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by

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Abstract

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Although the obesity epidemic in the United States appears to be leveling off, over half of all reproductive aged women in the U.S. are overweight [defined as body mass index (BMI) ≥25 kg/m^2] or obesity [defined as body mass index (BMI) ≥30 kg/m^2]. Overweight and obese women are more likely to suffer from a variety of reproductive complications, including adverse perinatal outcomes; overweight and obesity are also well-established risk factors for gestational diabetes and type 2 diabetes. Gestational diabetes mellitus (GDM), defined as carbohydrate intolerance leading to hyperglycemia with first onset or recognition during pregnancy, complicates 7% to 10% of pregnancies in the U.S. Type 2 diabetes mellitus (T2DM) is emerging as a leading cause of death and disability in the U.S. and currently straining the health care system.

This goal of this dissertation is to investigate the effects of periconceptual and prenatal maternal glucose levels on immediate and longer-term offspring outcomes. Three studies were undertaken, comprising three chapters, to complete this dissertation; all utilize a cohort study design, with data obtained from several sources. The study described in the first chapter examines the association between periconceptual maternal glycaemia and newborn sex ratio in a large data set from Kaiser Permanente Northern California. Women were categorized into the following groups: overt pregravid diabetes, gestational diabetes, mild pregnancy hyperglycemia and normoglycemic pregnancies. It has long been hypothesized that natural selection would favor a reproductive strategy biased towards females under adverse circumstances and males under favorable conditions in order to maximize the number of surviving grandchildren. Thus, I hypothesized that women with overt pregravid diabetes would exhibit the lowest newborn sex ratio (ratio of males to females at birth, i.e. more girls) due to the unfavorable state of chronic disease and women with gestational diabetes would exhibit the highest sex ratio (i.e. more boys) due to the presence of excessive fuel substrates early in pregnancy.

The study described in the second chapter explores programming for childhood obesity by maternal pregnancy glucose levels in women without recognized diabetes or gestational diabetes; the study comprising the third chapter considers the association between pregnancy glucose levels in these same women and cardiometabolic risk factors in their children at 7 years of age. Data for chapters 2 and 3 come from the CHAMACOS (Center for the Health Assessment of Mothers and Children of Salinas) longitudinal birth cohort. Several studies have demonstrated an increased risk for childhood obesity and adverse cardiometabolic profiles among children exposed to maternal diabetes or gestational diabetes *in utero*. Yet no study has considered the risk of childhood obesity across several ages or examined the childhood growth trajectory associated with increasing maternal glucose levels among children who were not exposed to maternal diabetes or gestational diabetes *in utero*. Likewise, little is known regarding the association between increasing maternal glucose levels and childhood cardiometabolic risk factors among children who were not exposed to maternal diabetes or gestational diabetes *in utero*. There appears to be a continuous association between increasing maternal glucose levels and the risk of several perinatal complications in infants born to women whose pregnancies were not complicated by diabetes, thus it is plausible that increasing pregnancy glucose levels below those diagnostic of disease could also be associated with longer-term adverse outcomes in the offspring.

In the first study, examination of the crude sex ratio across categories of maternal glycemia suggested a trend consistent with my hypothesis, but the odds ratio estimates did not attain statistical significance. The second study found a significant association between maternal pregnancy glucose levels in women without recognized diabetes or gestational diabetes and increased BMI z-score at 7 years of age in their children. The third study discovered that maternal pregnancy glucose levels in the same population were significantly associated with increased cardiometabolic risk in the children, specifically increased blood pressure and waist circumference. The results of studies two and three extend upon research in women with overt, recognized diabetes or gestational diabetes during pregnancy and lend additional support to the developmental origins of disease hypothesis. The findings of this dissertation indeed suggest that periconceptual and prenatal maternal glucose levels effect immediate and longer-term offspring outcomes. Of particular concern are the findings of studies two and three, which suggest programming for adverse childhood outcomes in women without recognized, overt disease. Given the epidemic of obesity in the U.S. and the relationship between obesity and increased levels of glycemia, these findings suggest the need for lifestyle interventions targeting maternal pregravid obesity and mildly increased levels of pregnancy glycemia in order to improve the health of the next generation.

Dedication

In memory of Maggie Farm
I love you more than words can tell

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INTRODUCTION

I. **The obesity epidemic and periconceptional and prenatal maternal glucose levels**

Although the obesity epidemic in the United States appears to be leveling off, in 2007-2008, the prevalence of overweight [defined as body mass index (BMI) ≥25 kg/m^2] and obesity [defined as body mass index (BMI) ≥30 kg/m^2] in adult women, 20-39 years of age, was 59.5% (1). The prevalence of overweight and obesity varies by racial-ethnic group and is highest among Non-Hispanic Black and Mexican American (78.0% and 70.3%, respectively). Overweight and obese women are more likely to suffer from a variety of reproductive complications, including adverse perinatal outcomes (2). Overweight and obesity are also well-established risk factors for gestational diabetes (3) and type 2 diabetes (4).

Gestational diabetes mellitus (GDM), defined as carbohydrate intolerance leading to hyperglycemia with first onset or recognition during pregnancy, complicates 7% to 10% of pregnancies in the U.S. (5). The prevalence of GDM in the U.S. increased 35-90% in the last decade (6,7). GDM is diagnosed during the third trimester of pregnancy and associated with an increased risk of several perinatal complications (8,9); women with GDM are also at high risk of developing type 2 diabetes later in life (10). Risk factors for GDM include advanced maternal age, non-white race-ethnicity, and a family history of diabetes (3,9). Pregravid cardiometabolic risk factors, including elevated random glucose levels, impaired fasting glucose, elevated fasting insulin, overweight and obesity, are also associated with the subsequent development of GDM (11,12). The second half of pregnancy is characterized by progressive insulin resistance, hyperinsulinemia and mild postprandial hyperglycemia. Most women are able to increase their insulin secretion to compensate for the insulin-resistant state that accompanies normal pregnancy and maintain regular glucose tolerance (13). Women with pregravid metabolic abnormalities require the hypersecretion of insulin to compensate for pregnancy-induced insulin resistance and are therefore more likely to develop GDM.

Type 2 diabetes mellitus (T2DM) is emerging as a leading cause of death and disability in the U.S. and currently straining the health care system (14-16). As for GDM, overweight and obesity are well-established risk factors for T2DM (4), which accounts for the majority of cases of diabetes in the U.S. Approximately 2% of women 20-39 years of age in the U.S. have been diagnosed with diabetes, with a higher prevalence reported in minority groups: 2.6% of Mexican American women of reproductive age and 4.7% of Non-Hispanic black women. Population-based data continues to demonstrate excess rates of congenital malformations as well as perinatal morbidity and mortality in women with diabetes. Therefore, it is currently recommended that women with diabetes be educated on the need for good pregravid glucose control and monitored by a multidisciplinary medical team throughout pregnancy and the postpartum period (17).

This goal of this dissertation is to investigate the effects of periconceptual and prenatal maternal glucose levels on immediate and longer-term offspring outcomes. The three papers (chapters) that make up this dissertation all utilize cohort study designs and the data come from several sources. The first chapter examines the association between periconceptual maternal glycaemia and offspring sex ratio in a large data set from Kaiser Permanente Northern California. The second chapter explores programming for childhood obesity by maternal pregnancy glucose level in women without recognized diabetes or GDM, the third considers pregnancy glucose levels in these same women and cardiometabolic risk factors in their children.

Data for chapters 2 and 3 come from the CHAMACOS (Center for the Health Assessment of Mothers and Children of Salinas) longitudinal birth cohort.

II. **An Immediate Effect of Periconceptual Glucose Levels: the Secondary Sex Ratio**

The human secondary sex ratio, or the ratio of male to female infants at birth, demonstrates small increases and decreases over time. Although many of these changes are statistically significant, they remain largely unexplained (18). A secondary sex ratio of 106 males per 100 females is considered "normal" for newborns of European ancestry (19,20), but variation in the magnitude of male excess has been observed between ethnic groups, with newborns of African ancestry exhibiting a lower secondary sex ratio of approximately 103 males per 100 females (20). Small but significant decreases in the human secondary sex ratio have been reported during periods of extreme stress experienced on a population level, such as times of war and natural and human-made disasters. Zorn et al. (21) hypothesized that acute psychological stress resulting from a short war in Slovenia was responsible for a decrease in the secondary sex ratio 6 to 9 months later. Fukuda et al. (22) similarly reported that acute stress caused by the Kobe earthquake in Japan in January 1995 might have been the cause of a lower secondary sex ratio observed 9 months later. Work by Catalano et al. (23,24) also support the hypothesis that population-level stress, whether induced by a declining economy (23) or terrorist attacks (24), reduce the human secondary sex ratio.

The reproductive efficiency of the population as a whole is believed to be quite low; in healthy adults with previous reproductive success, less than 25% of human fertilizations actually make it to term delivery (19). The majority of losses that occur between fertilization and term delivery take place early in pregnancy: two-thirds of embryos fail before the clinical recognition of pregnancy (25).

The work of Catalano et al. (24,26) suggests that differential selection *in utero* drives observed differences in the human secondary sex ratio. The authors used time serious analyses to examine the sex ratios of infants at various gestational ages in the state of California after the September 11th terrorist attacks. Based on the premise that population-level disasters have coincided with a reduction in the proportion of male births in previous studies, the authors sought to tease part whether the decrease in male births was due to excess male fetal deaths, a reduction in the conception of male embryos, or a combination of the two. The authors reported that the fetal death sex ratio exhibited its highest value (i.e. more boys) of the entire 72 month test period in October 2001 and remained significantly elevated in the following month. The results support the differential *in utero* selection hypothesis, at the expense of the decreased conception of male embryos hypothesis, for the association between ambient, population-level stressors and alterations to the human secondary sex ratio.

The differential selection *in utero* theory dates back to the 1970s, when Trivers and Willard (27) proposed that mechanisms contributing to the loss of human conceptions were likely to include the spontaneous abortion of those conceptions that were the least likely to produce grandchildren. Such a maternal screening mechanism assumes a ranking of gestations based on the expected yield of grandchildren, as well as a rank threshold below which a woman would spontaneously terminate pregnancy (28). The ranking system would reflect the probability that a conception, should it be born, survives to reproductive age, while also accounting for the

maternal cost of sustaining the child through that period. In accordance with this theory, male conceptions would holder a lower rank than females, since, if born, they are more likely to die before reaching reproductive age (29). Extending this logic, female twins would be the most desirable, followed by female singletons, male singletons and lastly, male twins in terms of the expected yield of grandchildren.

Catalano et al. (26) set out to test this differential maternal selection *in utero* theory, hypothesizing that an increase in the threshold for spontaneous abortion would cull male twins before male singletons, and result in a decrease in the odds of a male twin. A higher threshold was also hypothesized to result in the culling of female singletons before female twins, thereby increasing in the odds of female twins. The researchers demonstrated that deviations from the expected odds of male twinning predicted opposite movements in the odds of female twinning. In addition, they found that the higher threshold effected the odds of a male twin more than that of a female twin (1% versus 0.16%, respectively), which corresponds to the bottom rank assigned male twins in terms of the expected yield of grandchildren.

Periconceptional Maternal Glucose Levels and the Secondary Sex Ratio

Only a few studies have examined the sex ratio in women with diabetes (30) or GDM (31,32); a study by Rjasanowski et al. (30) examined the sex ratio among 181 families with 268 children for whom the father, the mother or both had insulin-dependent diabetes, inspiring several response letters to *the Lancet* (33-35). The authors found that the sex ratio was only disturbed in families with diabetic mothers: they had more female than male children. Responses letters presented several data sources (35-38); the smaller datasets, examining cohorts of 1,500 or less (35-37), suggested no sex ratio disturbances among women with diabetes, while data from a larger cohort of 5,875 mother-child pairs (38) confirmed this female excess. It should be noted that these (38) and other (34) data presented in response to the Rjasanowski et al. study were secondary analyses and the percentage of women with diabetes *prior* to the index pregnancy was not reported.

Only two studies examining the sex ratio in women with GDM were identified. In an Israeli cohort of 108,995 women, Sheiner et al. (31) reported that 51.2% of women without GDM were carrying a boy versus 52.8% of those with GDM; the increased odds of GDM among women carrying a male fetus attained statistical significance. A review article, Di Renzo et al. (32) presented an analysis of 5,994 deliveries in Italy: GDM occurred in 3.0% of women carrying males and 1.8% of patients carrying females, this difference also attained statistical significance. Neither of author controlled for confounding factors in their analyses and diagnoses of GDM were obtained from either a hospital databases (31) or unspecified (32). No study has considered the sex ratio across a range of maternal glycemic categories simultaneously. Larger studies utilizing objective laboratory tests and medical history data obtained prior to the index pregnancy to classify maternal periconceptual hyperglycemia would greatly contribute to our understanding of the association between maternal glucose levels and offspring sex ratio.

Aim of Chapter 1

The aim of the first chapter is examine deviations in the human secondary sex ratio associated with indicators of periconceptional glucose tolerance. Women were categorized into the following groups: overt pregravid diabetes, gestational diabetes, mild pregnancy hyperglycemia

and normoglycemic pregnancies. The study setting was Kaiser Permanente Northern California (KPNC), a large health maintenance organization. We utilized the KPNC Diabetes Registry (39) to identify women with recognized, overt diabetes prior to pregnancy. Women with GDM who were subsequently diagnosed with diabetes from 6 weeks to 12 months postpartum were considered to have unrecognized diabetes prior to pregnancy (40). These women were identified through the KPNC Diabetes Registry and by the results of their postpartum glucose tests. Women with GDM and mild pregnancy hyperglycemia were identified through the KPNC Gestational Diabetes and Pregnancy Glucose Tolerance Registry (6); both classifications were based on the results of objective laboratory tests. In this setting, routine prenatal care includes GDM screening in the form of a 50g, 1-hour glucose challenge test. This test is performed at any time of day, regardless of last intake. Women with abnormal values on the screening test go on to receive a diagnostic test, the 100g, 3-hour oral glucose tolerance test (OGTT); GDM diagnoses are made based on the results of the OGTT, which is performed after an overnight fast. Women with abnormal values on the screening test who did not meet the diagnostic criteria for GDM (41) on the OGTT comprised the mild pregnancy hyperglycemia group. Those without recognized diabetes or GDM during the index pregnancy comprised the normoglycemic group; these women would likely have also been normoglycemic throughout the periconceptual period.

III. Longer-term Effects of Maternal Glucose Levels during Pregnancy: the Developmental Origins of Childhood Obesity and Cardiometabolic Risk

Childhood Obesity and Cardiometabolic Risk

The trend of increasing childhood obesity appears to have leveled off in the past decade (42), but the prevalence of childhood overweight and obesity remains alarming. In 2007-2008, 21.2% of American children 2-5 years of age and 35.5% of children 6-11 years of age were overweight (defined as $\geq 85^{th}$ percentile for BMI) or obese (defined as $\geq 95^{th}$ percentile for BMI) (42). In ethnic minority groups, the prevalence is even higher: among Mexican American children, 27.7% of 2-5 year olds and 41.7% of 6-11 year olds are overweight or obese (42). Childhood obesity tracks into adulthood (43-45), thus these children are at greater risk for obesity and comorbidities of obesity as adults. Mexican Americans are the largest and fastest immigrant group in the U.S. (46,47); a greater understanding of the determinants of obesity and comorbidities of obesity in this population are needed to inform prevention efforts.

Overweight and obese youth are more likely to present with additional cardiometabolic risk factors, including as dyslipidemia, glucose intolerance, and hypertension (48). In adults, the metabolic syndrome is the clustering of specific risk factors for cardiovascular disease and type 2 diabetes. Several diagnostic criteria for the metabolic syndrome have been proposed for adult populations; recently, the International Diabetes Federation, National Heart, Lung and Blood Institute, American Heart Association, World Heart Federation, International Atherosclerosis Society and International Association for the Study of Obesity agreed on a unifying diagnostic criterion (49). An individual must have at least 3 of the following metabolic abnormalities to meet the criteria: elevated triglycerides or drug treatment for elevated triglycerides, reduced high-density lipoprotein cholesterol or drug treatment for the condition, elevated blood pressure or antihypertensive drug treatment in those with a history of hypertension, elevated fasting plasma glucose or drug treatment for elevated glucose levels, and elevated waist circumference

according to population and country-specific definitions. However, no standard definition of the metabolic syndrome for use in pediatric populations currently exists (50); research on cardiometabolic risk in children and adolescents have used a variety of criteria, largely modifications of those proposed for adult populations.

The Developmental Origins of Disease

Barker's hypothesis of the developmental origins of disease is based on the premise that fetal life, particularly fetal growth at a critical periods, influences later disease incidence, particularly coronary heart disease and type 2 diabetes in adulthood (51). These phenomena are credited to phenotypic plasticity, the ability of one genotype to result in a range of different physiological states in response to environmental conditions; this gene-environment interaction optimizes the fetuses' chance of survival after birth. The work of Barker and others suggests that low birth weight, birth length, body proportions and placental weight are markers of inadequate nutrition in fetal life, which leads to lifelong alterations in metabolism, hormonal balance, and the structure and function of key organs, thereby programming an individual for subsequent disease or disease risk factors (52,53). A poorly nourished mother signals a harsh environment and the fetus responds, attaining a smaller body size by altering its metabolism, thereby preparing itself for a suboptimal nutritional environment. Evidence from cross breeding and embryo transplant experiments support the premise that size at birth is largely determined by the maternal uterine environment, with limited influence of parental genotypes (54). Barker's more recent work has also suggested that compensatory growth, or rapid childhood growth, particularly following small size at birth, leads to cardiovascular disease and type 2 diabetes in later life (51).

Research on the fetal origins of disease has focused on nutrient supply to the fetus (53). The fetal nutrient supply regulates growth late in gestation (55) through its effects on insulin and insulin–like growth factors, the major hormonal mediators of fetal growth (53). The central role of nutrition in regulating fetal growth makes it a worthy candidate for fetal programming. Barker's work has focused primarily on under nutrition during pregnancy, exerting a strong influence on this growing field of research. Yet the excessive availability of nutrients may also have adverse effects on offspring development, as first suggested by Freinkel three decades ago, when he introduced the hypothesis of "fuel-mediated teratogenesis" (56). Freinkel proposed that developing fetal structures adapted to maternal fuel economy and pregnancies complicated by diabetes faced additional challenge due to an exaggeration of the metabolic oscillations that occur between fed and fasted states in a normal pregnancy. The resulting alterations to the processes of differentiation and proliferation of fetal cells were hypothesized to result in long-term effects on behavioral, anthropometric and metabolic functions.

In support of Freinkel's hypothesis, a growing body of evidence has linked excess maternal fuel substrates, manifested as maternal diabetes or GDM, to fetal overgrowth (57), as well as obesity and diabetes in the offspring (58-60). An elegant study by Dabelea et al. (61) demonstrated that intrauterine exposure to maternal diabetes conveys a higher risk for offspring obesity, independently of shared genetic factors. The researchers compared siblings born before and after their mother developed diabetes and reported that mean BMI was 2.6 kg/m^2 higher in those offspring exposed to maternal diabetes *in utero* as compared to their unexposed siblings. This could be a consequence of fetal exposure to several pathologies associated with diabetes,

including systemic inflammation and several hormonal abnormalities, and perhaps not the result of programming by fetal over nutrition alone.

Only one previous study has examined the association between over nutrition due to elevated maternal plasma glucose values during pregnancy and subsequent obesity in the children of women without recognized diabetes or GDM (58). No previous studies examining the association between increasing pregnancy plasma glucose values in women without recognized diabetes or GDM and subsequent cardiometabolic risk in their children were identified. Freinkel (56) suggested that even the "mildest limitations in insulin reserve, i.e., gestational diabetes" could result in long-term effects on the offspring. Studies among women with even milder limitations to insulin reserves, without recognized, overt disease during pregnancy, could lend further support to the role played by nutrition in developmental programming.

Maternal Glucose Levels below those Diagnostic of Overt Disease and Subsequent Childhood Obesity

Only one previous study has examined the association between maternal pregnancy glucose values and offspring obesity in women without recognized diabetes or GDM: Hillier et al. (58) reported a trend of increasing offspring weight-for-age across increasing quartiles of pregnancy glucose in women free of recognized, overt disease. Specifically, the researchers examined the association between plasma glucose values assessed 1 hour after a 50-g glucose challenge test and offspring weight-for-age from 5 to 7 years of age among 7,609 mother-child pairs belonging to health maintenance organizations; none of the 7,609 women had been diagnosed with diabetes or GDM. In this analysis, women in the upper quartile of plasma glucose values had significantly increased risks of offspring weight-for-age >85th percentile and >95th percentile based on U.S. standards.

These findings are consistent with studies examining the association between fetal exposure to maternal diabetes or GDM and subsequent childhood or adolescent obesity (58-62). Offspring exposed to maternal diabetes *in utero* are larger at birth but similar to the general population by 1 year of age; they begin demonstrating increases in weight relative to height by 5 years of age and are more likely to be overweight or obese by 8 years of age (63). The National Collaborative Perinatal Project (59) recently reported that the offspring of women with GDM had higher offspring BMI z-scores at 7 years of age compared to the offspring of women without GDM.

Hillier et al. (58) also considered the relationship between fetal exposure to varying severities of GDM and childhood weight-for-age in the study described previously. During the study period, the diagnosis and treatment of GDM was based exclusively on the National Diabetes Data Group (NDDG) criteria (64) [2 or more plasma glucose values on the 100-g, 3-hour OGTT at or higher than the NDDG thresholds: fasting 5.8 mmol/L; 1-hour 10.5 mmol/L; 2-hour 9.1 mmol/L; 3-hour 8.0 mmol/L]. Among women who did not meet the NDDG criteria (64), some had GDM by American Diabetes Association (ADA) criteria (41) only [2 or more plasma glucose measurements on the diagnostic 100-g, 3-hour OGTT at or higher than the following: fasting 5.3 mmol/L; 1-hour 10.0 mmol/L; 2-hour 8.6 mmol/L; 3-hour 7.8 mmol/L]; women who met only the lower thresholds of the ADA criteria did not receive treatment during

the study period. Interestingly, the risk of increased offspring weight-for-age among women with GDM by NDDG criteria (treated) was attenuated compared to that for women with GDM by the ADA criteria, a lesser degree of hyperglycemia (untreated).

No previous studies examining the trajectories of childhood growth by pregnancy glucose value among women without recognized diabetes or GDM were identified. A study examining the association between exposure to gestational diabetes and BMI trajectory from birth to 13 years of age (65) found that the overall sex and race-ethnicity adjusted growth trajectory was higher from 27 months through 13 years of age among youth exposed to GDM *in utero*; the difference was primarily driven by higher growth velocity from 10 to 13 years of age in those exposed to GDM.

Aim of Chapter 2

The aim of chapter 2 is to determine the relationship between pregnancy glucose levels and offspring BMI z-score at 2, 3.5, 5 and 7 years of age, the analysis only includes women without recognized diabetes or GDM, thereby contributing to gaps in the literature on fetal programming for obesity by maternal glycaemia. Chapter 2 also examines the rate of increase in offspring BMI z-score across the age range associated with maternal pregnancy glucose levels.

Pregnancy Glucose Levels Below those Diagnostic of Overt Disease and Cardiometabolic Risk in the Child at 7 years of age

Previous studies have reported significant deviations in several cardiometabolic risk factors among youth exposed to maternal GDM or diabetes *in utero*, including increases in diastolic blood pressure (DBP) (66), systolic blood pressure (SBP) (62,66-68), mean arterial blood pressure (62), waist circumference (60,68,68), and biomarkers of adverse endothelium perturbation (68), as well as decreases in high-density lipoprotein cholesterol levels (62,66,67). A retrospective cohort study of mother-child pairs belonging to an health maintenance organization in Colorado reported that youth 6 to 13 years of age exposed to maternal GDM *in utero* had a significantly greater waist circumference than their unexposed peers (60). A prospective cohort study in China compared youth 7 to 10 years of age who had been exposed to maternal GDM or gestational impaired glucose tolerance (IGT) *in utero* to youth whose mothers had normal glucose tolerance during pregnancy; no differences in triglyceride and cholesterol levels, or waist circumference, were found between groups but children exposed to maternal GDM or IGT *in utero* had significantly higher SBP and DBP and lower concentrations of high-density lipoprotein cholesterol (66).

Another retrospective cohort study in Colorado examined children 6 to 13 years of age belonging to a health maintenance organization who were exposed to pre-existing maternal diabetes or GDM (primarily GDM) *in utero* and compared them to unexposed children; after adjustment for age, sex, race-ethnicity, and maternal pre-pregnancy BMI, children exposed to maternal diabetes or GDM *in utero* had significantly greater waist circumferences and increased circulating levels of the cellular adhesion molecules E-selectin and vascular adhesion molecule 1 (VCAMI1); these biomarkers of adverse endothelium perturbations may be related to the early preclinical stages of atherosclerosis and diabetes (68). A retrospective study of Pima Indian youth 7 to 11 years of age likewise reported that the children of women with diabetes during

pregnancy had significantly higher SBP and glycosylated hemoglobin (HbA1c), but lower concentrations of high-density lipoprotein cholesterol than youth born to women without diabetes during pregnancy (67). The Diabetes in Pregnancy Center at Northwestern University reported that youth 10 to 16 years of age exposed to either maternal diabetes or GDM (almost exclusively type 1 diabetes) *in utero* had higher SBP and mean arterial BP as compared to unexposed youth, and lower levels of high-density lipoprotein cholesterol and total cholesterol (62).

Only one previous study has considered the association between mild impairments in maternal glucose regulation during pregnancy and subsequent cardiometabolic risk in the children; this study combined Chinese women with GDM and those with gestational IGT, defined as fasting plasma glucose less than 7.0 mmol/L and 2 hour plasma glucose between 7.8-11.1 mmol/L; 95% of women in the exposed group met the criteria for gestational IGT (66). The results of this study suggest that programming for increased cardiometabolic risk may also occur in the children of women with only mildly increased levels of pregnancy glycaemia. In women without recognized diabetes or GDM, there appears to be a continuous association between increasing maternal glucose levels and the risk of several perinatal complications (57), thus it is plausible that increasing glucose levels, including levels below those diagnostic for overt recognized disease or impairment, could also be associated with longer-term outcomes.

In the fetal origins of disease literature, including adjustment for current body size in analyses of subsequent hypertension has been debated (69). Several studies, including studies among children and adolescents, have demonstrated that blood pressure is correlated with concurrent measures of BMI, as well as glucose, insulin, and lipid profiles (62,70), leading to the hypothesis that current body size lies on the causal pathway from measures of size at birth to subsequent hypertension (69). Despite the epidemiologic tenant that variables on the causal pathway should not be considered confounders, some investigators (67) continue to adjust for measures of current body size in analyses examining the association between exposure to maternal glucose intolerance *in utero* and subsequent offspring hypertension; such adjustment may serve to mask a true association.

Aim of Chapter 3

The aim of Chapter 3 is to examine the association between increasing pregnancy glucose values in women without recognized diabetes or GDM during pregnancy and subsequent childhood cardiometabolic risk factors at 7 years of age, specifically: non-fasting cholesterol and triglyceride levels, blood pressure and waist circumference.

IV. Closing Remarks

The goal of this dissertation is to investigate the effects of periconceptual and prenatal maternal glucose levels on immediate and long-term offspring outcomes: the secondary sex ratio, as well as childhood BMI z-score and cardiometabolic risk in the offspring of women who are free of overt, recognized disease. The results of these analyses will fill important gaps in the literature. No study has examined the secondary sex ratio across a spectrum of maternal glycemic categories, although maternal glycaemia, an indicator of the maternal condition, is an excellent candidate to either support or refute the Trivers and Willard hypothesis (27). Several studies have reported increases in measures of childhood obesity and cardiometabolic risk among

children exposed to maternal diabetes or GDM *in utero*, but there is a paucity of data on this association in women free of recognized disease. If significant associations are found among women without recognized pre-gestational diabetes or GDM, the results would lend additional support to the development origins hypothesis and suggest a dose-response relationship, as opposed to a threshold effect, for maternal pregnancy glycaemia as an exposure.

Chapter 1: Sex ratio variations among the offspring of women with diabetes in pregnancy

1.a. Abstract

Introduction: It has long been hypothesized that natural selection would favor a reproductive strategy biased towards females under adverse circumstances in order to maximize the number of surviving grandchildren. An excess of daughters in women with type 1 diabetes and a greater likelihood of gestational diabetes in women carrying male fetuses have also been reported. This study aims to compare the sex ratio across categories of maternal glycemia.

Methods: Among 288,009 mother-infant pairs delivering at Kaiser Permanente Northern California in 1996-2008, sex ratios were calculated for the following categories: pregravid diabetes, gestational diabetes, mild pregnancy hyperglycemia (defined as an abnormal screening but normal diagnostic test for gestational diabetes) and normoglycemia. Odds ratios for delivering a male were estimated with logistic regression; normoglycemic pregnancies comprised the reference.

Results: Women with pregravid diabetes delivered the fewest males (ratio male/female= 1.01), followed by women with normoglycemic pregnancies and those with an abnormal screening only (both sex ratios= 1.05); women with gestational diabetes delivered the most males (sex ratio= 1.07). Odds ratio estimates suggested the same pattern, but none attained significance.

Conclusions: The crude sex ratios in this cohort suggest a possible gradient by category of maternal glycemia. Women with gestational diabetes, a condition characterized by excessive fuel substrates, appear to deliver more males. Women with pregravid diabetes delivered the fewest males, possibly reflecting the unfavorable state of chronic disease.

1.b. Introduction

In populations of European ancestry, 106 male newborn infants per 100 females are typically observed (19,20), yet in populations of African ancestry, the ratio is typically 103 male newborn infants per 100 females (20). Otherwise, there is very little fluctuation in the sex ratio across populations, except under extraordinary circumstances. Trivers and Willard (71) hypothesized that the sex ratio would be altered in difficult times, with natural selection favoring a reproductive strategy biased towards females under adverse circumstances to maximize the number of surviving grandchildren. Males have lower future reproductive success than their female counterparts, largely because they are less likely to reach reproductive age (29).

As a potential mechanism for the Trivers and Willard hypothesis, Catalano et al. provide empirical evidence for a maternal screening mechanism that ranks gestations by the expected yield of grandchildren and a corresponding rank threshold below which a woman would spontaneously terminate the pregnancy (26,28). In contrast, James hypothesizes that abnormal hormonal profiles in either parent at the time of conception is the cause of sex ratio perturbances (72). Mammalian (excluding human) sex ratio studies demonstrate that excess maternal glucose levels *in utero* favor the development of male blastocysts during early cell division (73). *In vitro* bovine blastocyst exposure to glucose-containing medium also results in significantly fewer female embryos able to progress to more advanced stages of development (74,75). These findings suggest that, in the absence of chronic disease, an abundance of nutritional substrates may result in more male embryos.

Previously, an excess of female births was reported among women with type 1 diabetes (30). One study has since considered the association between fetal sex and gestational diabetes mellitus (31), defined as glucose intolerance with onset or first recognition during pregnancy (76), and found that women carrying male fetuses were more likely to have gestational diabetes.

To better understand the relationship between maternal diabetes and hyperglycemia and fetal sex, we compared the infant sex ratio across several glycemic categories: women with pregravid diabetes, laboratory confirmed gestational diabetes or mild pregnancy hyperglycemia, and normoglycemic pregnancies.

1.c. Methods

This study utilized the Gestational Diabetes and Pregnancy Glucose Tolerance Registry (6) and the Diabetes Registry (39) of Kaiser Permanente Northern California (KPNC), a large group-practice, prepaid health plan that provides comprehensive medical services to approximately 3.2 million members residing in a 14 county region. Approximately 30% of the population that resides in the area served by the KPNC is enrolled in the health plan, which is representative of the underlying population.

Women with recognized diabetes prior to the index pregnancy were identified in the KPNC Diabetes Registry (39), which identifies patients from four data sources: primary hospital discharge diagnoses of diabetes mellitus; two or more outpatient visit diagnoses of diabetes; any prescription for a diabetes-related medication; or any record of an abnormal HbA1c test (greater than 50 mmol/mol or 6.7%). Diabetes type was defined by the inpatient or outpatient diagnosis occurring closest to date of conception (calculated as the delivery date minus gestational age at

delivery). Diagnoses of diabetes type were identified according to the International Classification of Disease (ICD-9) codes as follows, type 1: 250.x1 and 250.x3, type 2: 250.x0 and 250.x2. Of the 2,261 women with recognized diabetes prior to pregnancy, 88% were thus classified as type 1 or type 2.

In addition to women with recognized diabetes prior to pregnancy, women with gestational diabetes who were subsequently diagnosed with diabetes from 6 weeks to 12 months postpartum were also considered to have pregravid diabetes. These women were identified in the KPNC Diabetes Registry or by the following postpartum laboratory tests: 75-g, 2-hour oral glucose tolerance test with fasting value greater than or equal to 7.0 mmol/L or 2 hour value greater than or equal to 11.1 mmol/L; stand-alone fasting value greater than or equal to 7.0 mmol/L; 2 hour post-prandial value greater than or equal to 11.1 mmol/L; random value greater than or equal to 11.1 mmol/L; or HbA1c greater than or equal to 48 mmol/mol (6.5%) (40). Among women diagnosed with gestational diabetes in this cohort (n= 18,285), 44% performed a glucose screening between 6 weeks and 12 months postpartum. Based on the postpartum glucose screening results, there were 271 women who met the criteria for unrecognized pregravid diabetes (77). Therefore, these women were combined with those who had been diagnosed with diabetes prior to pregnancy, resulting in a total of 2,532 women classified as having pregravid diabetes.

The KPNC Gestational Diabetes and Pregnancy Glucose Tolerance Registry (6) was used to classify the pregnancy glucose tolerance of women without pregravid diabetes. In this setting, among women without diabetes diagnosed prior to pregnancy, 94% underwent the recommended 50-g, 1-hour glucose challenge test to screen for gestational diabetes (78) (hereafter, referred to as the screening test) during a routine prenatal visit. Women with plasma glucose values greater than or equal to 7.8 mmol/L on the screening test went on to receive a diagnostic 100-g, 3-hour oral glucose tolerance test (hereafter referred to as the diagnostic test). All plasma glucose measurements were performed using the hexokinase method at the KPNC regional laboratory, which participates in the College of American Pathologists' accreditation and monitoring program. Gestational diabetes was defined according to the American Diabetes Association (ADA) plasma glucose thresholds (41) for the diagnostic test, or two or more values meeting or exceeding the following cut points: fasting 5.3 mmol/L; 1-hour 10.0 mmol/L; 2-hour 8.6 mmol/L; 3-hour 7.8 mmol/L.

Comprising another glycemic category were women who had an abnormal screening test (plasma glucose $\geq$ 7.8 mmol/L) but whose diagnostic test results did not meet the ADA (41) criteria for gestational diabetes (hereafter referred to as those with only abnormal screening test results). This group was considered to have mild pregnancy hyperglycemia. Women in the normoglycemic category were those who had a normal screening test result during pregnancy.

Newborn sex was obtained by linking the KPNC database with birth certificate data from the State of California (99% successful linkage). Maternal race-ethnicity, age at delivery, and educational attainment were also obtained through linkage with the birth certificate database.

To estimate the ratio of males to females at birth in each maternal glycemic category, we began with all members of KPNC who delivered liveborn singletons between 1996 and 2008 and were 15 to 45 years of age at delivery. The cohort was then restricted to the first liveborn

singleton delivered to a woman within the study period (n= 313,698); 21,014 women that had not been diagnosed with diabetes prior to pregnancy and did not perform the screening or the diagnostic tests for gestational diabetes were subsequently excluded. An additional 4,675 women who were not screened but performed a diagnostic test and did not meet the diagnostic thresholds for gestational diabetes were also excluded from the final analytic cohort (n= 288,009); however, these 4,675 mother-infant pairs were considered further in sensitivity analyses. Data are presented for a final analytic cohort of 288,009 mother-infant pairs (92%).

We first examined the crude sex ratio for each category of maternal glycemia; the Cochran-Armitage test for trend tested the null hypothesis of no linear trend in the proportion of male infants across the following categories: pregravid diabetes, normoglycemic pregnancies, abnormal screening test only and gestational diabetes. Logistic regression analyses were then used to examine the association between maternal glycemic category and delivering a live born, singleton male. The odds of delivering a male in women with pregravid diabetes, gestational diabetes and those with an abnormal screening test only were compared to that in women with normoglycemic pregnancies. Unadjusted estimates, as well as estimates adjusted for maternal race-ethnicity (model 1) (79) and maternal race-ethnicity, education, and age (model 2) (80) are presented. Further adjustment for parity (79) did not alter the odds ratio estimates (data not shown). The Hosmer and Lemeshow chi-square test statistic was used to assess model fit (81).

Maternal race-ethnicity was categorized as non-Hispanic Caucasian, African American, Asian, Hispanic, and Other. Educational attainment was classified as elementary or secondary school only, high school graduate, some college (1 to 3 years of college), college graduate (4 years of college), or graduate studies (5+ years of college). Maternal age was modeled as a continuous variable.

Modification of the association between maternal glycemic category and fetal sex by maternal race-ethnicity and age were further explored. Cross-products for these variables and the maternal glycemic categories were entered into logistic regression models, the results of which offered no evidence for interaction on the multiplicative scale (all *P values* > 0.20). Stratum specific ORs were also calculated for each racial-ethnic group, which again revealed no modification of effect.

SAS 9.1 (SAS Institute Inc., Cary, NC) was used for all analyses. This study was approved by the human subjects committees of KPNC, the University of California, Berkeley, and the State of California.

1.d. Results

The characteristics of the cohort and stratified sex ratios are displayed in Table 1.1. The mean age at delivery was 28.6 years (SD 6.0 years) and over half of the women were primiparous. Forty percent were non-Hispanic Caucasian, 29% Hispanic, 22% Asian and 9% African American; 12% of the cohort had less than a high school education. The crude sex ratio (male/female) for the entire cohort was 1.05. The sex ratio varied by race-ethnicity: African American women exhibited the lowest sex ratio (1.02), next highest were Hispanic women (1.04), followed by non-Hispanic Caucasian women (1.05) and Asian women (1.07); those reporting Other as their race-ethnicity demonstrated the highest sex ratio (1.10).

The number of women in each category of maternal glycemia, along with the crude sex ratio for that category, is displayed in Table 1.2. Women with gestational diabetes had the highest sex ratio (1.07), followed by women with abnormal screening values only (1.05) and normoglycemic pregnancies (1.05). Women with pregravid diabetes, who constituted the smallest category, exhibited the lowest sex ratio (1.01; Cochran-Armitage test for trend across groups, $P = 0.22$). In women with pregravid diabetes, the 1,742 women with type 2 diabetes had a sex ratio of 1.05, the 271 women identified postpartum exhibited a sex ratio of 0.88, and the 245 women with type 1 diabetes a sex ratio of 0.87.

The 4,675 women who were not screened for gestational diabetes during pregnancy but did perform the diagnostic test were considered further in sensitivity analyses; the sex ratio estimates remained identical to those presented in Table 1.2 when these women were included, categorized either as having abnormal screening values only or as normoglycemic pregnancies.

Results of the logistic regression models are presented in Table 1.3; the 95% confidence intervals for all estimates included the null value, thus none attained statistical significance. Compared to women with normoglycemic pregnancies, those with gestational diabetes were more likely to deliver males. Women with abnormal screening values only did not differ from those with normoglycemic pregnancies. Women with pregravid diabetes were more likely to deliver females compared to women with normoglycemic pregnancies.

There were 2,480 women missing data on race-ethnicity and 5,482 missing data on educational attainment; these women were excluded from the adjusted multiple regression models. Unadjusted odds ratios estimates among those with complete data only were identical to those presented in Table 1.3.

Adjustment for maternal race-ethnicity, education, and age did not appreciably alter the odds ratio estimates. For all models, there was no suggestion of lack of fit based on the Hosmer and Lemeshow chi-square statistic (all $P > 0.75$).

1.e. Discussion

Despite the large cohort investigated in this study, the odds of delivering a liveborn male singleton across several categories of maternal glycemia did not vary significantly, even after adjustment for covariates. Yet the crude sex ratios suggest a possible gradient by category of maternal glycemia: women with pregravid diabetes delivered the fewest males, followed by women with normoglycemic pregnancies and those with an abnormal screening values only (or mild pregnancy hyperglycemia), and women with a gestational diabetes delivering more males than any other group. Women with abnormal screening values only did not appear to differ from women with normoglycemic pregnancies. U.S. national vital statistics (79) confirm the variation by race-ethnicity described in these data: Asian women had the highest sex ratio (most males), next highest were Non-Hispanic women of Caucasian ancestry, followed by Hispanic women, and lastly African American women, who exhibited the lowest sex ratio.

The observed sex ratio trend across maternal glycemic categories supports the original Trivers and Willard hypothesis (71), whereby the spontaneous abortion of those conceptions with the lowest probability of producing grandchildren is believed to contribute to fetal loss. A

maternal screening mechanism that ranks gestations based on their expected yield of grandchildren, along with a corresponding rank threshold below which a woman would spontaneously terminate a pregnancy, have been proposed (26,28). This rank threshold would account for a mother's ability to sustain her offspring through reproductive age and reflect the probability that a given conception would reach reproductive age, thus male conceptions would hold a lower rank than females (29). Extending this logic further, female twins would be the most desirable, followed by female singletons, male singletons and lastly, male twins in terms of the yield of grandchildren (26).

Pregravid diabetes is characterized by insufficient metabolic regulation due to either insulin deficiency, as in type 1 diabetes mellitus, or increased insulin resistance, as in type 2 diabetes mellitus. The resulting state of pathologic hyperglycemia is also associated with oxidative and metabolic stress. These unfavorable conditions may lead to a higher rank threshold for fetal loss to maximize reproductive success, thereby resulting in the loss of male fetuses and a lower overall sex ratio in this group. In these data, women with more severe disease, such as those with type 1 diabetes recognized prior to pregnancy and those identified with diabetes postpartum (who would not have received monitoring or treatment for their disease around the time of conception) demonstrated the greatest sex ratio perturbances.

Gestational diabetes, on the other hand, is a condition associated with increased metabolic substrates that results in fetal over nutrition. According to the Trivers and Willard hypothesis (71), women with gestational diabetes should have more males. As compared to women with normoglycemic pregnancies, those who develop gestational diabetes are more likely to have experienced higher glycemic levels around the time of conception due to a predisposition for insulin resistance prior to pregnancy (11,12). The maternal state of abundant fuel, although not high enough to constitute an unfavorable state of overt disease, may signal an increased maternal ability to sustain offspring and thus lower the rank threshold for fetal loss, thereby resulting in more males overall and a higher sex ratio in this group.

Gestational diabetes is diagnosed late in the second or early in the third trimester in women exhibiting hyperglycemia despite the increased insulin response to oral glucose that accompanies normal pregnancy (82). Thus, it cannot be determined whether some pre-pregnancy manifestation of gestational diabetes affects early sex selection *in utero* or conversely, if the sex of the fetus impacts the later development of gestational diabetes. A meta-analysis of mammalian sex ratio studies (excluding human) found that studies examining maternal body condition, weight or food availability assessed or manipulated around the time of conception demonstrated the most consistent and significant support for the Trivers and Willard hypothesis of increased maternal investment in male gestations under favorable conditions (73). Mammalian reproductive research demonstrates that high concentrations of glucose have detrimental effects on early embryonic development *in vitro* (83,84); specifically, glucose supplementation of culture media results in the preferential loss of female bovine blastocysts (74,75).

Previous work has similarly reported an association between gestational diabetes and delivering a male. Sheiner et al. (31) found that Israeli women carrying males were ten percent more likely to have gestational diabetes in a sample of 108,995 mother-infant pairs, with the odds ratio estimate attaining statistical significance. Rjasanowski et al. (30) reported more female than male offspring (ratio= 0.45) among children born to a small sample (n= 112) of women with

type 1 diabetes in Germany. The sample of women with type 1 diabetes in the current study was twice the size (n= 245); in our data, women with pregravid diabetes delivered fewer males than any other glycemic category, with females in absolute excess among women with type 1 diabetes and those identified postpartum (ratio male to female < 1.00). It should be noted that KPNC health plan members with recognized diabetes prior to pregnancy receive medical treatment and monitoring of their disease. It is possible that we did not observe sex ratio perturbances in women with type 1 diabetes as extreme as those reported by Rjasanowski et al. due to secular trends of improved treatment for patients with diabetes. Population differences may also play a role, as the Rjasanowski et al. study included primarily Caucasians.

Near universal pregnancy glucose screening and the availability of plasma glucose values for the identification of laboratory confirmed cases of gestational diabetes and mild pregnancy hyperglycemia are strengths of the current study. Limitations include the small number of women with pregravid diabetes. In women with pregravid diabetes, suboptimal glycemic control pre-conception increases the risk of miscarriage. Unfortunately, we lacked data on perinatal deaths; we also lacked data on paternal glucose tolerance and diabetes status. Lastly, less than half of the women with gestational diabetes were screened for diabetes in the postpartum period, thus some women with pregravid diabetes were likely misclassified as having gestational diabetes, suggesting that the sex ratio difference between women with pregravid diabetes and those with gestational diabetes may be larger than observed.

The findings of this study suggest that sex ratio at birth may vary by category of maternal glycemia. Our findings also demonstrate the stability of the sex ratio, as only diminutive differences were observed between categories of maternal glycemia, as well as between racial-ethnic groups. Although this cohort contained over 250,000 mother-infant pairs, data from even larger cohorts, particularly data on glucose control around the time of conception in women with pregravid diabetes, would address several questions raised by these findings.

Table 1.1. Characteristics and Corresponding Sex Ratios (Male/Female) for Women Delivering a Liveborn singleton at Kaiser Permanente Northern California, 1996-2008.

	n (%)	Sex Ratio
All women	**288,009**	**1.05**
Age (years)		
15-19	22,214 (7.7)	1.03
20-24	53,876 (18.7)	1.05
25-29	83,698 (29.1)	1.05
30-34	77,685 (27.0)	1.05
35-39	40,580 (14.1)	1.06
40-45	9,956 (3.5)	1.05
Parity[a] (n= 287,790)		
0	166,756 (57.9)	1.05
1	71,677 (24.9)	1.04
2	33,437 (11.6)	1.05
3+	15,920 (5.5)	1.04
Education* (n= 282,527)		
Elementary or secondary school only	34,785 (12.3)	1.04
High school graduate	79,188 (28.0)	1.04
Some college	79,923 (28.3)	1.06
College graduate	50,985 (18.1)	1.06
Graduate studies	37,646 (13.3)	1.04
Race-ethnicity[a] (n= 285,529)		
Non-Hispanic Caucasian	114,838 (40.2)	1.05
Hispanic	83,009 (29.1)	1.04
African American	24,256 (8.5)	1.02
Asian	62,284 (21.8)	1.07
Other	1142 (0.4)	1.10
Year of Delivery		
1996-1997	51,309 (17.8)	1.04
1998-1999	47,462 (16.5)	1.03
2000-2001	42,258 (14.7)	1.05
2002-2003	43,087 (15.0)	1.05
2004-2005	40,449 (14.0)	1.04
2006-2007	42,553 (14.8)	1.07
2008	20,891 (7.3)	1.06

[a] n differs due to missing values

Table 1.2. Sex Ratio (Male/Female) by Category of Maternal Glycemia for 288,009 Women Delivering Liveborn Singletons at Kaiser Permanente Northern California, 1996-2008.

Maternal glycemic category	n	Sex Ratio
Pregravid diabetes	2,532	1.01
Identified postpartum	271	0.88
Type 1	245	0.87
Type 2	1,742	1.05
Normoglycemic pregnancy	232,106	1.05
Abnormal screening and normal diagnostic test	35,357	1.05
Gestational diabetes	18,014	1.07

Table 1.3. Odd Ratios and 95% Confidence Intervals for Delivering a Male Infant Among 288,009 Women Delivering Liveborn Singletons at Kaiser Permanente Northern California, 1996-2008.

	Unadjusted n= 288,009		Model 1[a] n= 285,529		Model 2[b] n= 282,236	
	OR	**95% CI**	**OR**	**95% CI**	**OR**	**95% CI**
Maternal glycemic category						
Pregravid diabetes	0.97	0.90, 1.05	0.97	0.90, 1.05	0.96	0.89, 1.04
Normoglycemic pregnancy	Ref		Ref		Ref	
Abnormal screening and normal diagnostic test	1.00	0.98, 1.02	1.00	0.98, 1.02	1.00	0.98, 1.02
Gestational diabetes	1.02	0.99, 1.05	1.02	0.99, 1.05	1.02	0.99, 1.05
Race-ethnicity						
African American			0.98	0.95, 1.01	0.98	0.95, 1.01
Hispanic			0.99	0.97, 1.01	0.99	0.97, 1.01
Non-Hispanic Caucasian			Ref		Ref	
Asian			1.02	1.00, 1.04	1.02	1.00, 1.04
Other			1.05	0.93, 1.18	1.06	0.94, 1.19
Educational attainment						
Less than High School					Ref	
High School graduate					1.00	0.98, 1.03
Some College					1.02	0.99, 1.04
College graduate					1.01	0.98, 1.04
Graduate studies					1.00	0.96, 1.03
Age					1.00	1.00, 1.00

[a] Adjusted for maternal race-ethnicity

[b] Adjusted for maternal race-ethnicity, educational attainment, and age

Chapter 2: Programming of childhood obesity by pregnancy glycemia among Mexican-American women without diabetes or gestational diabetes.

2.a. Abstract

Introduction: To estimate, among Mexican-American women without diabetes or gestational diabetes (GDM), the association between pregnancy glucose and offspring BMI z-score at 2, 3.5, 5 and 7 years of age, as well as BMI z-score trajectory.

Methods: The Center for the Health Assessment of Mothers and Children of Salinas (CHAMACOS Study) prospectively followed pregnant women and their offspring through 7 years of age (n= 266). Plasma glucose values obtained 1-hour after a 50-g oral glucose load comprised the exposure. Offspring body mass index (BMI) was compared to national sex and age-specific data to calculate z-score. Linear regression and linear mixed effects (LME) models were adjusted for maternal obesity, soda consumption and smoking during pregnancy, gestational weight gain, poverty level, infant birth weight and the child's absolute age at the follow up measurements.

Results: There was a significant association between increasing glucose and greater BMI z-score at 7 years of age; each mmol/L increase in glucose corresponded to an increase of 0.12 BMI z-score units (SD= 0.045, p-value< 0.01). In non-obese women only, mean BMI z-score over the age range appeared to increase with increasing glucose (p-value = 0.06).

Conclusions: In the offspring of Mexican-American women without recognized pregestational diabetes or GDM, exposure to higher maternal glucose levels in the second trimester was significantly associated with increased BMI z-score at 7 years of age. Obesity prevention efforts for children of Mexican descent may consider lifestyle interventions to reduce pregnancy glucose values, even if below levels diagnostic of disease.

2.b. Introduction

Mexican-American children in the United States (U.S.) are more likely to be overweight or obese than non-Hispanic white children and, in some studies, than non-Hispanic black children (42,85,86). Among Mexican-American children, close to 30% of 2- to 5-year olds and 43% of 6- to 11-year olds are overweight or obese; the corresponding prevalence in non-Hispanic whites is 17% and 35%, respectively (42). Obese children are more likely than their normal weight peers to become obese adults (43-45) and Mexican-American adults are at increased risk for comorbidities of obesity such as diabetes (87-90). As the largest and fastest growing immigrant group in the U.S. (46,47), research is urgently needed to understand the determinants of obesity in this population.

A growing body of research suggests that the intrauterine environment may influence later development and morbidity (91). For example, gestational diabetes (GDM) has been associated with childhood obesity in several studies of primarily non-Hispanic white (58,59) or multiethnic populations (60). However, it is less clear whether elevated pregnancy glucose values that are below the diagnostic levels for GDM also have an effect on offspring obesity. Only one study has reported an increasing trend in offspring weight-for-age across increasing quartiles of maternal glucose in women without recognized pregestational diabetes or GDM, but that study did not present analyses of body mass index (BMI) (58).

In the present study, in women without recognized pregestational diabetes or GDM, we sought to determine the relationship between pregnancy glucose levels and offspring BMI z-score at 2, 3.5, 5 and 7 years of age. Since rapid weight gain in infancy and early childhood is a risk factor for adult obesity (92), we also examined the association between pregnancy glucose levels and the rate of increase, or velocity, of childhood BMI z-score across this age range. The mothers and children were participants in the Center for the Health Assessment of Mothers and Children of Salinas (CHAMACOS Study), a longitudinal birth cohort of low-income Mexican-Americans.

2.c. Methods

Pregnant women were eligible for the CHAMACOS study if they sought prenatal care at one of six participating health clinics between October 1999 and October 2000, were less than 20 weeks gestation (mean gestational age= 13.8 weeks, SD= 5.1), 18 years of age or older, eligible for state-sponsored health care (Medi-Cal) and intended to deliver at Natividad Medical Center (Monterey County, CA, USA). A total of 601 women were enrolled and 485 were followed until the delivery of a full term ($\geq$ 37 weeks gestation), liveborn singleton.

Plasma glucose was measured at the end of the second trimester in conjunction with routine prenatal care. Measurements of plasma glucose, as well as diabetes and GDM diagnoses, were abstracted from the medical record by a registered nurse. Included in this analysis are women without diabetes (type 1 and type 2 diabetes) or GDM that had a plasma glucose value measured 1-hour after a 50-g oral glucose challenge test (screening test) performed within the recommended window of 24 to 28 weeks gestation (64). We excluded 11 women with recognized pregestational diabetes and one with unrecognized pregestational diabetes (any glucose level >11.1 mmol/L on more than one occasion during pregnancy). Five cases of GDM,

according to the results of the screening test and a diagnostic 100-g, 3-hour oral glucose tolerance test, were excluded; during this period in this setting, the diagnosis of GDM was based on the National Diabetes Data Group criteria (64). Also excluded was one woman with an abnormal value on the screening test (11.1 mmol/L) but no follow up glucose testing, 23 women with diagnoses of GDM in their medical record who did not meet the diagnostic criteria (since these women likely received treatment for pregnancy hyperglycemia), and 113 woman whose screening tests were not performed within the recommended window of 24 to 28 weeks gestation (64). None of the remaining 331 women met the lower thresholds of the American Diabetes Association criteria for GDM (41). Of these 331 women, 266 had offspring anthropometric data, including weight and height, available at 2, 3.5, 5 or 7 years of age.

Children were weighed and measured without jackets and shoes using a calibrated electronic scale (Tanita Mother-Baby Scale Model 1582 or TBF-300A Body Composition Analyzer, Tanita Corp.) and stadiometer. Body mass index (BMI) was calculated as mass in kilograms divided by height in meters squared. BMI Z-scores were calculated from sex-specific, BMI-for-age data issued by the Centers for Disease Control and Prevention (CDC) in 2000 (93).

Mothers were interviewed during pregnancy to obtain information on: smoking status (yes or no), poverty level (above versus at or below the poverty line) (94) and soda consumption; and abstracted from the medical record were data on: gestational weight gain, gestational age at the prenatal weight measurements (weeks), maternal height, child birthweight (grams) and gestational age at birth (weeks). Maternal soda consumption prior to the screening test was used as an indicator of dietary added sugars (95) and was ascertained at the end of the second trimester (mean gestational age= 26.7 weeks, SD= 1.9); women were asked how often they drank a 12 ounce can of Coca Cola or other soft drinks (non-diet) during the last three months and the frequency of consumption coded times per week. Pre-pregnancy weight was obtained from several sources, according to the following hierarchy: 1) as reported in the medical record, 2) self-reported on the pregnancy questionnaire, 3) from an early prenatal weight measurement (<13 weeks gestational age), or 4) calculated by a regression line that utilized all prenatal weight measurements and corresponding gestational ages. In the sub-set of women with pre-pregnancy weight data available in the medical record, the pregnancy questionnaire and from an early prenatal weight measurement (n= 139), pre-pregnancy weight from the medical record was significantly and positively correlated with the early prenatal weight (Spearman's rho =0.96, *p*-value < 0.0001); self-reported pre-pregnancy weight was also significantly and positively correlated with the early prenatal weight (Spearman's rho =0.95, *p*-value < 0.0001). Since the amount of gestational weight gained after the screening and diagnostic tests could be affected by the test results, pre-pregnancy weight was subtracted from the nearest prenatal weight measurement taken prior to the glucose test to calculate the amount of weight gained (in pounds) up until the time of glucose testing.

Statistical Analyses

Multiple linear regression analyses were used to estimate the association between pregnancy glucose level, measured 1-hour after a 50-g oral glucose load, and offspring BMI z-score at 2, 3.5, 5 and 7 years of age. We also estimated the association between pregnancy

glucose and BMI z-score velocity over time using linear mixed effects (LME) models. Pregnancy glucose was examined as a continuous variable.

A directed acyclic graph (DAG) (96) guided the selection of adjustment variables. Multiple linear regression models were adjusted for pre-pregnancy obesity (BMI $\geq$ 30 kg/m^2), times per week soda was consumed prior to the glucose test (continuous), gestational weight gained prior to the glucose test (continuous), gestational age at the weight measurement (continuous), smoking during pregnancy, poverty, infant birth weight (continuous), gestational age at birth (continuous) and the child's absolute age, in months (continuous), at the follow-up measurements. Models that included pre-pregnancy BMI (continuous) instead of pre-pregnancy obesity gave equivalent results; models that excluded birth weight and gestational age at birth gave comparable results, thus models containing these variables are presented. We verified that these data met the assumptions of linear regression and investigated potential effect modification in the association between pregnancy glucose and BMI z-score by pre-pregnancy obesity, gestational weight gained prior to the glucose test and birth weight. Each cross product was added, one at a time, to a fully adjusted model; no cross products were statistically significant in any model (all *p-values*> 0.10).

The linear mixed effects (LME) models of longitudinal data account for intrasubject correlation between repeated measurements and allow for a different number of follow up observations per child (97). Maximum likelihood was the method of estimation and an unstructured working covariance matrix for the random effects parameters (intercept and slope) was chosen. Pregnancy glucose, in addition to the adjustment variables previously described, were modeled as fixed effects.

We hypothesized differential growth velocities for the offspring of women who were obese prior to pregnancy and those who were not (98); therefore, separate LME models were constructed among obese (BMI $\geq$ 30 kg/m^2; n= 52) and non-obese women (n= 214). The Akaike information criterion (AIC) statistic (99), a measure of the relative goodness of model fit, facilitated model selection; the final LME models for obese and non-obese women differed only in that the model for non-obese women included a quadratic term for age (*p*-value< 0.0001). Locally weighted scatterplot smoothing (LOEWESS) was used to graphically display, separately for non-obese and obese women, BMI z-score trajectories by glucose tertile.

In sensitivity analyses, we assessed whether missing follow-up data on some children resulted in bias by conducting analyses weighted by the inverse probability that a mother-child pair would remain in the cohort for at least one follow-up measurement. SuperLearner (100), a prediction algorithm, was used to predict whether a pair remained in the cohort.

All analyses were conducted in SAS (version 9.1, SAS Institute Inc., Cary, NC) and plots produced in STATA (version 10.1, StataCorp, College Station, Texas). SuperLearner was run in R (version 2.12.1, The R Foundation for Statistical Computing, Vienna, Austria). Study participants provided written informed consent and all research activities were approved by the University of California–Berkeley Committee for the Protection of Human Subjects.

2.d. Results

Characteristics of the 266 mother-child pairs are displayed in Table 2.1. Only 20% of the mothers had completed high school and 63% of households had incomes at or below the federal poverty threshold. Mothers tended to be young (mean maternal age = 26.0 years, SD= 5.0) and the majority were overweight (mean pre-pregnancy BMI = 26.8 kg/m^2, SD= 5.0). Mothers consumed an average 1.6 (SD 2.3) servings of non-diet soda per week prior to the glucose test and gained an average of 11.4 pounds (SD 9.5) prior to the glucose test (mean gestational age at the weight measurement= 22.3 weeks; SD 5.3). In the offspring, the mean BMI z-score was 0.49 (SD 1.09) at 2 years, 1.09 (SD 1.05) at 3.5 years, 1.21 (SD 1.03) at 5 years and 1.16 (SD 1.03) at 7 years of age.

Pregnancy glucose values significantly predicted offspring BMI z-score at 7 years of age independently of pre-pregnancy obesity, pregnancy soda consumption, gestational weight gained prior to the glucose test and infant birth weight (model R-Square = 0.15): each mmol/L increase in glucose value corresponded to an increase of 0.12 BMI z-score units (SE= 0.045, *p*-value< 0.01; Table 2.2). The association between pregnancy glycemia and BMI z-score at 5 years of age achieved borderline significance; no associations were observed at younger ages. Adjustment for pre-pregnancy obesity, the amount soda consumed and gestational weight gained prior to the glucose test, smoking, poverty, infant birth weight and the child's absolute age at the follow-up measurements did not appreciably modify coefficient estimates (Table 2.2). Pre-pregnancy obesity, significantly and independently of pregnancy glucose, predicted increases in BMI z-score at 5 and 7 years of age; the association between pre-pregnancy obesity and BMI z-score at 2 years of age achieved borderline significance. The coefficient for infant birth weight did not attain statistical significance at any age.

Figure 2.1 presents smoothed scatterplots of offspring BMI z-score trajectory across the age range, demonstrating differences in growth pattern for the offspring of non-obese and obese women. For non-obese women, the stratum specific glucose tertile ranges were 2.9-4.9, 5.0-6.3, and 6.4-10.1 mmol/L; the corresponding ranges for obese women were 3.3-5.3, 5.4-6.4, and 6.8-9.8 mmol/L. In the fully adjusted LME for non-obese women (n= 213), the observed increase in mean BMI z-score across the age range with increasing glucose did not attain statistical significance (*p*-value = 0.06); the estimated average BMI z-score at 4.5 years of age increased by 0.11 BMI z-score units (SE 0.059) for each mmol/L increase in glucose. In the fully adjusted LME for obese women (n= 49), the observed increase in BMI z-score velocity with increasing glucose was also non-significant (*p*-value = 0.07); the estimated rate of increase was 0.035 (SE 0.019) BMI z-score units per year for each mmol/L increase in glucose.

The linear regression results obtained from the inverse probability weighted analyses were similar to the results of the unweighted analyses; however, the association between pre-pregnancy obesity and childhood BMI z-score at 2 years of age attained statistical significance, but the association between pregnancy glycemia and BMI z-score at 5 years of age did not (Table 2.3). The results of the inverse probability weighted LME models were also similar; in the fully adjusted LME for non-obese women, the coefficient for increasing mean BMI z-score across the age range with increasing glucose was numerically identical to the unweighted estimate but achieved borderline significance (*p*-value = 0.05).

2.e. Discussion

In this population of Mexican-American women without recognized pregestational diabetes or GDM, we found a significant association between increasing pregnancy plasma glucose values, assessed during a single 50-g oral glucose challenge test in mid-pregnancy, and increasing offspring BMI z-score at 7 years of age. Increasing pregnancy glucose was nearly associated with a higher average BMI z-score from ages 2 to 7 years in the children of non-obese women, although the estimate achieved only borderline significance. These findings suggest that in women without recognized pregestational diabetes or GDM, *in utero* exposure to increasing glucose levels is associated with obesity at age 7 and that the offspring of non-obese women exposed to higher glucose levels may demonstrate increased adiposity, on average, from 2 to 7 years of age. This study fills important gaps in the literature on developmental origins of obesity in a population that is at high risk for childhood obesity.

To our knowledge, only one previous study has examined whether increasing levels of maternal pregnancy glucose are associated with childhood anthropometrics in the offspring of women without recognized pregestational diabetes or GDM. This multiethnic study of over 7,000 mother-child pairs enrolled in an HMO reported a positive trend in weight-for-age greater than the 85th and 95th percentiles across increasing quartiles of maternal glucose, assessed 1 hour after a 50-g oral glucose load (58). However, data on children's height were not universally available, thus analyses of BMI were not presented. Youth 5 to 7 years of age were combined for analyses, so associations could not be estimated separately by age; the study also did not examine growth trajectories.

Our results are consistent with previous reports suggesting that the association between exposure to maternal diabetes *in utero* and increased offspring adiposity does not become apparent until later childhood or in puberty. Offspring exposed to maternal diabetes *in utero* are larger at birth but similar to the general population by 1 year of age, they begin demonstrating increases in weight relative to height by 5 years of age and are more likely to be overweight or obese by 8 years of age (63). The National Collaborative Perinatal Project (59) has similarly reported that the offspring of women with GDM had higher offspring BMI z-scores at 7 years of age compared to the offspring of women without GDM.

Although we identified no previous studies examining the trajectories of childhood growth by pregnancy glucose value and pre-pregnancy obesity in women without recognized pregestational diabetes or GDM, our results are consistent with a study examining the association between *in utero* exposure to GDM and BMI growth trajectory from birth to 13 years of age (65) which found that the overall sex and race-ethnicity adjusted BMI growth trajectory was significantly higher between the ages of 27 months and 13 years among youth exposed to GDM; this difference was primarily driven by an increased BMI growth velocity from 10 to 13 years of age in those exposed to GDM. No differences were observed in infancy or early childhood.

In our analyses of BMI z-score trajectory, among non-obese women, there was a near significant association between increasing pregnancy glucose and higher offspring BMI z-score, on average, across the age range. The effect of *in utero* exposure to increasing glucose levels would likely be easier to detect in the offspring of non-obese women, who are not exposed to excessive fuel substrates over the course of gestation as a result of maternal obesity (98). In obese women, the association between increasing pregnancy glucose and an increased rate of

BMI z-score increase over time almost attained statistical significance. In fetuses already exposed to excessive fuel substrates due to maternal obesity, *in utero* exposure to higher glucose levels would likely compound over nutrition and exacerbate programming for subsequent obesity. Significant associations between pregnancy glucose and BMI z-score velocity may not have been recognized if, similar to the offspring of women with diabetes and GDM, the effects of pregnancy glucose are not detectable until later childhood or puberty. Data from larger pregnancy cohorts with longer follow up would contribute greatly to answering these questions.

The prospective design is a clear strength of the current study and essential for examining the effect of any *in utero* exposure on subsequent childhood obesity. There are several limitations to consider. We lacked data on physical activity during pregnancy and pre-pregnancy weight, as in most studies, was self-reported for some women (101). Physical activity during pregnancy likely influences pregnancy glucose values and later childhood obesity through shared lifestyle characteristics, thus our estimates likely contain some amount of bias due to residual confounding. Pre-pregnancy weight was most likely underreported, thus some non-obese women may have been misclassified. This suggests that our estimates of the association between pre-pregnancy obesity and offspring BMI z-scores may have been underestimated. Unfortunately, the number of women in the current study was insufficient for linear regression analyses to be stratified by pre-pregnancy obesity at each time point over the course of follow-up.

Our findings are biologically plausible. Women with mildly high pregnancy glucose levels who are free of recognized, overt disease may have children with increased body mass as a result of similar mechanisms to those hypothesized for the offspring of women with diabetes and GDM. Increasing levels of maternal glycemia are associated with increasing fetal hyperinsulinemia (57) and neonatal adiposity (102). The third trimester of pregnancy is known to be a critical period for adipose cell hyperplasia (103) and increased maternal glycemia may also result in fetal exposure to increased amounts of lipid substrates during this critical period (104). It has been hypothesized that maternal hyperglycemia and fuel metabolism in pregnant women may have long-term effects on offspring by modifying phenotypic gene expression in terminally differentiated cells during intrauterine development (56). It is therefore possible that similar gene expression modification may also occur in women without overt disease that display only mildly elevated glucose levels.

Our findings lend additional support to the hypothesis of developmental programming in women without recognized pregestational diabetes or GDM. In an at-risk cohort of Mexican descent, we found that exposure to higher levels of plasma glucose during pregnancy was associated with increased offspring adiposity at 7 years of age. In non-obese women, there was the suggestion of an association between higher levels of pregnancy plasma glucose and increased average offspring BMI z-score from 2 to 7 years of age. Therefore, obesity prevention efforts targeting children of Mexican descent may begin *in utero* or prior to pregnancy. Lifestyle interventions aimed at improving maternal pregnancy glucose levels and reducing pregravid obesity could potentially be effective in reducing offspring adiposity in late childhood. As the largest and fastest growing minority group in the U.S., it is of critical public health importance to develop multifaceted strategies to prevent and reduce obesity in children of Mexican descent.

Table 2.1. Cohort characteristics of 266 Mexican-American mother-child pairs from the CHAMACHOS cohort, 1999-2000.

Characteristic	n	%
Pre-pregnancy BMI		
Underweight (<18.5 kg/m^2)	2	0.8
Normal (18.5-24.9 kg/m^2)	105	39.5
Overweight (25.0-29.9 kg/m^2)	107	40.2
Obese (≥30.0 kg/m^2)	52	19.6
Years in the U.S.		
≤ 5 years	140	52.6
> 5 years	126	47.4
Maternal education		
≤ 6th grade	121	45.5
7-12th grade	91	34.2
≥ high school graduate	54	20.3
At or below the poverty line	168	63.2
Parity		
0	88	33.1
1	85	32.0
2	54	20.3
3+	39	14.7
Smoked during pregnancy	16	6.0
Soda consumed prior to the glucose		
Never	105	39.9
1-3 times per month	29	11.0
1-2 times per week	76	28.9
3-6 times per week	18	7.9
Every day	32	12.2
Maternal age at delivery		
18-24 years	121	45.5
25-29 years	90	33.8
30-34 years	34	12.8
35-45 years	21	7.9
Offspring BMI z-score ≥ 95%		
2 years of age (n= 240)	41	17.1
3.5 years of age (n= 213)	72	33.8
5 years of age (n= 213)	75	35.2
7 years of age (n= 217)	82	37.8

	mean	SD
Glucose screening value (mmol/L)	5.9	1.5
Gestational age at screening test	26.4	1.1
Gestational age at soda consumption	26.7	1.9
Gestational weight gained prior to the	11.4	9.5
Gestational age at weight	22.3	5.3
Birth weight (grams)	3,496.7	440.0
Gestational age at delivery (weeks)	39.2	1.2

Table 2.2. Linear regression coefficients, standard errors and p-values for the association between pregnancy glucose and pre-pregnancy obesity (≥ 30 kg/m^2) with offspring BMI z-score at 2 years, 3.5 years, 5 years and 7 years of age.

	Unadjusted	**Adjusted***
2 years	n= 236	n= 232
Glucose β (SE)	0.080 (0.047)	0.080 (0.046)
p-value	0.09	0.09
Pre-pregnancy obesity β (SE)	-	**0.37 (0.18)**
p-value		**0.05**
3.5 years	n= 203	n= 201
Glucose β (SE)	0.050 (0.049)	0.069 (0.051)
p-value	0.30	0.18
Pre-pregnancy obesity β (SE)	-	0.29 (0.20)
p-value		0.16
5 years	n= 206	n= 204
Glucose β (SE)	**0.097 (0.047)**	**0.097 (0.048)**
p-value	**0.04**	**0.05**
Pre-pregnancy obesity β (SE)	-	**0.46 (0.19)**
p-value		**0.02**
7 years	n= 217	n= 214
Glucose β (SE)	**0.13 (0.046)**	**0.12 (0.045)**
p-value	**<0.01**	**<0.01**
Pre-pregnancy obesity β (SE)	**-**	**0.57 (0.17)**
p-value		**<0.01**

*Adjusted for soda consumed prior to the glucose test (times per week, continuous), gestational weight gained prior to the glucose test (lbs, continuous), gestational age at the prenatal weight measurement (continuous), smoking (yes/no), poverty (at/below poverty line vs. above), infant birth weight (continuous), gestational age at birth (continuous) and child's absolute age at the follow-up measurements (continuous).

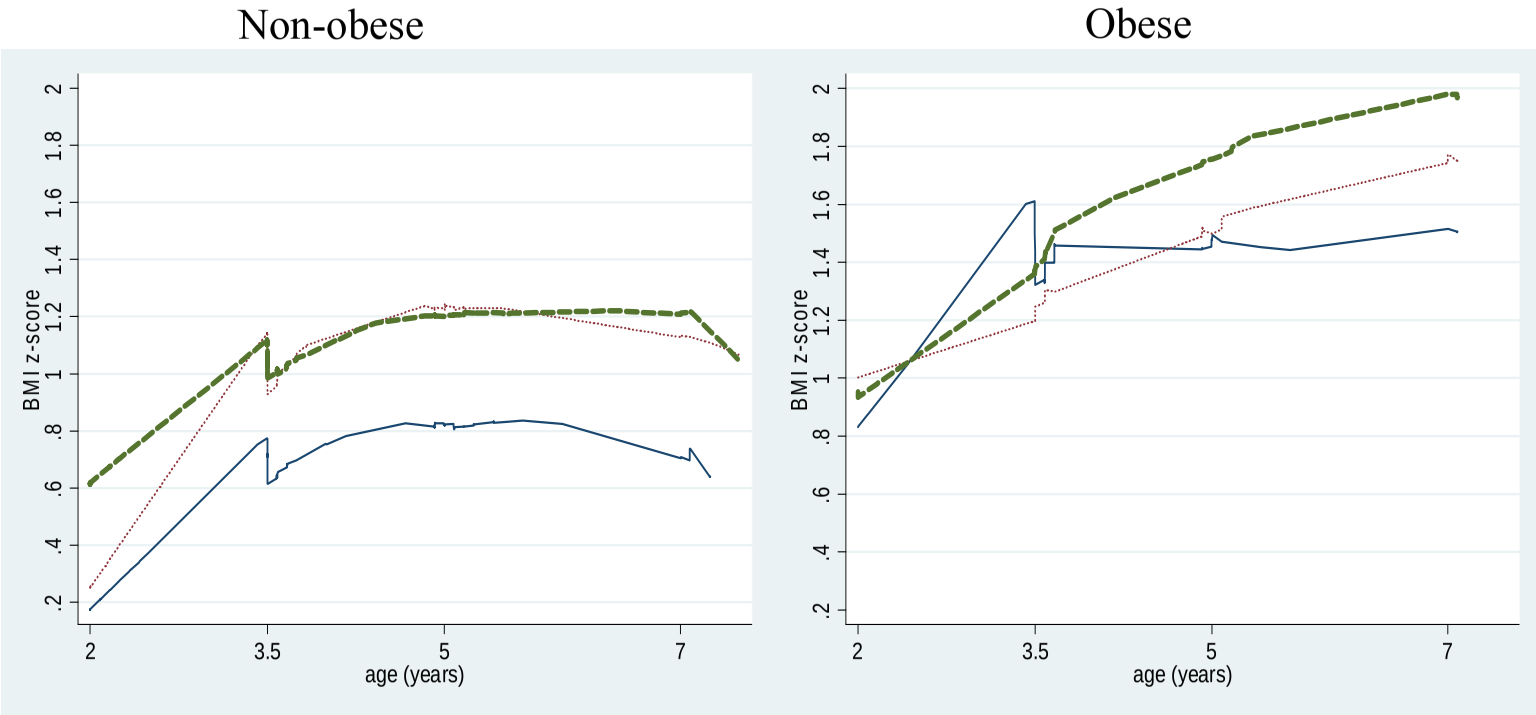
Table 2.3. Inverse weighted linear regression coefficients, standard errors and p-values for the association between pregnancy glucose and pre-pregnancy obesity (≥ 30 kg/m^2) with offspring BMI z-score at 2 years, 3.5 years, 5 years and 7 years of age.

	Unadjusted^	**Adjusted^***
2 years		
Glucose β (SE)	0.082 (0.047)	0.067 (0.046)
p-value	0.08	0.15
Pre-pregnancy obesity β (SE)	-	**0.38 (0.19)**
p-value		**0.04**
3.5 years		
Glucose β (SE)	0.034 (0.049)	0.050 (0.051)
p-value	0.49	0.33
Pre-pregnancy obesity β (SE)	-	0.26 (0.21)
p-value		0.22
5 years		
Glucose β (SE)	0.089 (0.048)	0.089 (0.049)
p-value	0.06	0.07
Pre-pregnancy obesity β (SE)	-	**0.49 (0.19)**
p-value		**0.01**
7 years		
Glucose β (SE)	**0.14 (0.045)**	**0.12 (0.045)**
p-value	**<0.01**	**0.01**
Pre-pregnancy obesity β (SE)	**-**	**0.58 (0.17)**
p-value		**<0.01**

^Linear regression weighted by the inverse probability of remaining in the CHAMACOS cohort for at least one follow-up visit.

*Adjusted for soda consumed prior to the glucose test (times per week, continuous), gestational weight gained prior to the glucose test (lbs, continuous), gestational age at weight measurement (continuous), smoking (yes/no), poverty (at/below poverty line vs. above), infant birth weight (continuous), gestational age at birth (continuous), and child's absolute age at the follow-up measurements (continuous).

Figure 2.1. Smoothed scatter plots of offspring BMI z-score trajectory from 2 to 7 years of age for non-obese (BMI< 30 kg/m^2) and obese (BMI≥ 30 kg/m^2) women, by pregnancy glucose tertile.



Non-obese

Solid line (blue) is lowest tertile of pregnancy glucose: 2.9-4.9 mmol/L; Dotted line (red) is middle tertile of pregnancy glucose: 5.0-6.3 mmol/L; Dashed line (green) is upper tertile of pregnancy glucose: 6.4-10.1 mmol/L

Obese

Solid line (blue) is lowest tertile of pregnancy glucose: 3.3-5.3 mmol/L; Dotted line (red) is middle tertile of pregnancy glucose: 5.4-6.4 mmol/L; Dashed line (green) is upper tertile of pregnancy glucose: 6.8-9.8 mmol/L

Chapter 3: Pregnancy glucose levels in women without diabetes or gestational diabetes and childhood cardiometabolic risk at 7 years of age

3.a. Abstract

Introduction: To estimate the association between pregnancy glucose values in women without recognized pregestational diabetes or gestational diabetes (GDM) and cardiometabolic risk in their children.

Methods: Longitudinal cohort study of 211 Mexican-American mother-child pairs participating in the Center for the Health Assessment of Mothers and Children of Salinas (CHAMACOS) Study. Multiple logistic regression analyses estimated the children's risk of cholesterol, triglycerides, blood pressure and waist circumference $\geq 75^{th}$ percentile at 7 years of age associated with an 18 mg/dl (or 1 mmol/L) increase in maternal pregnancy glucose level, measured 1-hour after a 50-g oral glucose load.

Results: The odds ratios for children belonging to the upper quartile of diastolic blood pressure (DBP), systolic blood pressure (SBP), and waist circumference associated with an 18 mg/dl (or 1 mmol/L) increase in pregnancy glucose level were 1.38 (95% CI 1.09-1.74), 1.38 (95% CI 1.10-1.74) and 1. 25 (95% CI 1.01-1.53), respectively. For SBP, there was also an association with increasing maternal soda consumption (OR= 1.16 [95% CI 1.01 - 1.34]).

Conclusions: We found a significant, positive association between increasing pregnancy glucose values in women without recognized pregestational diabetes or GDM and children's waist circumference, DBP and SBP at 7 years of age.

3.b. Introduction

Similar to other ethnic groups in the United States (U.S.), cardiovascular disease is the leading cause of death among Mexican-Americans (87). Compared to non-Hispanic Whites, Mexican-American adults are at greater risk of cardiovascular and coronary heart disease mortality (88) in addition to several cardiovascular disease risk factors, including metabolic syndrome (88), diabetes (87), and uncontrolled hypertension (105), which are likely related to the high prevalence of obesity in this population (87).

Mexican-American children are more likely to be overweight or obese than non-Hispanic white children and, in some reports, than non-Hispanic black children (42,86). Among Mexican-American children, close to 30% of 2- to 5-year olds and over 42% of 6- to 11-year olds are overweight or obese, with the corresponding prevalence in non-Hispanic whites at 17% and 35%, respectively (42). Although obesity is associated with increased cardiometabolic risk, no previous study has considered the potential origins of cardiometabolic risk in a predominantly Mexican-American population. As the largest and fastest growing immigrant group in the U.S. (46), research is urgently needed to understand the determinants of cardiometabolic risk in this population.

A widely accepted hypothesis is that exposure to abnormal maternal fuel metabolism *in utero*, resulting from maternal diabetes at one end of the spectrum and maternal under-nutrition at the other, programs a fetus for later life morbidity, including obesity, diabetes, hypertension and heart disease (52,56). Several studies have reported a significant increase in cardiometabolic risk factors, including diastolic blood pressure (DBP) (66), systolic blood pressure (SBP) (66-68) and waist circumference (60,68), among youth exposed to maternal diabetes or gestational diabetes *in utero*. There is a paucity of data on the association between *in utero* exposure to levels of maternal glycemia below those diagnostic of disease and childhood cardiometabolic risk. In women free of gestational diabetes, there appears to be a continuous association between increasing maternal glucose levels and the risk of several perinatal complications (57,106), thus it is plausible that increasing pregnancy glucose levels below those diagnostic of disease could also be associated with longer-term adverse outcomes in the offspring.

Among Mexican-American women without recognized pregestational diabetes or gestational diabetes (GDM), we previously reported an association between increasing pregnancy glucose levels and increased BMI z-score in the children at 7 years of age (manuscript under review). The current study examines the association between increasing pregnancy glucose levels in women without recognized pregestational diabetes or GDM and cardiometabolic risk factors in the children at 7 years of age, specifically: waist circumference, diastolic blood pressure, systolic blood pressure and non-fasting cholesterol and triglyceride levels.

3.c. Methods

The mothers and children were participants in the Center for the Health Assessment of Mothers and Children of Salinas (CHAMACOS Study), a longitudinal birth cohort of low-income Mexican-Americans. Pregnant women were eligible for the CHAMACOS study if they sought prenatal care at six health clinics between October 1999 and October 2000, were less than 20 weeks gestation, 18 years of age or older, eligible for state-sponsored health care (Medi-Cal)

and intended to deliver at Natividad Medical Center (a county hospital in Monterey County, CA, USA). A total of 601 women were enrolled and 485 followed until the delivery of a full term (≥ 37 weeks gestation), liveborn singleton.

Non-fasting blood samples were collected from the children at 7 years of age between March 2007 and November 2008. Blood samples were immediately processed, with sera stored at -80° C until shipment on dry ice to the U.S. Centers for Disease Control and Prevention (CDC) (Atlanta, GA), where they were analyzed. Measurement of triglycerides (mg/dl) and cholesterol (mg/dl) in serum were made using standard enzymatic methods (Roche Chemicals, Indianapolis, IN) (107).

Blood pressure measurements (BP; mmHg) were made after the child had been sitting quietly for a minimum of 2 minutes; children were sitting with their arm relaxed either in their lap or on a low table. BP was measured up to 4 times on the same arm using a Dinamap 9300 (Critikon Corp., Tampa, FL), an automatic blood pressure machine that allows inflation pressure to be set at an appropriate level for children. One child had only one BP measurement. Trials were averaged for children with 2 BP measurements (n= 3); we averaged the last two trials for those with 3 BP measurements (n= 174). If any readings were unusually high (for boys: SBP >115 mmHg or DBP >76 mmHg, for girls: SBP >113 mmHg or DBP >75 mmHg), the cuff was removed and the child rested for at least 5 minutes prior to a fourth measurement. For children with 4 measurements available (n= 11), we excluded the first measurement and averaged the 2 trials in which mean arterial pressure (MAP) values were closest to each other.

Waist circumference (cm) was measured with a tape against the skin at the crest of the ileum while the children were standing upright. Measurements were recorded to the nearest 0.1 cm after the child exhaled. Waist circumference was measured in triplicate, with the tape loosened prior to repeating each measurement; we took the mean of the 3 waist circumference trials.

From questionnaires administered to the mother during pregnancy, we obtained data on: smoking status (yes or no), poverty level [above versus at or below the poverty line (94)], and soda consumption. Soda consumption during pregnancy, prior to the screening test was used as an indicator of dietary added sugars and ascertained from the women at the end of the second trimester (mean gestational age= 26.7 weeks, SD= 2.0); women were asked how often they drank a 12-ounce can of Coca Cola or other soft drinks (non-diet) during the last three months and the frequency of consumption was coded in times per week. Pre-pregnancy weight was obtained from several sources, according to the following hierarchy: 1) as recorded in the medical record (n= 189), 2) self-reported on the pregnancy questionnaire (n= 16), 3) from an early prenatal weight measurement (<13 weeks gestational age; n= 2), or 4) calculated by a regression line that utilized all prenatal weight measurements and corresponding gestational ages (n= 4). In the sub-set of women with pre-pregnancy weight data available in the medical record, the pregnancy questionnaire and from an early prenatal weight measurement (n= 108), pre-pregnancy weight from the medical record was significantly and positively correlated with the early prenatal weight (Spearman's rho =0.96, p-value < 0.0001); self-reported pre-pregnancy weight was also significantly and positively correlated with the early prenatal weight (Spearman's rho =0.96, p-value < 0.001). From the medical record, we abstracted gestational weight gained prior to glucose test and gestational age at this pregnancy weight measurement (weeks), as well as infant

birth weight (grams) and gestational age at birth (weeks); maternal height was measured by study staff. The amount of gestational weight gained after the screening and diagnostic tests for GDM could be affected by the test results, therefore, pre-pregnancy weight was subtracted from the nearest prenatal weight measurement taken prior to the glucose test to calculate the amount of weight gained (in pounds) up until the time of the glucose test.

Since there is no standard definition for abnormal cardiometabolic risk factors in children, we classified each cardiometabolic risk factor as increased if it was at or above the upper quartile for that outcome. The 75th percentile was determined from sex- and age-specific, nationally representative waist circumference data for Mexican-American youth (108) and the study cohort sex-specific distributions; for all other cardiometabolic risk factors, the study cohort distributions were used to determine the 75th percentile.

Measurements of pregestational and gestational plasma glucose, as well as diabetes and GDM diagnoses, were abstracted from the medical record by a registered nurse. Of the 485 women delivering full term, liveborn singletons, we excluded 11 with recognized pregestational diabetes; one with unrecognized diabetes (any glucose level >200 mg/dl [11.1 mmol/L] on more than one occasion during pregnancy); five cases of GDM, according to the results of the screening test followed by a diagnostic 100-g, 3-hour oral glucose tolerance test [the diagnosis of GDM was based on the National Diabetes Data Group criteria (64) during the study period]; one woman with an abnormal value on the screening test (200 mg/dl) but no follow up diagnostic test; 23 women with diagnoses of GDM in their medical record who did not meet the diagnostic criteria (since these women likely received treatment for pregnancy hyperglycemia); and 113 woman whose screening tests were not performed within the recommended window of 24 to 28 weeks gestation (64). None of the remaining 331 women met the lower plasma glucose thresholds for GDM of the American Diabetes Association (41). Among these 331 women, 211 of their children had measurements at 7 years of age of non-fasting total cholesterol and triglyceride levels, blood pressure or waist circumference.

Statistical Analyses

We used separate multiple logistic regression models to estimate the children's risk of each cardiometabolic risk factor, defined as ≥75th percentile, associated with an 18 mg/dl (the equivalent of 1 mmol/L) increase in pregnancy glucose, measured 1-hour after a 50-g oral glucose tolerance load. A directed acyclic graph (DAG) (96) guided the selection of adjustment variables. Logistic regression models were adjusted for pre-pregnancy obesity (BMI ≥ 30 kg/m^2), soda consumption (times per week) prior to the glucose test (continuous), gestational weight gained prior to the glucose test (continuous), gestational age at the weight measurement (continuous), smoking during pregnancy, poverty, infant birth weight (continuous) and gestational age at birth (continuous). All models, except for models for waist circumference, included additional adjustment for sex. In the fetal origins of disease literature, including adjustment for current body size in analyses of subsequent hypertension has been debated (69). Thus, we conducted the BP analyses with and without additional adjustment for current waist circumference, BMI, and BMI z-score (93) (in separate models). Since we hypothesized that current body size was on the causal pathway from *in utero* glucose exposure to subsequent

hypertension, we focus on the results of models that excluded any adjustment for current body size.

We assessed potential effect modification in the relationship between pregnancy glucose level and each childhood cardiometabolic outcome by maternal pre-pregnancy obesity, birth weight and child's sex. Cross products were added, one at a time, to fully adjusted models; no cross products were statistically significant (all p-values ≥ 0.10).

To assess whether missing follow-up data for some children resulted in bias, we conducted analyses weighted by the inverse probability that a mother-child pair would remain in the cohort and attend the 7-year follow-up visit. SuperLearner (100), a prediction algorithm, was used to predict whether a pair was assessed at 7 years.

All analyses were conducted in SAS (version 9.1, SAS Institute Inc., Cary, NC); SuperLearner was run in R (version 2.12.1, The R Foundation for Statistical Computing, Vienna, Austria). Study participants provided written informed consent and all research activities were approved by the University of California–Berkeley Committee for the Protection of Human Subjects.

3.d. Results

Characteristics of the 211 mother-child pairs are presented in Table 1. Over three-quarters of the women attained less than a high school education. Half of the women had been in the U.S. for 5 years or less when they became pregnant and 64% were at or below the poverty line. The mean glucose value at the screening test was 107.1 mg/dl (SD 27.0) and mean pre-pregnancy BMI 26.9 kg/m^2 (SD 4.9). The average gestational age at the weight measurement prior to the screening test was 22.1 weeks (SD= 5.5 weeks) and women gained, on average, 10.8 pounds prior to the glucose test (SD= 9.3 lbs). Thirty-eight percent of the children were obese at age 7 years [with BMI z-scores $\geq 95^{th}$ percentile (93)].

The 75th percentiles used to define each cardiometabolic risk factor are displayed in Table 2. The study cohort-specific 75th percentiles for waist circumference were 71.5 cm and 75.3 cm for boys and girls, respectively, thereby exceeding the nationally representative 75th percentiles for 7-year old Mexican-Americans by 8.1 cm and 12.3 cm, respectively; 51% of the boys and 55% of the girls met or exceeded the nationally representative cut points for Mexican-American children.

Odds Ratios and 95% confidence intervals for the association between an 18 mg/dl increase in pregnancy glucose level and the presence of each childhood cardiometabolic risk factor are presented in Table 3. The odds ratios for children belonging to the upper quartile of DBP and SBP associated with an 18 mg/dl increase in pregnancy glucose level were 1.38 (95% CI 1.09-1.74) and 1.38 (95% CI 1.10-1.74), respectively. Using the nationally representative cut point, the odds of children in the upper quartile of waist circumference were 1. 25 (95% CI 1.01-1.53) times higher for those exposed to an 18 mg/dl increase in maternal glucose level; with the cohort-specific cut point, the estimate for waist circumference did not attain statistical significance [1.19 (95% CI 0.95-1.49)].

Adjustment for child's sex, infant birth weight and gestational age at birth; maternal pre-pregnancy obesity, soda consumption prior to the glucose test, gestational weight gained prior to the glucose test, gestational age at weight measurement, smoking, and poverty did not appreciably alter the results of any of the risk estimates (Table 3). In the model for waist circumference defined by the nationally representative cut point, the association between maternal pre-pregnancy obesity and childhood waist circumference attained statistical significance (OR= 3.05 [95% CI 1.39-6.71]); this association was also significant when waist circumference was defined by the cohort-specific cut point (OR= 2.77 [95% CI 1.24 - 6.20]). In the model for SBP, there was a significant association with increasing maternal soda consumption prior to the glucose test (OR= 1.16 [95% CI 1.01 - 1.34]).

Odds ratio estimates for the associations between maternal glucose levels and childhood DBP and SBP were almost identical in models that included additional adjustment for waist circumference, BMI or BMI z-score at 7 years of age (Table 4).

Estimates obtained from the inverse probability weighted analyses were similar to those presented in Tables 2 (data not shown).

3.e. Discussion

In this cohort of Mexican-American women without recognized pregestational diabetes or GDM, increasing pregnancy glucose values, assessed during a 50-g 1-hour oral glucose challenge test, were significantly associated with having a child in the upper quartile of DBP, SBP and waist circumference at 7 years of age. Pregnancy glucose values were predictive of DBP, SBP, and waist circumference independently of pre-pregnancy obesity, as well as soda consumption and the amount of gestational weight gained prior to the glucose test, suggesting that *in utero* exposure to increasing maternal glucose levels has long-term effects, even in the absence of overt disease. To our knowledge, this is also the first study to report a significant association between maternal prenatal soda consumption and having a child in the upper quartile of SBP at 7 years of age. No associations were found for total cholesterol and triglycerides measured in non-fasting blood samples. Nevertheless, our results provide additional support for the developmental origins of cardiometabolic risk, specifically, that maternal glucose levels during pregnancy within the normal range are related to central obesity and elevated blood pressure in the children at 7 years of age.

Our results are consistent with the findings of previous studies among women with diabetes and GDM (60,66,67). A retrospective cohort study of mother-child pairs belonging to an HMO in Colorado reported that youth, 6 to 13 years of age, exposed to maternal GDM *in utero* had a significantly larger waist circumference than their unexposed peers (60). Analyses stratified by Hispanic ethnicity were also presented: Hispanic youth exposed to maternal GDM *in utero* had a significantly higher waist circumference (7.1 cm) as compared to Hispanic youth that were not exposed to GDM, yet the comparable estimate among non-Hispanic white youth (3.4 cm) did not attain statistical significance. A prospective cohort study in China compared youth 7 to 10 years of age who had been exposed to either maternal gestational impaired glucose tolerance (defined as fasting plasma glucose <7.0 mmol/L and 2 hour plasma glucose level ≥7.8-

11.1 mmol/L) or GDM *in utero* to youth whose mothers had normal glucose tolerance during pregnancy (66); youth exposed to maternal GDM or IGT *in utero* had significantly higher SBP and DBP. Previous studies have also found significant associations between maternal pregnancy hyperglycemia and lower levels of high-density lipoprotein cholesterol in the children, but not total cholesterol or triglycerides (66,67). Given that our results for total cholesterol and triglycerides are consistent with these findings, we speculate that the lack of association in the current study was not due to the use of non-fasting blood samples.

The developmental origins of disease hypothesis stems largely from associations between low birth weight and chronic diseases in adulthood, including hypertension (52), yet the excess availability of nutrients, as seen in women with diabetes or GDM, have also been proposed to exert long-term effects on offspring (56). Increased pregnancy glucose levels result in larger, not smaller, size at birth (57), yet in the present study, were related to increasing BP in the children. In Pima Indians over 10 years of age, a U-shaped relationship between birth weight and increasing glucose levels has been reported (109), suggesting that fetal programming for increased cardiometabolic risk may also pertain to the upper end of the birth weight spectrum.

Increasing levels of maternal glycemia are associated with increased neonatal adiposity (102) and this disproportionately high fat mass relative to lean body mass is likely to persist, as fetal development is the critical period for muscle growth (110). The unfavorable body composition that accompanies exposure to increased glucose levels *in utero* likely leads to the increased cardiometabolic risk observed in this and other studies (60,66,67). The increased growth velocity observed among children exposed to increasing pregnancy glucose levels *in utero* (65) could also partially explain observations of higher BP in childhood. The U.S. Collaborative Perinatal Project reported that children who had crossed into higher weight percentiles throughout their childhood growth displayed significantly higher BP at 7 years of age (111). Therefore, developmental programming for increased childhood fat mass and growth velocity could potentially lie on the causal pathways connecting pregnancy glucose levels to increased blood pressure in late childhood.

Given the high prevalence of cardiovascular disease and cardiovascular disease mortality in Mexican-American adults (87,88), our investigation of this high-risk cohort of children has important public health implications. Previous studies demonstrate that high BP in childhood tracks across the lifespan (112), thereby suggesting an increased risk of future cardiovascular disease among children exposed to mildly high glucose levels *in utero*. We previously reported a significant association between increasing pregnancy glucose levels in women without recognized pregestational diabetes or GDM and increased overall adiposity in the children at 7 years age (manuscript under review). The current analysis further demonstrates that the increased adiposity stemming from *in utero* exposure to increasing maternal glucose values is centralized in the abdominal region; abdominal obesity has been associated with several components of the metabolic syndrome in children and waist circumference has been proposed as a tool for identifying children at risk of developing metabolic and cardiovascular complications (113).

The prospective design is a clear strength of the current study and essential for examining the effect of any *in utero* exposure on subsequent childhood obesity. The amount of bias incurred from the use of self-reported pre-pregnancy weight in a few women also appears to be minimal.

Yet there are several limitations to consider. We lacked data on physical activity during pregnancy. Physical activity during pregnancy likely influences pregnancy glucose values and later childhood obesity through shared lifestyle characteristics; thus our estimates likely contain some amount of bias due to residual confounding. Triglyceride and cholesterol levels were measured in non-fasting blood samples, which may have masked a significant association, but previous studies of pregnancy glycemia and children's total cholesterol and triglyceride levels also did not find significant associations (66,67).

In this cohort of Mexican-American women without recognized pregestational diabetes or GDM, we found a significant, positive association between increasing pregnancy glucose values and increased DBP, SBP, and waist circumference at 7 years of age. Our results suggest that maternal glucose levels below those diagnostic of overt disease may result in increased cardiometabolic risk that is detectable in late childhood and lend additional support to the hypothesis of the developmental origins of disease. Our data also demonstrate that the children in this low-income cohort have greater central adiposity compared to a nationally representative cohort of their Mexican-American peers, especially the girls, and are therefore at increased risk of developing metabolic and cardiovascular complications. These findings suggest the need for lifestyle interventions promoting healthy diet and physical activity to improve maternal pregnancy glucose levels, as well as obesity and elevated blood pressure in late childhood, and prevent the future development of cardiometabolic disease.

Table 3.1. Cohort characteristics of 211 Mexican-American mother-child pairs from the CHAMACHOS cohort, 1999-2000.

Characteristic	n	%
Pre-pregnancy BMI		
Underweight (<18.5 kg/m^2)	2	1.0
Normal (18.5-24.9 kg/m^2)	81	38.4
Overweight (25.0-29.9 kg/m^2)	85	40.3
Obese (≥30.0 kg/m^2)	43	20.4
Years in the U.S.		
≤ 5 years	109	51.7
> 5 years	102	48.3
Maternal education		
≤ 6th grade	97	46.0
7-12th grade	68	32.2
≥ high school graduate	46	21.8
At or below the poverty line	135	64.0
Parity		
0	67	31.8
1	67	31.8
2	50	23.7
3+	27	12.8
Smoked during pregnancy	8	3.8
Soda consumed prior to the glucose test (n= 208)		
Never	80	38.5
1-3 times per month	23	11.1
1-2 times per week	64	30.8
3-4 times per week	14	6.7
5-6 times per week	3	1.4
Every day	24	11.5
Maternal age at delivery		
18-24 years	94	44.6
25-29 years	77	36.5
30-34 years	24	11.4
35-45 years	16	7.6
Offspring BMI z-score ≥ 95%* (n= 210)	79	37.6
Offspring sex		
Boy	99	46.9
Girl	112	53.1

	mean	SD
Glucose screening value (mg/dl)	107.1	27.0
Gestational age at screening test (weeks)	26.3	1.1
Gestational age at soda consumption assessment (n= 201;	26.7	2.0
Gestational weight gained prior to the glucose test (lbs)	10.8	9.3
Gestational age at weight measurement (weeks)	22.1	5.5
Birth weight (grams)	3,495.0	440.7
Gestational age at delivery (weeks)	39.2	1.2

* BMI z-score calculated from sex-specific, BMI-for-age cut points issued by the CDC (National Center for Health Statistics. CDC growth charts. 2005. United States.)

Table 3.2. Cut points for meeting or exceeding the 75th percentile of each cardiometabolic risk factor.

Cardiometabolic risk factor	Cut Point (75th Percentile)	n	n meeting criteria
Total non-fasting cholesterol[a]	≥186 mg/dl	174	44
Non-fasting triglycerides[a]	≥ 160 mg/dl	174	44
Waist Circumference[a]			
Boys	≥ 71.5 cm	99	24
Girls	≥ 75.3 cm	112	28
Waist Circumference[b]			
Boys	≥ 63.4 cm	98	50
Girls	≥ 63.0 cm	112	62
Diastolic blood pressure[a]	≥ 56.5 mmHg	211	48
Systolic blood pressure[a]	≥ 101 mmHg	211	56

[a] Based on the study cohort distribution

[b] Based on a nationally representative sample of Mexican-American children, age and sex specific

Table 3.3. Odds Ratios (95% Confident Intervals) for each offspring cardio-metabolic risk factor meeting or exceeding the 75th percentile at 7 years of age associated with an 18 mg/dl (1 mmol/L) increase in maternal pregnancy glucose level, CHAMACOS study, 1999-2008.

Cardio-metabolic risk factor ≥ 75th percentile	Unadjusted OR (95% Confidence Interval)	Adjusted° OR (95% Confidence Interval)
Total cholesterol	1.06 (0.85 - 1.33)	1.13 (0.88 – 1.45)
Triglycerides	1.16 (0.93 - 1.45)	1.15 (0.90 – 1.47)
Waist Circumference[a]	1.17 (0.95-1.44)	1.19 (0.95-1.49)
Waist Circumference[b]	**1.25 (1.04 - 1.52)**	**1.25 (1.01 - 1.53)**
Diastolic blood pressure	**1.29 (1.04 - 1.60)**	**1.38 (1.09 - 1.74)**
Systolic blood pressure	**1.31 (1.07 - 1.61)**	**1.38 (1.10 - 1.74)**

° Adjusted for child's sex, infant birth weight (continuous) and gestational age at birth (continuous); maternal pre-pregnancy obesity (BMI ≥ 30 kg/m^2), soda consumption during pregnancy (times per week, continuous), gestational weight gained prior to the glucose test (lbs, continuous), gestational age at weight measurement (continuous), smoking (yes/no), and poverty (at/below poverty line vs. above).

[a] Based on the study cohort sex-specific distributions; child's sex dropped from the adjusted model

[b] Based on a nationally representative sample of Mexican-American children, age- and sex-specific; child's sex dropped from the adjusted model

Table 3.4. Fully adjusted Odds Ratios (95% Confident Intervals) for diastolic and systolic blood pressure meeting or exceeding the 75th percentile at 7 years of age associated with an 18 mg/dl (1 mmol/L) increase in maternal pregnancy glucose level with additional adjustment for measures of current body size, CHAMACOS study, 1999-2008.

Measures of current body size	Diastolic blood pressure ≥ 75th percentile	Systolic blood pressure ≥ 75th percentile
Waist circumference	1.37 (1.08-1.74)	1.37 (1.07-1.77)
BMI	1.36 (1.08-1.73)	1.33 (1.04-1.71)
BMI z-score	1.35 (1.06-1.71)	1.30 (1.01-1.66)

° Adjusted for child's sex, infant birth weight (continuous) and gestational age at birth (continuous); maternal pre-pregnancy obesity (BMI ≥ 30 kg/m^2), soda consumption during pregnancy (times per week, continuous), gestational weight gained prior to the glucose test (lbs, continuous), gestational age at weight measurement (continuous), smoking (yes/no), and poverty (at/below poverty line vs. above).

CONCLUSION

The goal of this dissertation was to investigate the effects of periconceptual and prenatal maternal glucose levels on immediate and longer-term offspring outcomes: offspring sex, as well as childhood obesity and cardiometabolic risk. Over half of all reproductive-aged women in the U.S. are overweight or obese (1). As a consequence of the high prevalence of overweight and obesity among reproductive-aged women in the U.S., a greater proportion of pregnant women are experiencing T2DM, GDM and mildly elevated pregnancy glucose levels than ever before. The second half of pregnancy is characterized by progressive insulin resistance, hyperinsulinemia and mild postprandial hyperglycemia, even among women entering pregnancy free of recognized pregravid diabetes that do not go on to develop GDM (13). Women with pregravid metabolic abnormalities, including overweight and obesity, require the hypersecretion of insulin to compensate for pregnancy-induced insulin resistance and are therefore more likely to develop GDM or mildly elevated glucose levels during pregnancy. Although it is widely acknowledged that overweight and obese women (2) and women with GDM (8,9) and pregravid diabetes (17) are at increased risk for adverse perinatal outcomes, questions remain as to the immediate and longer-term effects on the children, particularly in women with only mildly elevated pregnancy glucose levels. Thus, this dissertation aimed to inform clinicians and public health practitioners about whether mildly elevated pregnancy glucose levels may necessitate intervention.

I. Chapter 1: An Immediate Effect of Periconceptual Glucose Levels: the Secondary Sex Ratio

Significant deviations to the human secondary sex ratio, the ratio of males to females at birth, have been reported in times of extreme stress experienced on a population level, such as times of war (21) and natural (22) and human-made disasters (23,24). Several hypotheses attempting to explain the mechanisms responsible for these deviations have been proposed (26,28,72), yet evolutionary advantage is largely recognized as the impetus (71). Males have lower reproductive success than females, largely because they are less likely to reach reproductive age (29); thus, as outlined by Trivers and Willard (71), natural selection would favor a reproductive strategy biased towards females under adverse circumstances in order to maximize the number of surviving grandchildren.

The current obesogenic environment of the U.S. is mismatched to the circumstances that have molded much of human evolution (114). In an environment of constant caloric abundance, the ability to store fat in anticipation of future scarcity is no longer advantageous and the conditions comprising ‘adverse circumstances’ have been turned upside down in a relatively short period of time. Changes to the maternal metabolic condition have outpaced human evolution, and in conjunction with medical advances, resulted in increases in the prevalence of maternal diabetes and GDM. Increases in the prevalence of these conditions allows one to address the following question: do maternal metabolic abnormalities around the time of conception, as defined by maternal diabetes and GDM, result in sex ratio deviations consistent with the reproductive strategy outlined by Trivers and Willard (71)? From an evolutionary standpoint, varying degrees of maternal glycemia represent adaptations to a spectrum of environmental conditions, with maternal diabetes representing maladaptation to the environment and GDM resulting from an environment of excessive fuel substrates.

Despite the large cohort investigated in chapter 1, the odds of delivering a liveborn male singleton across several categories of maternal glycemia did not vary significantly, even after adjustment for maternal race-ethnicity, education, and age. Yet the crude sex ratios suggested a possible gradient by category of maternal glycemia: women with diabetes delivered the fewest males (ratio of males/females= 1.01), followed by women with normoglycemic pregnancies and those with an abnormal screening values only (or mild pregnancy hyperglycemia; both ratios= 1.05), and women with a gestational diabetes delivering more males than any other group (ratio= 1.07). Women with abnormal screening values only did not appear to differ from women with normoglycemic pregnancies. Given inherent limitations in the sensitivity and specificity of the prenatal screening test for GDM [79% and 87%, respectively (115)] and the general stability of the secondary sex ratio, the similarity observed between normoglycemic women and those with only an abnormal screening value was not surprising.

According to the Trivers and Willard (27) hypothesis, as compared to normoglycemic women, women with overt pregravid diabetes should have more girls and women with GDM more boys. Chronic, overt diabetes is characterized by insufficient metabolic regulation due to either insulin deficiency, as in type 1 diabetes mellitus, or increased insulin resistance, as in type 2 diabetes mellitus. The resulting state of pathologic hyperglycemia in both types is associated with oxidative and metabolic stress. To maximize future reproductive success under such unfavorable conditions, female gestations would be favored through a raising of the rank threshold below which a woman would spontaneously terminate pregnancy (28). GDM, on the other hand, is a condition associated with increased metabolic substrates in the periconceptual period (11,12). As compared to normoglycemic women, those who develop GDM are likely to have experienced higher glycemic levels in the periconceptual period due to their predisposition for insulin resistance. The maternal state of abundant fuel is not high enough to constitute overt disease, but is likely to signal a higher probability of survival for all conceptions. Under such conditions of abundance, the rank threshold below which a woman would spontaneously terminate a pregnancy would be lowered and, as compared to normoglycemic women, more male fetuses would be observed.

To the best of my knowledge, this is the first study to examine the secondary sex ratio across a range of maternal glycemic categories, yet there are several limitations to be noted. Maternal periconceptual glycemic category, the exposure of interest, was based largely on laboratory tests or medical diagnoses occurring outside of the periconceptual period and served as proxies for maternal periconceptual glycemic status. Women who develop GDM are more likely to have experienced higher glycemic levels around the time of conception due to their predisposition for insulin resistance prior to pregnancy (11,12), but these findings are from studies examining pregravid insulin resistance or glucose intolerance, thus measurements did not occur in the periconceptual period per se. Women with chronic, overt diabetes were members of a health maintenance organization, who, for the most part, received treatment for their disease. Women, who were aware of their disease status and trying to get pregnant, were likely counseled by their providers on the importance of good glycemic control. Assuming these women followed their providers' advice, the sex ratio deviation observed in this group could have been diminished. Data on the quality of glycemic control during the periconceptual period in women with overt diabetes, as well as disease duration and severity, could address several questions raised by these findings. Yet routine screening for diabetes is not usual care for young,

reproductive aged women, making such data difficult to come by, particularly for those who were not diagnosed with diabetes until the postpartum period. Approximately half of all women with GDM are screened for diabetes in the postpartum period (40), thus some women with overt diabetes were likely to have been misclassified as having GDM, suggesting that the difference in sex ratio between these two groups may actually be larger than observed in the current study. Lastly, although this cohort contained over 250,000 mother-infant pairs, data from even larger cohorts of women are needed to confirm or refute these findings.

II. Chapters 2 & 3: Longer-term Effects of Maternal Glucose Levels during Pregnancy, the Developmental Origins of Childhood Obesity and Cardiometabolic Risk

In light of the obesity epidemic in the U.S., more women of reproductive-age are likely to enter pregnancy with recognized pregravid diabetes, be subsequently diagnosed with GDM or experience mildly elevated pregnancy glucose levels. A growing body of research also suggests that the intrauterine environment may influence later development and morbidity (91). Exposure to maternal diabetes *in utero* has been associated with childhood obesity (61) and an adverse offspring cardiometabolic risk profile (67,116). Studies examining *in utero* exposure to GDM have reported significant associations with childhood obesity in studies of primarily non-Hispanic white (58,59) or multiethnic populations (60). However, it is less clear whether elevated pregnancy glucose values that are below the diagnostic levels for GDM have an effect on offspring obesity and cardiometabolic risk. One study previously reported an increasing trend in offspring weight-for-age across increasing quartiles of maternal glucose in women without recognized pregestational diabetes or GDM (58). Youth exposed to maternal GDM or IGT *in utero* (i.e. those with and without overt disease combined into a single exposed group) also had significantly higher SBP and DBP then their unexposed counterparts. In general, there is a paucity of data on the association between increasing pregnancy glucose levels in women without recognized diabetes or GDM and programming for childhood obesity and cardiometabolic risk.

In a population of Mexican-American women without recognized diabetes or GDM, we found a significant association between increasing pregnancy plasma glucose values, assessed during a single 50-g oral glucose challenge test in mid-pregnancy, and increasing offspring BMI z-score at 7 years of age. Increasing pregnancy glucose was also nearly associated with a higher average BMI z-score from ages 2 to 7 years in the children of non-obese women, although the estimate only achieved borderline significance. These findings suggest that in women without recognized pregestational diabetes or GDM, *in utero* exposure to increasing glucose levels is associated with obesity at age 7 and that the offspring of non-obese women exposed to higher glucose levels may demonstrate increased BMI z-score, on average, from 2 to 7 years of age.

Increasing pregnancy glucose values, assessed during a 50-g 1-hour oral glucose challenge test, were also significantly associated with having a child in the upper quartile of diastolic blood pressure, systolic blood pressure and waist circumference at 7 years of age. Pregnancy glucose values were predictive of diastolic blood pressure, systolic blood pressure, and waist circumference independently of pre-pregnancy obesity, soda consumption and the amount of gestational weight gained prior to the glucose test, demonstrating that *in utero* exposure to increasing maternal glucose levels have long term offspring effects in the absence of

overt maternal disease. These two studies fill important gaps in the literature on developmental origins of childhood obesity and increased blood pressure, a comorbidity of obesity.

Women with mildly high pregnancy glucose levels who are free of recognized, overt disease may have children with increased body mass as a result of similar mechanisms to those hypothesized for the offspring of women with diabetes and GDM. Increasing levels of maternal glycemia are associated with increasing fetal hyperinsulinemia (57) and neonatal adiposity (102). The third trimester of pregnancy is known to be a critical period for adipose cell hyperplasia (103) and increased maternal glycemia may result in fetal exposure to increased amounts of lipid substrates during this critical period (104). It has been hypothesized that maternal hyperglycemia and fuel metabolism in pregnant women may have long-term effects on offspring by modifying phenotypic gene expression in terminally differentiated cells during intrauterine development (56). It is therefore possible that similar gene expression modification may also occur in women without overt disease that display only mildly high glucose levels. In women free of recognized pregestational diabetes and GDM, there appears to be a continuous association between increasing maternal glucose levels and the risk of several infant complications (57,78,106), furthering the plausibility of an association between increasing pregnancy glucose levels below those diagnostic of disease and longer-term adverse outcomes in the children.

The increased neonatal adiposity (102) associated with increasing levels of maternal glycemia results in a disproportionately high amount of fat relative to lean body mass, which is likely to persist since fetal development is the critical period for muscle growth (110). The unfavorable body composition that accompanies exposure to increased glucose levels *in utero* likely leads to the increased cardiometabolic risk, including higher diastolic and systolic blood pressure and greater waist circumference, observed in this and other studies (60,66,67). The increased growth velocity observed among children exposed to increasing pregnancy glucose levels *in utero* (65) may also partially explain observations of higher blood pressure in childhood. The U.S. Collaborative Perinatal Project reported that children who had crossed into higher weight percentiles throughout their childhood growth displayed significantly higher blood pressure at 7 years of age (111). Therefore, developmental programming for increased childhood fat mass and growth velocity could potentially lie on the causal pathways connecting pregnancy glucose levels to increased blood pressure in late childhood.

The developmental origins of disease hypothesis stems largely from associations between low birth weight and chronic diseases in adulthood, including hypertension (52), yet the excess availability of nutrients, as seen in women with recognized pregestational diabetes or GDM, have also been proposed to exert long-term effects on offspring (56). Increasing pregnancy glucose levels result in a larger, not smaller size, at birth (57), yet in the chapter 3, were related to increasing BP in the children. In Pima Indians over 10 years of age, a U-shaped relationship between birthweight and increasing glucose levels has been reported (109), suggesting that fetal programming for increased cardiometabolic risk may also pertain to the upper end of the birth weight spectrum.

As in all studies of the developmental origins of disease, direct measures of the content and quantity of the fetal nutrient supply were not available, necessitating the use of proxy measures. Maternal diet, although challenging to assess in epidemiological studies, is more amenable to measurement than fetal nutrition, yet the quality and quantity of nutrients that are

directly available to the fetus likely play a larger role in development programming. In much of Barker's work, measurements made at birth, including infant birthweight, length, body proportions and placental weight (53), have been used to estimate the quality of the fetal nutrient supply. Freinkel's hypothesis (56) was centered on alterations to maternal fuel economy, in the form of maternal diabetes, influencing the fetal nutrient supply. Gaps in knowledge surrounding the form and function of the placenta, a key organ in the fetal supply line, necessitate the continued use of such proxy measures in this field (53), with much of what we currently know obtained from studies on sheep, cows and rats.

The placenta plays many important roles in regulating the fetal nutrient supply, the most obvious being the transport of nutrients from maternal to fetal circulation. This transfer capacity is dependent on the placental surface area and the availability of nutrient transporters on placental cell membranes (53). In light of evidence that the availability of placental nutrient transporters may be affected by the maternal nutritional environment (117,118), prenatal measurements of the maternal nutritional environment, particularly glucose levels, may serve as a better proxy for the fetal nutrient supply than infant measurements made at birth. The placenta also affects fetal nutrition through its role in the metabolism and synthesis of key nutrients and competes directly with the fetus for available nutrients (53). Lastly, the placenta produces a variety of hormones that influence both the fetal and maternal nutrient supplies (53). In addition to residual confounding due to our inability to assess placental function, data on physical activity during pregnancy was missing; pregnancy physical activity influences pregnancy glucose values and later childhood obesity and cardiometabolic risk through shared lifestyle characteristics. Despite these limitations, these analyses make important contributions to the literature on the developmental origins of disease by demonstrating an association in women free of overt, recognized disease during pregnancy.

The implications of these findings are great; the results suggest the need for lifestyle interventions promoting healthy diet and physical activity to improve maternal pregnancy glucose levels, as well as pregravid obesity, in all women in order to prevent development programming for increased body mass, central adiposity and blood pressure in late childhood. Currently, only women with recognized pregravid diabetes or those with GDM receive treatment for hyperglycemia during pregnancy. For the majority of women with GDM, treatment in the form of counseling on healthy diet and physical activity is enough to reduce serious perinatal morbidity (119). The success of these interventions is likely related to the life stage at which they are initiated. Women are concerned about the health of their babies during pregnancy; their frequent contact with the healthcare system during this period also makes pregnancy an especially powerful "teachable moment" for the promotion of healthy eating and physical activity, even among women free of overt disease (120). There is some evidence that lifestyle intervention during pregnancy has the potential to prevent excessive pregnancy weight gain, thereby lowering postpartum weight retention and reducing the future risk of obesity (120). Such interventions leave women better prepared for a subsequent pregnancy, but the findings of chapters 2 and 3, particularly the significant associations found with maternal obesity, reveal the need for pregravid obesity prevention efforts. Pregravid lifestyle interventions promoting healthy diet and physical activity would be more appropriate for reproductive-aged women than pharmacological weight loss interventions, which have the potential to adversely affect a developing fetus. In the research setting, such lifestyle interventions have been found to

effectively promote modest weight loss among overweight and obese adults, but there is some question as to the applicability of these findings in a clinical setting, particularly since loss to follow up is a common problem (121). Entering pregnancy at a healthier BMI is likely to reduce levels of pregnancy glycemia in women free of overt disease, but it also remains to be seen whether lifestyle interventions that are initiated late in pregnancy, after the identification of women with mildly elevated levels of glycemia, have any impact on programming for increased body mass, central adiposity and blood pressure in late childhood and if so, at what glucose thresholds such interventions should be initiated.

III. Concluding Remarks

The findings presented in this dissertation suggest that periconceptual and prenatal maternal glucose levels affect immediate and longer-term offspring health. A gradient in the crude secondary sex ratio by category of maternal glycemia was revealed, as well as a significant association between pregnancy glucose levels in women without recognized pregravid diabetes or GDM and increased BMI z-score in their children at 7 years of age. Also in women without recognized pregravid diabetes or GDM, a significant association was identified between pregnancy glucose levels and increased cardiometabolic risk in the children, specifically increased blood pressure and waist circumference. The results of the logistic regression analyses presented in chapter 1 were not statistically significant, and the outcome, although of interest from an evolutionary standpoint, are not an immediate public health concern. Yet the findings of chapters 2 and 3 have clear public health implications; they demonstrate the need for lifestyle interventions promoting healthy diet and physical activity to improve maternal pregnancy glucose levels and reduce pregravid obesity in order to prevent development programming for increased body mass, central adiposity and blood pressure in late childhood. Further epidemiological research is needed to identify additional adverse offspring outcomes associated with maternal prenatal glucose levels, as well as psychosocial and behavioral research for the development and successful implementation of lifestyle interventions for healthy diet and physical activity in pregnant and reproductive-aged women.

Reference List

1. Flegal KM, Carroll MD, Ogden CL, Curtin LR: Prevalence and trends in obesity among US adults, 1999-2008. *JAMA* 303:235-241, 2010

2. Siega-Riz AM, Siega-Riz AM, Laraia B: The implications of maternal overweight and obesity on the course of pregnancy and birth outcomes. *Matern Child Health J* 10:S153-S156, 2006

3. Solomon CG, Willett WC, Carey VJ, Rich-Edwards J, Hunter DJ, Colditz GA, Stampfer MJ, Speizer FE, Spiegelman D, Manson JE: A prospective study of pregravid determinants of gestational diabetes mellitus. *JAMA* 278:1078-1083, 1997

4. Hu FB, Manson JE, Stampfer MJ, Colditz G, Liu S, Solomon CG, Willett WC: Diet, lifestyle, and the risk of type 2 diabetes mellitus in women. *N Engl J Med* 345:790-797, 2001

5. American Diabetes Association: Gestational Diabetes Mellitus. *Diabetes Care* 23:S77-S79, 2001

6. Ferrara A, Kahn HS, Quesenberry C, Riley C, Hedderson MM: An increase in the incidence of gestational diabetes mellitus: Northern California, 1991-2000. *Obstet Gynecol* 103:526-533, 2004

7. Ferrara A: Increasing prevalence of gestational diabetes mellitus: a public health perspective. *Diabetes Care* 30 Suppl 2:S141-S146, 2007

8. Kjos SL, Buchanan TA: Gestational diabetes mellitus. *N Engl J Med* 341:1749-1756, 1999

9. Jovanovic L, Pettitt DJ: Gestational diabetes mellitus. *JAMA* 286:2516-2518, 2001

10. O'Sullivan JB, Mahan CM: Criteria for the oral glucose tolerance test in pregnancy. *Diabetes* 13:278-285, 1964

11. Gunderson EP, Quesenberry CP, Jr., Jacobs DR, Jr., Feng J, Lewis CE, Sidney S: Longitudinal Study of Prepregnancy Cardiometabolic Risk Factors and Subsequent Risk of Gestational Diabetes Mellitus: The CARDIA Study. *Am J Epidemiol* 172:1131-1143, 2010

12. Hedderson MM, Darbinian JA, Quesenberry CP, Ferrara A: Pregravid cardiometabolic risk profile and risk for gestational diabetes mellitus. *Am J Obstet Gynecol* 205:e1-e7, 2011

13. Buchanan TA, Xiang AH: Gestational diabetes mellitus. *J Clin Invest* 115:485-491, 2005

14. Wild S, Roglic G, Green A, Sicree R, King H: Global prevalence of diabetes: estimates for the year 2000 and projections for 2030. *Diabetes Care* 27:1047-1053, 2004

15. Yach D, Stuckler D, Brownell KD: Epidemiologic and economic consequences of the global epidemics of obesity and diabetes. *Nat Med* 12:62-66, 2006

16. King H, Aubert RE, Herman WH: Global burden of diabetes, 1995-2025: prevalence, numerical estimates, and projections. *Diabetes Care* 21:1414-1431, 1998

17. Kitzmiller JL, Block JM, Brown FM, Catalano PM, Conway DL, Coustan DR, Gunderson EP, Herman WH, Hoffman LD, Inturrisi M, Jovanovic LB, Kjos SI, Knopp RH, Montoro MN, Ogata ES, Paramsothy P, Reader DM, Rosenn BM, Thomas AM, Kirkman MS: Managing preexisting diabetes for pregnancy: summary of evidence and consensus recommendations for care. *Diabetes Care* 31:1060-1079, 2008

18. James WH: Secular movements in sex ratios of adults and of births in populations during the past half-century. *Hum Reprod* 15:1178-1183, 2000

19. Boklage CE: The epigenetic environment: secondary sex ratio depends on differential survival in embryogenesis. *Hum Reprod* 20:583-587, 2005

20. James WH: The human sex ratio. Part 1: A review of the literature. *Hum Biol* 59:721-752, 1987

21. Zorn B, Sucur V, Stare J, Meden-Vrtovec H: Decline in sex ratio at birth after 10-day war in Slovenia: brief communication. *Hum Reprod* 17:3173-3177, 2002

22. Fukuda M, Fukuda K, Shimizu T, Moller H: Decline in sex ratio at birth after Kobe earthquake. *Hum Reprod* 13:2321-2322, 1998

23. Catalano RA: Sex ratios in the two Germanies: a test of the economic stress hypothesis. *Hum Reprod* 18:1972-1975, 2003

24. Catalano R, Bruckner T, Gould J, Eskenazi B, Anderson E: Sex ratios in California following the terrorist attacks of September 11, 2001. *Hum Reprod* 20:1221-1227, 2005

25. Boklage CE: Survival probability of human conceptions from fertilization to term. *Int J Fertil* 35:75, 79-75, 94, 1990

26. Catalano RA, Saxton K, Bruckner T, Goldman S, Anderson E: A sex-specific test of selection in utero. *J Theor Biol* 257:475-479, 2009

27. Trivers RL, Willard DE: Natural selection of parental ability to vary the sex ratio of offspring. *Science* 179:90-92, 1973

28. Catalano R, Bruckner T: Secondary sex ratios and male lifespan: damaged or culled cohorts. *Proc Natl Acad Sci U S A* 103:1639-1643, 2006

29. Wells JC: Natural selection and sex differences in morbidity and mortality in early life. *J Theor Biol* 202:65-76, 2000

30. Rjasanowski I, Kloting I, Kovacs P: Altered sex ratio in offspring of mothers with insulin-dependent diabetes mellitus. *Lancet* 351:497-498, 1998

31. Sheiner E, Levy A, Katz M, Hershkovitz R, Leron E, Mazor M: Gender does matter in perinatal medicine. *Fetal Diagn Ther* 19:366-369, 2004

32. Di Renzo GC, Rosati A, Sarti RD, Cruciani L, Cutuli AM: Does fetal sex affect pregnancy outcome? *Gend Med* 4:19-30, 2007

33. James WH: Sex ratio of offspring of diabetics. *Lancet* 351:1514, 1998

34. Moller H, Jacobsen R, Tjonneland A, Overvad K: Sex ratio of offspring of diabetics. *Lancet* 351:1514-1515, 1998

35. Paterson AD: Sex ratio of offspring of diabetics. *Lancet* 351:1515, 1998

36. Casson IF, Clarke CA, Howard CV, McKendrick O, Pennycook S, Pharoah PO, Platt MJ, Stanisstreet M, van Velszen D, Walkinshaw S: Outcomes of pregnancy in insulin dependent diabetic women: results of a five year population cohort study. *BMJ* 315:275-278, 1997

37. Janssen PA, Rothman I, Schwartz SM: Congenital malformations in newborns of women with established and gestational diabetes in Washington State, 1984-91. *Paediatr Perinat Epidemiol* 10:52-63, 1996

38. Simpson NE: Diabetes in the families of diabetics. *Can Med Assoc J* 98:427-432, 1968

39. Selby JV, Ray GT, Zhang D, Colby CJ: Excess costs of medical care for patients with diabetes in a managed care population. *Diabetes Care* 20:1396-1402, 1997

40. Ferrara A, Peng T, Kim C: Trends in Postpartum Diabetes Screening and Subsequent Diabetes and Impaired Fasting Glucose among Women with Histories of Gestational Diabetes Mellitus. A report from the Translating Research Into Action for Diabetes (TRIAD) Study. *Diabetes Care* 2008

41. Gestational Diabetes Mellitus. *Diabetes Care* 23:S77-S79, 2000

42. Ogden CL, Carroll MD, Curtin LR, Lamb MM, Flegal KM: Prevalence of high body mass index in US children and adolescents, 2007-2008. *JAMA* 20;303:242-249, 2010

43. Guo SS, Wu W, Chumlea WC, Roche AF: Predicting overweight and obesity in adulthood from body mass index values in childhood and adolescence. *Am J Clin Nutr* 76:653-658, 2002

44. Deshmukh-Taskar P, Nicklas TA, Morales M, Yang SJ, Zakeri I, Berenson GS: Tracking of overweight status from childhood to young adulthood: the Bogalusa Heart Study. *Eur J Clin Nutr* 60:48-57, 2006

45. Nader PR, O'Brien M, Houts R, Bradley R, Belsky J, Crosnoe R, Friedman S, Mei Z, Susman EJ: Identifying risk for obesity in early childhood. *Pediatrics* 118:e594-e601, 2006

46. US Census Bureau. The American Community, Hispanics: 2004. 2007. Washington DC. 5-1-2011.

47. US Census Bureau. Coming to America: A Profile of the Nation's Foreign Born. 2000. Washington DC. 5-1-2011.

48. Kranz S, Mahood LJ, Wagstaff DA: Diagnostic criteria patterns of U.S. children with Metabolic Syndrome: NHANES 1999-2002. *Nutr J* 6:38.:38, 2007

49. Alberti KG, Eckel RH, Grundy SM, Zimmet PZ, Cleeman JI, Donato KA, Fruchart JC, James WP, Loria CM, Smith SC, Jr.: Harmonizing the metabolic syndrome: a joint interim statement of the International Diabetes Federation Task Force on Epidemiology and Prevention; National Heart, Lung, and Blood Institute; American Heart Association; World Heart Federation; International Atherosclerosis Society; and International Association for the Study of Obesity. *Circulation* %20;120:1640-1645, 2009

50. Ford ES, Li C: Defining the metabolic syndrome in children and adolescents: will the real definition please stand up? *J Pediatr* 152:160-164, 2008

51. Barker DJ, Eriksson JG, Forsen T, Osmond C: Fetal origins of adult disease: strength of effects and biological basis. *Int J Epidemiol* 31:1235-1239, 2002

52. Barker DJ: The developmental origins of adult disease. *J Am Coll Nutr* 23:588S-595S, 2004

53. Harding JE: The nutritional basis of the fetal origins of adult disease. *Int J Epidemiol* 30:15-23, 2001

54. Snow MHL: Effect of genome on size at birth. In *Fetal Growth*. Sharp F, Fraser RB, Milner RDG, Eds. London, Royal College of Obstetricians and Gynaecologists, 1989, p. 3-12

55. Gluckman P, Harding J: The regulation of fetal growth. In *Human growth: Basic and clinical aspects*. Hernandez M, Argente J, Eds. Amsterdam, Excerpta Medica, 1992, p. 253-260

56. Freinkel N: Banting Lecture 1980. Of pregnancy and progeny. *Diabetes* 29:1023-1035, 1980

57. Metzger BE, Lowe LP, Dyer AR, Trimble ER, Chaovarindr U, Coustan DR, Hadden DR, McCance DR, Hod M, McIntyre HD, Oats JJ, Persson B, Rogers MS, Sacks DA: Hyperglycemia and adverse pregnancy outcomes. *N Engl J Med* 358:1991-2002, 2008

58. Hillier TA, Pedula KL, Schmidt MM, Mullen JA, Charles MA, Pettitt DJ: Childhood obesity and metabolic imprinting: the ongoing effects of maternal hyperglycemia. *Diabetes Care* 30:2287-2292, 2007

59. Baptiste-Roberts K, Nicholson WK, Wang NY, Brancati FL: Gestational Diabetes and Subsequent Growth Patterns of Offspring: The National Collaborative Perinatal Project. *Matern Child Health J* 2011

60. Crume TL, Ogden L, West NA, Vehik KS, Scherzinger A, Daniels S, McDuffie R, Bischoff K, Hamman RF, Norris JM, Dabelea D: Association of exposure to diabetes in utero with adiposity and fat distribution in a multiethnic population of youth: the Exploring Perinatal Outcomes among Children (EPOCH) Study. *Diabetologia* 54:87-92, 2011

61. Dabelea D, Hanson RL, Lindsay RS, Pettitt DJ, Imperatore G, Gabir MM, Roumain J, Bennett PH, Knowler WC: Intrauterine exposure to diabetes conveys risks for type 2 diabetes and obesity: a study of discordant sibships. *Diabetes* 49:2208-2211, 2000

62. Cho NH, Silverman BL, Rizzo TA, Metzger BE: Correlations between the intrauterine metabolic environment and blood pressure in adolescent offspring of diabetic mothers. *J Pediatr* 136:587-592, 2000

63. Silverman BL, Rizzo T, Green OC, Cho NH, Winter RJ, Ogata ES, Richards GE, Metzger BE: Long-term prospective evaluation of offspring of diabetic mothers. *Diabetes* 40 Suppl 2:121-125, 1991

64. Classification and diagnosis of diabetes mellitus and other categories of glucose intolerance. National Diabetes Data Group. *Diabetes* 28:1039-1057, 1979

65. Crume TL, Ogden L, Daniels S, Hamman RF, Norris JM, Dabelea D: The Impact of In Utero Exposure to Diabetes on Childhood Body Mass Index Growth Trajectories: The EPOCH Study. *J Pediatr* 2011

66. Tam WH, Ma RC, Yang X, Ko GT, Tong PC, Cockram CS, Sahota DS, Rogers MS, Chan JC: Glucose intolerance and cardiometabolic risk in children exposed to maternal gestational diabetes mellitus in utero. *Pediatrics* 122:1229-1234, 2008

67. Bunt JC, Tataranni PA, Salbe AD: Intrauterine exposure to diabetes is a determinant of hemoglobin A(1)c and systolic blood pressure in pima Indian children. *J Clin Endocrinol Metab* 90:3225-3229, 2005

68. West NA, Crume TL, Maligie MA, Dabelea D: Cardiovascular risk factors in children exposed to maternal diabetes in utero. *Diabetologia* 54:504-507, 2011

69. Tu YK, West R, Ellison GT, Gilthorpe MS: Why evidence for the fetal origins of adult disease might be a statistical artifact: the "reversal paradox" for the relation between birth weight and blood pressure in later life. *Am J Epidemiol* 161:27-32, 2005

70. Webber LS, Cresanta JL, Croft JB, Srinivasan SR, Berenson GS: Transitions of cardiovascular risk from adolescence to young adulthood--the Bogalusa Heart Study: II. Alterations in anthropometric blood pressure and serum lipoprotein variables. *J Chronic Dis* 39:91-103, 1986

71. Trivers RL, Willard DE: Natural selection of parental ability to vary the sex ratio of offspring. *Science* 179:90-92, 1973

72. James WH: Further evidence that mammalian sex ratios at birth are partially controlled by parental hormone levels around the time of conception. *Hum Reprod* 19:1250-1256, 2004

73. Cameron EZ: Facultative adjustment of mammalian sex ratios in support of the Trivers-Willard hypothesis: evidence for a mechanism. *Proc Biol Sci* 271:1723-1728, 2004

74. Larson MA, Kimura K, Kubisch HM, Roberts RM: Sexual dimorphism among bovine embryos in their ability to make the transition to expanded blastocyst and in the expression of the signaling molecule IFN-tau. *Proc Natl Acad Sci U S A* 98:9677-9682, 2001

75. Gutierrez-Adan A, Granados J, Pintado B, De La FJ: Influence of glucose on the sex ratio of bovine IVM/IVF embryos cultured in vitro. *Reprod Fertil Dev* 13:361-365, 2001

76. Metzger BE: Summary and recommendations of the Third International Workshop-Conference on Gestational Diabetes Mellitus. *Diabetes* 40 Suppl 2:197-201, 1991

77. American Diabetes Association: Diagnosis and classification of diabetes mellitus. *Diabetes Care* 28 Suppl 1:S37-S42, 2005

78. Ferrara A, Weiss NS, Hedderson MM, Quesenberry CP, Jr., Selby JV, Ergas IJ, Peng T, Escobar GJ, Pettitt DJ, Sacks DA: Pregnancy plasma glucose levels exceeding the American Diabetes Association thresholds, but below the National Diabetes Data Group thresholds for gestational diabetes mellitus, are related to the risk of neonatal macrosomia, hypoglycaemia and hyperbilirubinaemia. *Diabetologia* 50:298-306, 2007

79. Mathews TJ, Hamilton BE: Trend analysis of the sex ratio at birth in the United States. *Natl Vital Stat Rep* 53:1-17, 2005

80. Subbaraman MS, Goldman-Mellor SJ, Anderson ES, Lewinn KZ, Saxton KB, Shumway M, Catalano R: An exploration of secondary sex ratios among women diagnosed with anxiety disorders. *Hum Reprod* 25:2084-2091, 2010

81. Hosmer DW, Lemeshow S: *Applied Logistic Regression*. New York, John Wiley & Sons, 1989

82. Buchanan TA: Metabolic changes during normal and diabetic pregnancies. In *Diabetes mellitus in pregnancy. 2nd ed.* 2 ed. Reece EA, Coustan DR, Eds. New York, Churchill Livingstone, 1995, p. 59-77

83. Takahashi Y, First NL: In vitro development of bovine one-cell embryos: Influence of glucose, lactate, pyruvate, amino acids and vitamins. *Theriogenology* 37:963-978, 1992

84. Iwata H, Akamatsu S, Minami N, Yamada M: Effects of antioxidants on the development of bovine IVM/IVF embryos in various concentrations of glucose. *Theriogenology* 50:365-375, 1998

85. Ogden CL, Carroll MD, Curtin LR, McDowell MA, Tabak CJ, Flegal KM: Prevalence of overweight and obesity in the United States, 1999-2004. *JAMA* 295:1549-1555, 2006

86. Whitaker RC, Orzol SM: Obesity among US urban preschool children: relationships to race, ethnicity, and socioeconomic status. *Arch Pediatr Adolesc Med* 160:578-584, 2006

87. Sundquist J, Winkleby MA: Cardiovascular risk factors in Mexican American adults: a transcultural analysis of NHANES III, 1988-1994. *Am J Public Health* 89:723-730, 1999

88. Davidson JA, Kannel WB, Lopez-Candales A, Morales L, Moreno PR, Ovalle F, Rodriguez CJ, Rodbard HW, Rosenson RS, Stern M: Avoiding the looming Latino/Hispanic cardiovascular health crisis: a call to action. *J Cardiometab Syndr* 2:238-243, 2007

89. Hunt KJ, Resendez RG, Williams K, Haffner SM, Stern MP, Hazuda HP: All-cause and cardiovascular mortality among Mexican-American and non-Hispanic White older participants in the San Antonio Heart Study- evidence against the "Hispanic paradox". *Am J Epidemiol* 158:1048-1057, 2003

90. Winkleby MA, Fortmann SP, Rockhill B: Health-related risk factors in a sample of Hispanics and whites matched on sociodemographic characteristics. The Stanford Five-City Project. *Am J Epidemiol* 137:1365-1375, 1993

91. Barker DJ: The origins of the developmental origins theory. *J Intern Med* 261:412-417, 2007

92. Ekelund U, Ong K, Linne Y, Neovius M, Brage S, Dunger DB, Wareham NJ, Rossner S: Upward weight percentile crossing in infancy and early childhood independently predicts fat mass in young adults: the Stockholm Weight Development Study (SWEDES). *Am J Clin Nutr* 83:324-330, 2006

93. National Center for Health Statistics. CDC growth charts. 2005. United States.

94. US Census Bureau. Poverty Thresholds for 2000 by Size of Family and Number of Related Children Under 18 Years. 9-16-2010. Washington DC. 5-1-2011.

95. Vartanian LR, Schwartz MB, Brownell KD: Effects of soft drink consumption on nutrition and health: a systematic review and meta-analysis. *Am J Public Health* 97:667-675, 2007

96. Greenland S, Pearl J, Robins JM: Causal diagrams for epidemiologic research. *Epidemiology* 10:37-48, 1999

97. Finucane MM, Samet JH, Horton NJ: Translational methods in biostatistics, linear mixed effect regression models of alcohol consumption and HIV disease progression over time. *Epidemiol Perspect Innov* 4: 2007

98. King JC: Maternal obesity, metabolism, and pregnancy outcomes. *Annu Rev Nutr* 26:271-91.:271-291, 2006

99. Brown H, Prescott R: *Applied Mixed Models in Medicine*. New York, NY, John Wiley & Sons, Ltd, 1999

100. van der Laan MJ, Polley EC, Hubbard AE: Super learner. *Stat Appl Genet Mol Biol* 6:Article25, 2007

101. Gunderson EP, Abrams B: Epidemiology of gestational weight gain and body weight changes after pregnancy. *Epidemiol Rev* 21:261-274, 1999

102. Hyperglycemia and Adverse Pregnancy Outcome (HAPO) Study: associations with neonatal anthropometrics. *Diabetes* 58:453-459, 2009

103. Brook CG: Evidence for a sensitive period in adipose-cell replication in man. *Lancet* 2:624-627, 1972

104. Kitajima M, Oka S, Yasuhi I, Fukuda M, Rii Y, Ishimaru T: Maternal serum triglyceride at 24--32 weeks' gestation and newborn weight in nondiabetic women with positive diabetic screens. *Obstet Gynecol* 97:776-780, 2001

105. Ramirez AG: Hypertension in Hispanic Americans: overview of the population. *Public Health Rep* 111 Suppl 2:25-6.:25-26, 1996

106. Ehrlich SF, Crites YM, Hedderson MM, Darbinian JA, Ferrara A: The risk of large for gestational age across increasing categories of pregnancy glycemia. *Am J Obstet Gynecol* 204: 2011

107. Phillips DL, Pirkle JL, Burse VW, Bernert JT, Jr., Henderson LO, Needham LL: Chlorinated hydrocarbon levels in human serum: effects of fasting and feeding. *Arch Environ Contam Toxicol* 18:495-500, 1989

108. Fernandez JR, Redden DT, Pietrobelli A, Allison DB: Waist circumference percentiles in nationally representative samples of African-American, European-American, and Mexican-American children and adolescents. *J Pediatr* 145:439-444, 2004

109. Dabelea D, Pettitt DJ, Hanson RL, Imperatore G, Bennett PH, Knowler WC: Birth weight, type 2 diabetes, and insulin resistance in Pima Indian children and young adults. *Diabetes Care* 22:944-950, 1999

110. Widdowson EM, Crabb DE, Milner RD: Cellular development of some human organs before birth. *Arch Dis Child* 47:652-655, 1972

111. Hemachandra AH, Howards PP, Furth SL, Klebanoff MA: Birth weight, postnatal growth, and risk for high blood pressure at 7 years of age: results from the Collaborative Perinatal Project. *Pediatrics* 119:e1264-e1270, 2007

112. Lane DA, Gill P: Ethnicity and tracking blood pressure in children. *J Hum Hypertens* 18:223-228, 2004

113. Hirschler V, Aranda C, Calcagno ML, Maccalini G, Jadzinsky M: Can waist circumference identify children with the metabolic syndrome? *Arch Pediatr Adolesc Med* 159:740-744, 2005

114. Prentice AM, Rayco-Solon P, Moore SE: Insights from the developing world: thrifty genotypes and thrifty phenotypes. *Proc Nutr Soc* 64:153-161, 2005

115. O'Sullivan JB, Mahan CM, Charles D, Dandrow RV: Screening criteria for high-risk gestational diabetic patients. *Am J Obstet Gynecol* 116:895-900, 1973

116. Araneta MR, Grandinetti A, Chang HK: A1C and diabetes diagnosis among Filipino Americans, Japanese Americans, and Native Hawaiians. *Diabetes Care* 33:2626-2628, 2010

117. Illsley NP: Glucose transporters in the human placenta. *Placenta* 21:14-22, 2000

118. Das UG, Sadiq HF, Soares MJ, Hay WW, Jr., Devaskar SU: Time-dependent physiological regulation of rodent and ovine placental glucose transporter (GLUT-1) protein. *Am J Physiol* 274:R339-R347, 1998

119. Crowther CA, Hiller JE, Moss JR, McPhee AJ, Jeffries WS, Robinson JS: Effect of treatment of gestational diabetes mellitus on pregnancy outcomes. *N Engl J Med* 352:2477-2486, 2005

120. Phelan S: Pregnancy: a "teachable moment" for weight control and obesity prevention. *Am J Obstet Gynecol* 202:135-138, 2010

121. Douketis JD, Macie C, Thabane L, Williamson DF: Systematic review of long-term weight loss studies in obese adults: clinical significance and applicability to clinical practice. *Int J Obes (Lond)* 29:1153-1167, 2005