.atsdr.cdc.gov/substances/toxsubstance.asp?toxid=3). (Accessed January 16, 2012)

106. Durrleman S, Simon R. Flexible regression models with cubic splines. *Stat. Med.* 1989;8(5):551–561.

107. StataCorp. Stata Statistical Software: Release 12. College Station, TX: Statacorp LP; 2013.

108. Xiang AH, Peters RK, Trigo E, et al. Multiple metabolic defects during late pregnancy in women at high risk for type 2 diabetes. *Diabetes*. 1999;48(4):848–854.

109. Ben-Haroush A, Yogev Y, Hod M. Epidemiology of gestational diabetes mellitus and its association with Type 2 diabetes. *Diabet. Med. J. Br. Diabet. Assoc.* 2004;21(2):103–113.

110. Kahn SE. The relative contributions of insulin resistance and beta-cell dysfunction to the pathophysiology of Type 2 diabetes. *Diabetologia*. 2003;46(1):3–19.

111. Hectors TLM, Vanparys C, van der Ven K, et al. Environmental pollutants and type 2 diabetes: a review of mechanisms that can disrupt beta cell function. *Diabetologia*. 2011;54(6):1273–1290.

112. Lei L, Jin T, Zhou Y. [The effects of cadmium on the levels of insulin in smelters] (Abstract Only). *Chin. J. Ind. Hyg. Occup. Dis.* 2006;24(1):3–6.

113. Tseng C-H. The potential biological mechanisms of arsenic-induced diabetes mellitus. *Toxicol. Appl. Pharmacol.* 2004;197(2):67–83.

114. Institute of Medicine (U.S.) Panel on Micronutrients. Dietary reference intakes for vitamin A, vitamin K, arsenic, boron, chromium, copper, iodine, iron, manganese, molybdenum, nickel, silicon, vanadium, and zinc. Washington, D.C.: National Academy Press; 2001.

115. Shvetsov YB, Hernandez BY, Wong SH, et al. Intraindividual Variability in Serum Micronutrients. *Epidemiol. Camb. Mass*. 2009;20(1):36–43.

116. Siega-Riz A-M, Siega-Riz A-M, Laraia B. The implications of maternal overweight and obesity on the course of pregnancy and birth outcomes. *Matern. Child Health J.* 2006;10(5 Suppl):S153–156.

117. Reece EA, Leguizamón G, Wiznitzer A. Gestational diabetes: the need for a common ground. *Lancet*. 2009;373(9677):1789–1797.

118. Chen Y, Quick WW, Yang W, et al. Cost of gestational diabetes mellitus in the United States in 2007. *Popul. Heal. Manag.* 2009;12(3):165–174.

119. Kuromoto K, Watanabe M, Adachi K, et al. Increases in urinary creatinine and blood pressure during early pregnancy in pre-eclampsia. *Ann. Clin. Biochem.* 2010;47(Pt 4):336–342.

120. McElroy JA, Shafer MM, Hampton JM, et al. Predictors of urinary cadmium levels in adult females. *Sci. Total Environ.* 2007;382(2-3):214–223.

121. McElroy JA, Shafer MM, Trentham-Dietz A, et al. Urinary cadmium levels and tobacco smoke exposure in women age 20-69 years in the United States. *J. Toxicol. Environ. Health A*. 2007;70(20):1779–1782.

122. Gabbe S. Obstetrics : normal and problem pregnancies. 5th ed. Philadelphia PA: Churchill Livingstone/Elsevier; 2007.

123. Barker DJP, Eriksson JG, Forsén T, et al. Fetal origins of adult disease: strength of effects and biological basis. *Int. J. Epidemiol.* 2002;31(6):1235–1239.

124. Hodgson D. Perinatal programming : early life determinants of adult health & disease. London ;;New York: Taylor & Francis; 2006.

125. Ju H, Chadha Y, Donovan T, et al. Fetal macrosomia and pregnancy outcomes. *Aust. N. Z. J. Obstet. Gynaecol.* 2009;49(5):504–509.

126. Curhan GC, Willett WC, Rimm EB, et al. Birth weight and adult hypertension, diabetes mellitus, and obesity in US men. *Circulation*. 1996;94(12):3246–3250.

127. Tian J-Y, Cheng Q, Song X-M, et al. Birth weight and risk of type 2 diabetes, abdominal obesity and hypertension among Chinese adults. *Eur. J. Endocrinol. Eur. Fed. Endocr. Soc.* 2006;155(4):601–607.

128. Gu S, An X, Fang L, et al. Risk factors and long-term health consequences of macrosomia: a prospective study in Jiangsu Province, China. *J. Biomed. Res.* 2012;26(4):235–240.

129. Centers for Disease and Control Prevention (USA). VitalStats - Births. 2010;(/nchs/vitalstats.htm.). (Accessed June 16, 2013)

130. Pilgaard K, Færch K, Carstensen B, et al. Low birthweight and premature birth are both associated with type 2 diabetes in a random sample of middle-aged Danes. *Diabetologia*. 2010;53(12):2526–2530.

131. Boulet SL, Alexander GR, Salihu HM, et al. Macrosomic births in the United States: determinants, outcomes, and proposed grades of risk. *Am. J. Obstet. Gynecol.* 2003;188(5):1372–1378.

132. Harder T, Rodekamp E, Schellong K, et al. Birth Weight and Subsequent Risk of Type 2 Diabetes: A Meta-Analysis. *Am. J. Epidemiol.* 2007;165(8):849–857.

133. Hermann GM, Dallas LM, Haskell SE, et al. Neonatal Macrosomia Is an Independent Risk Factor for Adult Metabolic Syndrome. *Neonatology*. 2010;98(3):238–244.

134. Luo Z-C, Xiao L, Nuyt A-M. Mechanisms of developmental programming of the metabolic syndrome and related disorders. *World J. Diabetes*. 2010;1(3):89–98.

135. Mcmillen IC, Robinson JS. Developmental Origins of the Metabolic Syndrome: Prediction, Plasticity, and Programming. *Physiol. Rev.* 2005;85(2):571–633.

136. Kramer MS, Platt R, Yang H, et al. Are all growth-restricted newborns created equal(ly)? *Pediatrics*. 1999;103(3):599–602.

137. Barbier A, Boivin A, Yoon W, et al. New Reference Curves for Head Circumference at Birth, by Gestational Age. *Pediatrics*. 2013;131(4):e1158–e1167.

138. Cooke RW, Lucas A, Yudkin PL, et al. Head circumference as an index of brain weight in the fetus and newborn. *Early Hum. Dev.* 1977;1(2):145–149.

139. Roza SJ, Govaert PP, Vrooman HA, et al. Foetal growth determines cerebral ventricular volume in infants The Generation R Study. *Neuroimage*. 2008;39(4):1491–1498.

140. Barker DJ, Osmond C, Simmonds SJ, et al. The relation of small head circumference and thinness at birth to death from cardiovascular disease in adult life. *BMJ*. 1993;306(6875):422–426.

141. Risnes KR, Nilsen TIL, Romundstad PR, et al. Head size at birth and long-term mortality from coronary heart disease. *Int. J. Epidemiol.* 2009;38(4):955–962.

142. Tideman E, Marsál K, Ley D. Cognitive function in young adults following intrauterine growth restriction with abnormal fetal aortic blood flow. *Ultrasound Obstet. Gynecol. Off. J. Int. Soc. Ultrasound Obstet. Gynecol.* 2007;29(6):614–618.

143. Geva R, Eshel R, Leitner Y, et al. Neuropsychological Outcome of Children With Intrauterine Growth Restriction: A 9-Year Prospective Study. *Pediatrics*. 2006;118(1):91–100.

144. Veena SR, Krishnaveni GV, Wills AK, et al. Association of birthweight and head circumference at birth to cognitive performance in 9-10 year old children in South India: prospective birth cohort study. *Pediatr. Res.* 2010;67(4):424–429.

145. Gale CR, O'Callaghan FJ, Bredow M, et al. The influence of head growth in fetal life, infancy, and childhood on intelligence at the ages of 4 and 8 years. *Pediatrics*. 2006;118(4):1486–1492.

146. Heinonen K, Räikkönen K, Pesonen A-K, et al. Prenatal and postnatal growth and cognitive abilities at 56 months of age: a longitudinal study of infants born at term. *Pediatrics*. 2008;121(5):e1325–1333.

147. Broekman BFP, Chan Y-H, Chong Y-S, et al. The influence of birth size on intelligence in healthy children. *Pediatrics*. 2009;123(6):e1011–1016.

148. Hendrix N, Berghella V. Non-placental causes of intrauterine growth restriction. *Semin. Perinatol.* 2008;32(3):161–165.

149. Bernstein PS, Divon MY. Etiologies of fetal growth restriction. *Clin. Obstet. Gynecol.* 1997;40(4):723–729.

150. Cogswell ME, Yip R. The influence of fetal and maternal factors on the distribution of birthweight. *Semin. Perinatol.* 1995;19(3):222–240.

151. McCowan L, Horgan RP. Risk factors for small for gestational age infants. *Best Pract. Res. Clin. Obstet. Gynaecol.* 2009;23(6):779–793.

152. Ergaz Z, Avgil M, Ornoy A. Intrauterine growth restriction-etiology and consequences: what do we know about the human situation and experimental animal models? *Reprod. Toxicol. Elmsford N*. 2005;20(3):301–322.

153. Odell CD, Kotelchuck M, Chetty VK, et al. Maternal hypertension as a risk factor for low birth weight infants: comparison of Haitian and African-American women. *Matern. Child Health J.* 2006;10(1):39–46.

154. Fang J, Madhavan S, Alderman MH. The influence of maternal hypertension on low birth weight: differences among ethnic populations. *Ethn. Dis.* 1999;9(3):369–376.

155. Wu G, Imhoff-Kunsch B, Girard AW. Biological Mechanisms for Nutritional Regulation of Maternal Health and Fetal Development. *Paediatr. Perinat. Epidemiol.* 2012;26:4–26.

156. Abalos E, Merialdi M, Wojdyla D, et al. Effects of calcium supplementation on fetal growth in mothers with deficient calcium intake: a randomised controlled trial. *Paediatr. Perinat. Epidemiol.* 2010;24(1):53–62.

157. Imdad A, Bhutta ZA. Effects of Calcium Supplementation During Pregnancy on Maternal, Fetal and Birth Outcomes. *Paediatr. Perinat. Epidemiol.* 2012;26:138–152.

158. Peña-Rosas JP, De-Regil LM, Dowswell T, et al. Daily oral iron supplementation during pregnancy. *Cochrane Database Syst. Rev.* 2012;12:CD004736.

159. Haider BA, Bhutta ZA. Multiple-micronutrient supplementation for women during pregnancy. *Cochrane Database Syst. Rev.* 2012;11:CD004905.

160. Miller HC, Hassanein K, Hensleigh PA. Fetal growth retardation in relation to maternal smoking and weight gain in pregnancy. *Am. J. Obstet. Gynecol.* 1976;125(1):55–60.

161. Ehrenberg HM, Mercer BM, Catalano PM. The influence of obesity and diabetes on the prevalence of macrosomia. *Am. J. Obstet. Gynecol.* 2004;191(3):964–968.

162. Levy A, Wiznitzer A, Holcberg G, et al. Family history of diabetes mellitus as an independent risk factor for macrosomia and cesarean delivery. *J. Matern.-Fetal Neonatal Med. Off. J. Eur. Assoc. Perinat. Med. Fed. Asia Ocean. Perinat. Soc. Int. Soc. Perinat. Obstet.* 2010;23(2):148–152.

163. Dashe JS, McIntire DD, Lucas MJ, et al. Effects of symmetric and asymmetric fetal growth on pregnancy outcomes. *Obstet. Gynecol.* 2000;96(3):321–327.

164. Landmann E, Reiss I, Misselwitz B, et al. Ponderal index for discrimination between symmetric and asymmetric growth restriction: percentiles for neonates from 30 weeks to 43 weeks of gestation. *J. Matern.-Fetal Neonatal Med. Off. J. Eur. Assoc. Perinat. Med. Fed. Asia Ocean. Perinat. Soc. Int. Soc. Perinat. Obstet.* 2006;19(3):157–160.

165. Villar J, Belizan JM. The timing factor in the pathophysiology of the intrauterine growth retardation syndrome. *Obstet. Gynecol. Surv.* 1982;37(8):499–506.

166. Patterson RE, Kristal AR, Tinker LF, et al. Measurement characteristics of the Women's Health Initiative food frequency questionnaire. *Ann. Epidemiol.* 1999;9(3):178–187.

167. Report of the National High Blood Pressure Education Program Working Group on High Blood Pressure in Pregnancy. *Am. J. Obstet. Gynecol.* 2000;183(1):S1–S22.

168. Weight Gain During Pregnancy: Reexamining the Guidelines - Institute of Medicine. 2009;(http://www.iom.edu/Reports/2009/weight-Gain-during-pregnancy-Reexamining-the-Guidelines.aspx). (Accessed July 2, 2013)

169. Schakel SF. Maintaining a nutrient database in a changing marketplace: Keeping pace with changing food products-A research perspective. In: *Journal of food composition and analysis*. 2004:315–322.

170. Salgado-Ugarte IH, Shimizu M. Robust scatterplot smoothing: enhancements to Stata's ksm. *Stata Tech. Bull.* 1996;5(25).

171. Ricart W, López J, Mozas J, et al. Maternal glucose tolerance status influences the risk of macrosomia in male but not in female fetuses. *J. Epidemiol. Community Health*. 2009;63(1):64–68.

172. Lampl M, Gotsch F, Kusanovic JP, et al. Sex differences in fetal growth responses to maternal height and weight. *Am. J. Hum. Biol. Off. J. Hum. Biol. Counc.* 2010;22(4):431–443.

173. Wilcox MA, Chang AMZ, Johnson IR. The effects of parity on birthweight using successive pregnancies. *Acta Obstet Gynecol Scand*. 1996;75(5):459–463.

174. Juntunen KST, Läärä EMJ, Kauppila AJI. Grand grand multiparity and birth weight. *Obstet. Gynecol.* 1997;90(4, Part 1):495–499.

175. Himmelmann K, Himmelmann A, Niklasson A, et al. Hypertension in pregnancy and size at birth. *Blood Press.* 1996;5(5):278–284.

176. Ananth C, Savitz D, Luther E, et al. Preeclampsia and Preterm Birth Subtypes in Nova Scotia, 1986 to 1992. *Am. J. Perinatol.* 2008;14(01):17–23.

177. Churchill D, Perry IJ, Beevers DG. Ambulatory blood pressure in pregnancy and fetal growth. *Lancet*. 1997;349(9044):7–10.

178. Bamfo JEAK, Kametas NA, Chambers JB, et al. Maternal cardiac function in fetal growth-restricted and non-growth-restricted small-for-gestational age pregnancies. *Ultrasound Obstet. Gynecol. Off. J. Int. Soc. Ultrasound Obstet. Gynecol.* 2007;29(1):51–57.

179. Bonamy A-KE, Parikh NI, Cnattingius S, et al. Birth characteristics and subsequent risks of maternal cardiovascular disease: effects of gestational age and fetal growth. *Circulation*. 2011;124(25):2839–2846.

180. Gilbert JS, Ford SP, Lang AL, et al. Nutrient restriction impairs nephrogenesis in a gender-specific manner in the ovine fetus. *Pediatr. Res.* 2007;61(1):42–47.

181. Gilbert JS, Nijland MJ. Sex differences in the developmental origins of hypertension and cardiorenal disease. *Am. J. Physiol. - Regul. Integr. Comp. Physiol.* 2008;295(6):R1941–R1952.

182. Ballard JL, Rosenn B, Khoury JC, et al. Diabetic fetal macrosomia: significance of disproportionate growth. *J. Pediatr.* 1993;122(1):115–119.

183. Hindmarsh PC, Geary MPP, Rodeck CH, et al. Intrauterine growth and its relationship to size and shape at birth. *Pediatr. Res.* 2002;52(2):263–268.

184. Siega-Riz AM, Viswanathan M, Moos M-K, et al. A systematic review of outcomes of maternal weight gain according to the Institute of Medicine recommendations: birthweight, fetal growth, and postpartum weight retention. *Am. J. Obstet. Gynecol.* 2009;201(4):339.e1–14.

185. Clifton VL. Review: Sex and the human placenta: mediating differential strategies of fetal growth and survival. *Placenta*. 2010;31 Suppl:S33–39.

186. Uauy R, Casanello P, Krause B, et al. Conceptual basis for prescriptive growth standards from conception to early childhood: present and future. *Bjog Int. J. Obstet. Gynaecol.* 2013;

187. Eunice Kennedy Shriver National Institute of Child Health and Human Development. NICHD Fetal Growth Study. *Epidemiol. Branch Res. - Perinat. Epidemiol.* 2013;(http://www.nichd.nih.gov/about/org/despr/eb/research/pages/fetal-growth-study.aspx). (Accessed July 4, 2013)

188. Obesity: preventing and managing the global epidemic. Report of a WHO consultation. *World Health Organ. Tech. Rep. Ser.* 2000;894:i–xii, 1–253.

189. Thompson J, Bannigan J. Cadmium: toxic effects on the reproductive system and the embryo. *Reprod. Toxicol. Elmsford N*. 2008;25(3):304–315.

190. Satarug S, Moore MR. Adverse Health Effects of Chronic Exposure to Low-Level Cadmium in Foodstuffs and Cigarette Smoke. *Environ. Health Perspect.* 2004;112(10):1099–1103.

191. Kippler M, Ekström E-C, Lönnerdal B, et al. Influence of iron and zinc status on cadmium accumulation in Bangladeshi women. *Toxicol. Appl. Pharmacol.* 2007;222(2):221–226.

192. Akesson A, Berglund M, Schütz A, et al. Cadmium exposure in pregnancy and lactation in relation to iron status. *Am. J. Public Health*. 2002;92(2):284–287.

193. Chertok RJ, Kullgren B, Burbank D. The effects of CdCl2 on the maternal-to-fetal clearance of 67Cu and placental blood flow. *Proc. Soc. Exp. Biol. Med. Soc. Exp. Biol. Med. New York N*. 1984;176(2):138–142.

194. Levin, AA, Body, R, di Sant'Agnese, PA. Heavy metal alterations of placental function: A mechanism for the induction of fetal toxicity of cadmium. In: Clarkson, TW, Nordberg,

GR, Safer, PR, eds. *Reproductive and developmental toxicity of metals*. New York: Plenum Press; 1983:633–654.

195. Stasenko S, Bradford EM, Piasek M, et al. Metals in human placenta: focus on the effects of cadmium on steroid hormones and leptin. *J. Appl. Toxicol. Jat*. 2010;30(3):242–253.

196. Kippler M, Wagatsuma Y, Rahman A, et al. Environmental exposure to arsenic and cadmium during pregnancy and fetal size: a longitudinal study in rural Bangladesh. *Reprod. Toxicol. Elmsford N*. 2012;34(4):504–511.

197. Xu L, Yokoyama K, Tian Y, et al. Decrease in birth weight and gestational age by arsenic among the newborn in Shanghai, China. *Nihon Kōshū Eisei Zasshi Jpn. J. Public Heal.* 2011;58(2):89–95.

198. Hopenhayn C, Ferreccio C, Browning SR, et al. Arsenic exposure from drinking water and birth weight. *Epidemiol. Camb. Mass*. 2003;14(5):593–602.

199. Rahman A, Vahter M, Smith AH, et al. Arsenic exposure during pregnancy and size at birth: a prospective cohort study in Bangladesh. *Am. J. Epidemiol.* 2009;169(3):304–312.

200. Croghan C, Egeghy P. Methods of Dealing With Values Below the Limit of Detection Using SAS. 2003;(http://cfpub.epa.gov/ordpubs/nerlpubs/recordisplay.cfm?deid=62970)

201. Glass DC, Gray CN. Estimating mean exposures from censored data: exposure to benzene in the Australian petroleum industry. *Ann. Occup. Hyg.* 2001;45(4):275–282.

202. Bisai S. Maternal Height as an Independent Risk Factor for Neonatal Size Among Adolescent Bengalees in Kolkata, India. *Ethiop. J. Heal. Sci.* 2010;20(3):153–158.

203. Witter FR, Luke B. The effect of maternal height on birth weight and birth length. *Early Hum. Dev.* 1991;25(3):181–186.

204. UCLA: Statistical Consulting Group. Chapter 3 - Regression with Categorical Predictors. In: *Regression with Stata*. (Accessed May 23, 2013)(http://www.ats.ucla.edu/stat/stata/webbooks/reg/chapter3/statareg3.htm). (Accessed May 23, 2013)

205. Wier PJ, Miller RK, Maulik D, et al. Toxicity of cadmium in the perfused human placenta. *Toxicol. Appl. Pharmacol.* 1990;105(1):156–171.

206. Ronco AM, Urrutia M, Montenegro M, et al. Cadmium exposure during pregnancy reduces birth weight and increases maternal and foetal glucocorticoids. *Toxicol. Lett.* 2009;188(3):186–191.

207. Jaquet D. Ontogeny of leptin in human fetuses and newborns: effect of intrauterine growth retardation on serum leptin concentrations. *J. Clin. Endocrinol. Metab.* 1998;83(4):1243–1243–6.

208. Hossain MB, Vahter M, Concha G, et al. Low-level environmental cadmium exposure is associated with DNA hypomethylation in Argentinean women. *Environ. Health Perspect.* 2012;120(6):879–884.

209. Castillo P, Ibanez F, Guajardo A, et al. Impact of Cadmium Exposure during Pregnancy on Hepatic Glucocorticoid Receptor Methylation and Expression in Rat Fetus. *Plos One* [electronic article]. 2012;7(9). (http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3434215/). (Accessed May 28, 2013)

210. Clifton VL, Murphy VE. Maternal asthma as a model for examining fetal sex-specific effects on maternal physiology and placental mechanisms that regulate human fetal growth. *Placenta*. 2004;25 Suppl A:S45–52.

211. Yang K, Julan L, Rubio F, et al. Cadmium reduces 11β-hydroxysteroid dehydrogenase type 2 activity and expression in human placental trophoblast cells. *Am. J. Physiol. - Endocrinol. Metab.* 2006;290(1):E135–E142.

212. Brzóska MM, Moniuszko-Jakoniuk J. Bone metabolism of male rats chronically exposed to cadmium. *Toxicol. Appl. Pharmacol.* 2005;207(3):195–211.

213. Brzóska MM, Moniuszko-Jakoniuk J. Disorders in bone metabolism of female rats chronically exposed to cadmium. *Toxicol. Appl. Pharmacol.* 2005;202(1):68–83.

214. Bhattacharyya MH. Cadmium osteotoxicity in experimental animals: mechanisms and relationship to human exposures. *Toxicol. Appl. Pharmacol.* 2009;238(3):258–265.

215. Thomas LD MK. Dietary cadmium exposure and fracture incidence among men: a population-based prospective cohort study. *J. Bone Miner. Res. Off. J. Am. Soc. Bone Miner. Res.* 2011;26(7):1601–8.

216. Afkhami-Ardekani M, Rashidi M. Iron status in women with and without gestational diabetes mellitus. *J. Diabetes Complications*. 2009;23(3):194–198.

217. Chen X, Scholl TO, Stein TP. Association of elevated serum ferritin levels and the risk of gestational diabetes mellitus in pregnant women: The Camden study. *Diabetes Care*. 2006;29(5):1077–1082.

218. Lao TT, Chan PL, Tam KF. Gestational diabetes mellitus in the last trimester - a feature of maternal iron excess? *Diabet. Med. J. Br. Diabet. Assoc.* 2001;18(3):218–223.

219. Lao TT, Chan LY, Tam KF, et al. Maternal hemoglobin and risk of gestational diabetes mellitus in Chinese women. *Obstet. Gynecol.* 2002;99(5 Pt 1):807–812.

220. Sharifi F, Ziaee A, Feizi A, et al. Serum ferritin concentration in gestational diabetes mellitus and risk of subsequent development of early postpartum diabetes mellitus. *Diabetes Metab. Syndr. Obes. Targets Ther.* 2010;3:413–419.

221. Soubasi V, Petridou S, Sarafidis K, et al. Association of increased maternal ferritin levels with gestational diabetes and intra-uterine growth retardation. *Diabetes Metab.* 2010;36(1):58–63.

222. Delves H. Assessment of trace element status. *Clin. Endocrinol. Metab.* 1985;14(3):725–60.

223. ATSDR. Toxicological Profile for Zinc. U.S. Dept of Health and Human Services, Agency for Toxic Substances and Disease Registry; 2005 (Accessed January 31, 2012).(http://www.atsdr.cdc.gov/substances/toxsubstance.asp?toxid=54). (Accessed January 31, 2012)

224. Lowe NM, Fekete K, Decsi T. Methods of assessment of zinc status in humans: a systematic review. *Am. J. Clin. Nutr.* 2009;89(6):2040S–2051S.