CONSEQUENCES OF EXPOSURE TO MATERNAL DIABETES ON OFFSPRING PUBERTY AND ADIPOSITY

by

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Thesis directed by Professor Dana Dabelea

ABSTRACT

With the global increase in the prevalence of obesity and diabetes, including among youth, along with the decrease in age of pubertal timing, understanding how fetal over-nutrition may influence puberty and the development of adiposity in the offspring is an important area of focus for chronic disease prevention. This thesis uses innovative epidemiological approaches to investigate these relationships to provide a better understanding of the long-term effects of exposure to maternal diabetes on offspring adiposity, including the complex and critical role that puberty may play. We used epidemiologic data provided by the Exploring Prenatal Outcomes in Children (EPOCH) study, a historical prospective cohort comprised of 604 mother-offspring dyads followed during early and late puberty. For the first aim, using height measurements collected from age two years through adolescence, we estimated the pubertal timing, defined by age at peak height velocity (APHV), and speed of pubertal growth, defined by peak height velocity PHV), for each child. We found that the median APHV was reached ~3 months earlier in youth exposed to maternal diabetes compared to unexposed youth and that exposed females had 10% greater median PHV compared to unexposed females and exposed males had a 3.5% greater median PHV compared to unexposed males. Puberty is comprised of many different components that drive physical and hormonal changes. For the second aim, we investigated whether exposure to maternal diabetes alters hormones of puberty, specifically estradiol (E), testosterone (TT), dehydroepiandrosterone sulfate (DHEA-S), and luteinizing hormone (LH). We found that exposure to maternal diabetes was associated with increased concentrations of luteinizing hormone in exposed females, but not in males and that exposure to maternal diabetes did not influence estradiol, total testosterone or DHEA-S concentrations. The third aim built on the first aim and previous findings of increased adiposity in exposed youth in this cohort[1] and explored a hypothesized pathway from exposure to maternal

diabetes in utero to adolescent adiposity, measured by waist-to-height ratio (WHR). Our results suggest that the association between exposure to maternal diabetes and offspring adiposity is established early in life, likely before puberty, and tracks throughout puberty, with adiposity being both a possible driver and consequence of earlier pubertal timing. Taken together, this dissertation suggests that exposure to maternal diabetes during the intrauterine period influences puberty and adiposity in the offspring, which subsequently shape health outcomes later in life; however, further replication studies are needed. These findings are important to public health as they suggest a need for obesity and lifestyle interventions among young adults, specifically women of child-bearing age, which may help disrupt the intergenerational cycle of obesity.

The form and content of this abstract are approved. I recommend its publication.

Approved: Dana Dabelea

DEDICATION

This thesis is dedicated to my husband, Joey Hockett, my daughter, Isabelle Rose, and to my parents, Bonny Specker and Howard Wey. Thank you for your unconditional love, support and patience.

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CHAPTER I

INTRODUCTION

Background

The prevalence of obesity in children has increased significantly in the past 30 years and is a major public health concern. The percentage of obese adolescents aged 12-19 years in the U.S. increased from 5% to nearly 21% from 1980 to 2012[2]. Childhood and adolescent obesity is associated with an increased risk of adult obesity and other chronic diseases, such as type 2 diabetes and cardiovascular disease[3–6]. Concurrently, the age of pubertal onset has decreased over the last several decades[7], specifically in females, and has also been associated with an increased risk of chronic diseases[8,9]. Understanding how the intrauterine environment may influence puberty and the development of obesity is important. There is a growing understanding that even mild changes in the intrauterine environment influence offspring outcomes not only in the perinatal period, but also over the entire life course[10]. Several studies have explored the effects of fetal exposure to maternal diabetes on infant and childhood adiposity[11–16]; however, few have assessed whether intrauterine exposures, such as maternal diabetes, affects puberty[17,18] and if these effects explain the relationship between intrauterine exposure and increased adiposity in the offspring[9,10]**. The overall goal of this dissertation is to explore the effects of intrauterine exposure to maternal diabetes on different aspects of puberty and a hypothesized pathway from exposure to maternal diabetes to adolescent adiposity, looking at the contribution of pubertal timing along this pathway.**

The EPOCH (Exploring Perinatal Outcomes in CHildren) study is a historical prospective cohort study, with an ethnically diverse population of 604 children exposed and unexposed to maternal diabetes during pregnancy. This cohort was followed through puberty, a critical post-natal developmental period associated with rapid growth, alterations of fat distributions and hormonal changes. Data were collected on participating youth at two research visits: the first at 6-13 years (average 10.4 years) and the second visit about 6 years later when the youth were 12-19 years (average 16.9 years). These data enabled us to evaluate the relationships between exposure to

maternal diabetes and several different puberty measures, and to investigate the relationships along the hypothesized pathway from exposure to maternal diabetes to adolescent adiposity throughout puberty.

Specific aims and hypotheses

Specific aim 1

Using growth curves to estimate the timing and speed of puberty, we explored the impact of exposure to diabetes during pregnancy on pubertal timing, defined by age at peak height velocity, and speed of pubertal growth, defined by peak height velocity, in adolescents.

For hypothesis 1, we hypothesized that adolescents exposed to maternal diabetes would have a different age at peak height velocity compared to adolescents not exposed to maternal diabetes; specifically, the difference would be sex-specific with exposed females having an earlier pubertal timing and exposed males having no difference in pubertal timing.

For hypothesis 2, we hypothesized that adolescents exposed to maternal diabetes will have a faster speed of pubertal growth compared to adolescents not exposed to maternal diabetes.

Specific aim 2

There are many different aspects of puberty, as it is a complex process that occurs over several years. For the second aim, we investigated the relationship between exposure to maternal diabetes and hormone concentrations of puberty, specifically, estradiol, total testosterone, luteinizing hormone and dehydroepiandrosterone sulfate (DHEA-S).

We hypothesized that exposure to maternal diabetes would affect hormone concentrations in adolescents; specifically, the direction and magnitude would be sex-specific with (a) exposed females having increased levels of estradiol and luteinizing hormone and decreased levels of total testosterone and DHEA-S compared to unexposed females; and (b) exposed males having increased levels of estradiol and decreased levels of total testosterone, luteinizing hormone, and DHEA-S.

Specific aim 3

To further understand the effect of maternal diabetes on offspring puberty and adiposity, we explored a hypothesized pathway from exposure to maternal diabetes to adolescent adiposity, and the contributions of adiposity in early puberty and pubertal timing along this pathway.

We hypothesized that (1) youth exposed to diabetes would have a larger waist-to-height ratio in late puberty, (2) youth exposed to diabetes would have a larger waist-to-height ratio in early puberty, (3) youth with a larger waist-to-height ratio in early puberty would have earlier pubertal timing, and (4) youth with earlier pubertal timing, defined by APHV, would have a larger waist-to-height ratio in late puberty.

Overall impact and innovation

Understanding the consequences of fetal programming from over-nutrition is important for the health and well-being of future children. This research uses novel hypotheses and methods to explore these relationships to provide a better understanding of the long-term effects of intrauterine exposure to maternal diabetes on offspring adiposity during the complex postnatal critical period of puberty. This research furthers our understanding of the developmental origins of obesity and chronic disease, and lends support for the development of future lifestyle interventions in early life to help disrupt the intergenerational cycle of obesity.

CHAPTER II

LITERATURE REVIEW

Childhood obesity epidemic and its consequences

The prevalence of obesity in children has increased significantly in the past 30 years. The percentage of obese children aged 6-11 years in the United States increased from 7% in 1980 to nearly 18% in 2012. Similarly, the percentage of obese adolescents, aged 12-19 years, increased from 5% to nearly 21% over the same period[2]. This increase in the prevalence of childhood obesity has been shown worldwide in both developed and developing countries[19]. Childhood and adolescent obesity is associated with an increased risk of adult obesity[20] and in general, obesity is associated with other chronic diseases such as cardio-metabolic diseases, stroke, cancer, low quality of life, and mental illness[21–24]. The economic impact of obesity and its related health problems is substantial in the United States. The medical care costs of obesity in the US were estimated to be $147 billion in 2008[25], and the annual nationwide productive costs of obesity and obesity-related absenteeism range between $3.38 billion and $6.38 billion, which is $79 to $132 per obese individual[26].

Obesity begins *in utero*: the fetal over-nutrition programming hypothesis

Fetal metabolic programming occurs during the critical period of early development *in utero* when nutritional stressors or chemical stimuli are hypothesized to permanently alter the offspring's physiology and metabolism, though the consequences are often seen much later in life. Maternal diabetes, including gestational diabetes (GDM) and pre-gestational diabetes, are intrauterine exposures that are likely to result in fetal over-nutrition. Maternal diabetes is a result of pregnancy-induced insulin resistance, on a background of deficient compensatory insulin secretion and further enhanced by pre-existent obesity. Maternal insulin resistance results in a need for increased insulin secretion to compensate, but failure to compensate fully results in increased glucose levels and likely other nutrients such as lipids and amino acids in the maternal blood, which after placental transfer, leads to fetal overnutrition. Since maternal glucose freely crosses the placenta to the fetus, while maternal insulin does not, the developing fetal pancreas responds by producing additional anabolic

hormones and growth factors, such as insulin, which promote growth and adiposity[27,28]. The precise mechanisms responsible for these effects are not clearly understood, but considerable progress has been made in the last decades.

There is a growing understanding that even mild changes in the intrauterine environment influences the infant's outcome not only in the perinatal period, but also over the entire life course[10]. The role of exposure to maternal diabetes *in utero* on obesity has been examined in several studies. Focusing on obesity and growth, several studies found that offspring of diabetic mothers had an increased risk of obesity and glucose intolerance in late childhood, above and beyond genetic influences[29,30]. Offspring of Pima Indian women with pre-existing type 2 diabetes and GDM were larger for gestational age at birth and were heavier than the offspring of pre-diabetic or non-diabetic women after 5 years of age[31]. Previously, the EPOCH study found that exposure to maternal GDM was associated with higher BMI, waist circumference, and both visceral adipose tissue and subcutaneous adipose tissue in 6-13 year olds[32]. Similar, but less conclusive findings when adjusting for maternal pre-pregnancy BMI, were seen in other epidemiologic studies[11,13]. However, all of these studies focused on different age groups when examining the effect of maternal diabetes on obesity, which may explain the varying results. Crume et al. was the only study that looked at a range of ages by using BMI growth trajectories of the offspring. Early in life, BMI growth trajectories of children exposed to GDM were similar to those unexposed, until around the time of puberty when they began to diverge. By 10-13 years of age the mean BMI growth velocities of exposed children were significantly higher than those not exposed[33].

Puberty, its timing and progression

Puberty is a complex physiological process defined by a period of intense hormonal changes and rapid physical growth, leading to psychological and physical maturation. In a normal environment, puberty is centrally activated as the hypothalamic-pituitary-gonadal (HPG) axis initiates secretory pulses of the gonadotropin releasing hormone (GnRH) neurons. The sustained increase in pulsatile levels of GnRH induce gonadotropin (LH & FSH) synthesis and secretion from the pituitary

gland, which initiate gonad development, the synthesis and secretion of sex steroids and gamete maturation. For females, higher estrogen production promotes breast and uterine development and contributes to body fat redistribution during and after puberty. For males, androgen production promotes changes in the musculoskeletal system and spermatogenesis. These sex steroids interact with the production and secretion of pituitary growth hormone (GH) that in turn increases the production of insulin-like growth factor-1 (IGF-1). The characteristic pubertal growth spurt is due to both the indirect increase in sex steroids and the direct increase in GH stimulation[34].

Since puberty is comprised of many different components that drive the physical and hormonal changes, there are many measures that define these changes in one of two ways: the timing at which a pubertal milestone occurred (i.e. age at menarche in females or when the voice changes in a male) or where a child is in the pubertal process (i.e. Tanner stage for breast development or testicular growth). Almost five decades ago, Marshall & Tanner documented the clinical pubertal signs called Tanner stages[35,36]. The initial description of Tanner stages was by visual inspection alone, but due to the subjectivity of the assessment and the significant increase in obesity in pre-pubertal children, palpation for thelarche (breast development) and gonadarche (testicular growth) by an Endocrinologist is now considered the best method of Tanner stage assessment. However, this method is not typically performed during normal well-child visits and is hard to obtain in large observational epidemiological studies. Another measure of pubertal timing in females is self-reported age of menarche, which is accurate when reported prospectively and in detail (using month and year). A physical hallmark of puberty is the period of rapid growth[37]; as puberty approaches, the growth velocity slows ("preadolescent dip") before it suddenly accelerates ("peak height velocity (PHV)") during mid-puberty. For females, the age at which the accelerated growth occurs, also known as the age of peak height velocity (APHV), is typically earlier than males and during Tanner stage 3; while males usually reach peak height velocity during Tanner stage 4[38]. When pubertal hormone concentrations are not available or the self-reported Tanner stages are unreliable, linear growth can be used as a surrogate marker of pubertal timing if longitudinal height measurements are available**.**

The determinants of puberty are poorly understood

Determinants of puberty and its onset are not entirely established. While adequate nutrition is a key permissive factor for normal timing of pubertal development, it remains unclear whether pubertal onset is altered by relative over-nutrition leading to excessive adiposity. In 1970, Frisch and Revelle hypothesized that a critical body weight or % body fat had to be achieved to allow puberty to progress[39,40]. However, few longitudinal studies have evaluated the effect of pre-pubertal body composition on pubertal onset and progression[41–44]. Furthermore, some researchers hypothesize that early-life or prenatal exposures predispose children to earlier pubertal maturation and altered hormones of puberty, in addition to increasing the risk of future insulin resistance and obesity later in life[45,46]. For example, rapid early weight gain has been linked to elevated IGF-1 concentration, insulin resistance, elevated adrenal androgen concentrations, exaggerated adrenarche, obesity and elevated leptin[47]. Keim et al. (2011) found maternal obesity to be associated with a younger menarcheal age among their daughters, compared to normal weight mothers[48]. In an animal study, female offspring of rats fed a high-fat diet during pregnancy and lactation presented with earlier sexual maturation[15]. The impact of fetal over-nutrition on pubertal timing and hormones of puberty in the offspring are not well established.

Fetal over-nutrition, puberty and the development of obesity, what do we know?

The relationship between pubertal maturation and markers of obesity have been seen in several epidemiological cohort studies, in both males and females[8,9,51]. The Atherosclerosis Risk in Communities (ARIC) study found that early age at menarche, 8-11 years compared to 13 years, was associated with the development of T2D among White women, where the association between earlier age of menarche and T2D was slightly mediated by higher BMI, suggesting that early pubertal timing may directly and indirectly increase T2D risk. However, the self-reported age of menarche was retrospectively obtained several decades after the event. Widen et al (2012) found an association, independent of fetal and childhood growth, between pubertal timing and higher diastolic blood pressure, BMI, and waist circumference in both males and females; and higher serum insulin and

triglycerides and lower HDL cholesterol in males. However, few have investigated the impact of fetal over-nutrition on pubertal timing and hormones of puberty; to our knowledge no one to-date has looked at the relationships between fetal over-nutrition, puberty and risk of increased adiposity. Davis et al. found that GDM significantly modified the relationship between Tanner stages and fat mass, where children exposed to GDM had higher mean fat mass compared to those unexposed[52], but, this study was conducted in an overweight Latino population and may not be generalizable to normal-weight children, or other ethnic population.

The full consequences of fetal programming from over-nutrition still remain unclear. This dissertation aims to contribute novel evidence on potential relationships that may provide a better understanding of the long-term effects of *in utero* exposure to maternal diabetes on different aspects of puberty and adiposity. A better understanding of these associations may lend additional support for lifestyle interventions in early life to help stop the vicious transgenerational cycle of obesity.

CHAPTER III

METHODS

Overview

This study used data provided by the Exploring Perinatal Outcomes in CHildren (EPOCH) study (Grant #: 2R01-DK068001, PI Dr. Dana Dabelea). The EPOCH study is a historical prospective study that enrolled 604 mother-child dyads from the Kaiser Permanente of Colorado (KPCO) perinatal database. The goal of the prospective EPOCH cohort was to follow the youth for another six years, when they would be 12-19 years old, to determine if the detrimental effects of exposure to intrauterine over-nutrition become more evident during the transition through puberty.

Study population overview

Eligible EPOCH participants were children (males and non-pregnant females) exposed to maternal diabetes and a random sample of children not exposed to maternal diabetes. Participants were offspring of singleton pregnancies, born at a single hospital in Denver between 1992 and 2002, whose biological mothers were members of KPCO. Children and their mothers were invited to participate in two research visits at average ages of 10.5 (SD=1.5) and 16.7 (SD=1.2). Four hundred seventeen youth completed the second research visit, which was on average 6.2 years from the first research visit. This study population was sampled to reflect similar racial and ethnic distributions of Colorado and provides a unique and diverse population. Numbers and characteristics of participants that completed each research visit are shown in Table 1.

Preliminary data

Crume et al. showed that children exposed *in utero* to maternal diabetes had similar growth velocity in infancy and early childhood compared to unexposed children, until around the time of puberty, whereas by 10-13 years of age the mean BMI growth velocities of exposed children were significantly higher than those of youth unexposed[33]. This is consistent with current findings that showed no difference in BMI at 4-5 and 5-8 years of age between children exposed and unexposed to maternal diabetes[13,16], but significantly higher BMI in exposed siblings at 9-12 years of age, which

persisted throughout adolescents into young adulthood[16]. EPOCH provided novel and strong evidence that the long-term effects of exposure to maternal diabetes on offspring BMI growth becomes stronger as youth enter puberty.

By using a state of the art technique, magnetic resonance imaging (MRI), EPOCH was the first study to look at more sensitive measures of childhood adiposity and fat distribution in children exposed and not exposed to maternal diabetes. Using these measures, Crume et al. (2011) found that exposure to maternal diabetes is associated with higher BMI, waist circumference, both visceral adipose tissue (VAT) and subcutaneous adipose tissue (SAT) and more centralized fat distribution pattern in 6-13 year olds youth.

Data collection

Maternal data

Exposure to maternal diabetes was defined as a physician diagnosis of diabetes during the index pregnancy and was ascertained from the KPCO perinatal database, an electronic database that links prenatal and neonatal medical records. At 24-28 weeks, all pregnant women were offered screening using the 2-step standard protocol, a 1-h 50-g oral glucose tolerance test (OGTT) [53]. A value of 140 mg/dl or above identified mothers who needed to undergo a 3-h 100-g fasting diagnostic OGTT. Diagnosis of GDM was defined using the recommendations from the National Diabetes Data Group[54], glucose values exceeded >2 thresholds on the 3-hour, 100g oral glucose tolerance test[54]. Pre-pregnancy BMI was calculated from the KPCO measured weight before the last menstrual cycle preceding pregnancy and the mother's height collected at the first research visit. Socio-demographic, lifestyle and anthropometric variables were collected at the first research visit from the mother by a self-reported questionnaire (race/ethnicity, household income, education level, and parity) or a physical exam (current height, weight and blood pressure). Height and weight were assessed with a stadiometer and a calibrated scale. Table 2 summarizes the data collection and timing of measurements.

Offspring data

Questionnaires, anthropometrics, physical exam and an intravenous blood draw were collected at the first and second research visits. Physical pubertal development was assessed by a self-report 5-stage diagrammatic representation of Tanner pubic hair in both females and males, and breast development in girls[35,36]. Height was measured to the nearest 0.1 cm using a stadiometer, if the first two recorded measures were more than 0.5 cm apart then a third measure was taken, the measurements were then averaged. Weight was measured to the nearest 0.1 kg using an electronic, calibrated scale, if the first two recorded measures were more than 0.3 kg apart then a third measure was taken, the measurements were then averaged. Using the NHANES protocol, after normal expiration just above the right iliac crest at the midaxillary line, the waist circumference is measured twice to the nearest 0.1cm, if the first two recorded measures were more than 1.0 cm apart then a third measure was taken, the measurements were then averaged. Waist-to-height ratio (WHR) was calculated for each research visit as waist circumference divided by height, both measured in the same units (cm). Systolic and diastolic blood pressure was measured after 5 minutes of rest in a seated position using a standard mercury manometer in triplicate and the average was recorded[55]. Longitudinal heights and weights were collected using previously recorded measures of recumbent length (up to age 2 years), standing height (after the child is able to stand) and weight from pediatric office visits. These longitudinal measures were collected on all 604 youth from birth to the first research visit and 418 youth the first research visit to the second research visit. The median number of height measurements per participant from birth to the second research visit was 19 (range 4 to 52).

Laboratory measures

A fasting blood draw (~ 20 ml) occurred for all consenting children at both the first and second research visits. For hormones of puberty, estradiol, total testosterone, luteinizing hormone, and dehydroepiandrosterone sulfate (DHEA-S) were measured. Sera from the first research visit were frozen and stored at -80ºC for an average of 6.2 years, and then analyzed when the participant completed the second research visit. Sera from the second research visit were refrigerated and

analyzed within 24 hours of collection. All laboratory measurements were performed at the Colorado Clinical Translational Science Institute (CCSTI) Core Laboratories. Serum estradiol was measured by using a Beckman Coulter chemiluminescent with a sensitivity of 10.0 pg/mL. Serum testosterone (total) was measured by using a Beckman Coulter 1-step competative with a sensitivity of 17 ng/dL. Serum luteinizing hormone was determined by using a Beckman Coulter chemiluminescent with a sensitivity of 0.12 mIU/mL. Serum DHEA-S was measured by using a Beckman Coulter chemiluminescent with a sensitivity of 2 ug/dL. Additional details about assays methodology can be found at http://cctsi.ucdenver.edu/Research-Resources/CTRCs/Pages/Assays.aspx.

Aim 1

To explore the impact of intrauterine exposure to maternal diabetes on pubertal timing, defined by age at peak height velocity, and speed of pubertal growth, defined by peak height velocity, in adolescents.

Study design

The full prospective cohort of children in EPOCH who completed a second research visit includes 77 children exposed to maternal diabetes and 341 children unexposed to maternal diabetes (N=418). The main variable of interest was exposure to maternal diabetes. There were two outcomes of interest—APHV and PHV; each outcome was evaluated in separate survival models.

Statistical approach

We used a two-step analysis to assess this aim. First, we estimated pubertal timing, defined by age at peak height velocity, and speed of pubertal growth, defined by peak height velocity, using the SuperImposition by Translation and Rotation (SITAR) method[56]. The SITAR method uses a shape invariant spline curve and a nonlinear random-effects model to estimate an average growth curve for the entire sample and each individual's deviation from this average curve. There are three subject-specific parameters termed size, velocity (PHV), and timing (APHV); conceptually, these subject-specific parameters define how much bigger or smaller each child is compared to the population average (size, cm), how much faster and slower the child's growth velocity is compared to

the population average (velocity, cm/yr), and how much earlier or later the child experienced peak velocity compared to the population average (timing, yr). The models were fitted with the SITAR package in R[57]. The SITAR model allows for the flexibility in fitting the spline curves by adjusting the degrees of freedom, in doing so, the optimal models were selected by evaluating the BIC score. The lowest BIC score indicates the best fit model. Subject-specific parameters size, velocity, and timing were estimated as random effects. Second, accelerated failure time (AFT) models were used to evaluate the association between exposure to maternal diabetes and pubertal timing (APHV) and speed of pubertal growth (PHV).

Hypothesis 1.1

We hypothesized adolescents exposed to diabetes during pregnancy would have a different age of pubertal timing compared to adolescents not exposed to diabetes during pregnancy; specifically, the difference will be sex-specific with exposed females having an earlier pubertal timing and exposed males having no differences in pubertal timing. We calculated the ratio of the median time-to-event for exposed youth compared to unexposed youth, using an accelerated failure time model. The association between exposure to maternal diabetes and APHV was examined using two main models: an initial model including adjustment for child's sex and race/ethnicity; and a fully adjusted model including additional adjustment for potential socio-economic confounders (mother's education, total household income at birth). The potential effect modification by child's sex was evaluated using a cross-product term (child's sex*exposure status). As a secondary analysis in a subset of data, we explored the effect of the mother's pre-pregnancy BMI on the relationships of maternal diabetes with pubertal timing.

Hypothesis 1.2

We hypothesized adolescents exposed to diabetes during pregnancy would have a faster speed of pubertal growth compared to adolescents not exposed to diabetes during pregnancy. Similar to the first hypothesis, we used an accelerated failure time model to evaluate the association between exposure to maternal diabetes and PHV. We examined two main models: an initial model including

adjustment for child's sex and race/ethnicity; and a fully adjusted model including additional adjustment for potential socio-economic confounders (mother's education, total household income at birth). The potential effect modification by child's sex was evaluated using a cross-product term (child's sex*exposure status). As a secondary analysis in a subset of data, we explored the effect of the mother's pre-pregnancy BMI on the relationships of maternal diabetes with speed of pubertal timing.

Throughout this aim, R and SAS were used for analyses. Data checks were used to verify that the data remain unchanged when transferring back and forth between packages.

Power and sample size

For hypothesis 1.2, we estimated detectible HRs for 85 exposed and 425 unexposed children, using the powerSurvEpi package in R[58], to evaluate the effect of GDM (16.7% of the EPOCH population), for 80% power and a significance level of 0.05, with the proportion of participants to reach puberty at 90.3% and the regression of GDM with confounding variables having an R^2 of 0.30. Assuming proportional hazard assumptions are met, we estimate we will be able to detect HRs $\geq$ 1.52. This would allow use to detect a 52% or greater increase in the hazard of having an earlier pubertal timing.

Aim 2

To investigate the relationship between exposure to maternal diabetes and hormone concentrations of puberty, specifically, estradiol, total testosterone, luteinizing hormone and DHEA-S.

Study design

Four hundred fourteen youth completed two research visits and had at least one of the four hormone concentrations measured were included. The analytic sample of 414 was similar to the full cohort of 604 in all aspects, except the analytic sample had a higher percentage of mothers who completed more than a high school education (82.6 vs. 78.6%) and a higher percentage of non-Hispanic Whites (51.0% vs. 47.9%). The variable of interest was exposure to maternal diabetes and

the outcomes were hormones of puberty—estradiol, total testosterone, luteinizing hormone, and DHEA-S. Due to missing covariates and outcomes 8 youth were removed for the estradiol analysis, 7 youth were removed for the testosterone analysis, 2 youth were removed for the luteinizing hormone analysis, and 3 youth were removed for the DHEA-S analysis (Figure 1). The associations of exposure to maternal diabetes with offspring hormone concentrations were evaluated using separate linear regression models for each hormone. For hormones analyzed for only the second research visit (E2 and TT), general linear regression models were used for analyses. For hormones analyzed for both research visits (LH and DHEA-S), linear mixed models were used with repeated measures included in each model with time as the repeated effect.

Statistical approach

Hypothesis 2

We hypothesized that exposure to maternal diabetes would affect hormone concentrations in adolescents; specifically, the direction and magnitude will be sex-specific with (a) exposed females having increased levels of estradiol and luteinizing hormone and decreased levels of total testosterone and DHEA-S compared to unexposed females; and (b) exposed males having increased levels of estradiol and decreased levels of total testosterone, luteinizing hormone, and DHEA-S.

We used a three-step modeling procedure for each hormone outcome. First, the initial models included covariates—exposure status, child's sex, race, Tanner stage, and age, which remained in the model regardless of significance. We tested the following interactions—exposure-by-sex, sex-by-age and puberty-by-age. If the interactions were not significant, they were removed from the model. The second model was constructed using the previous initial model with the inclusion of perinatal characteristics (maternal education, household income and maternal smoking status). Lastly, the initial model was further adjusted for offspring BMI to evaluate whether associations of intrauterine exposure with offspring pubertal hormones were mediated by or independent of offspring body size, and an exposure-by-BMI interaction was also tested.

Aim 3

To further understand the effect of maternal diabetes on puberty and offspring adiposity, we studied a hypothesized pathway from exposure to maternal diabetes to adolescent adiposity, and the contributions of early pubertal adiposity and pubertal timing along this pathway.

Study design

We used data from 364 youth (69 exposed and 295 unexposed to maternal diabetes) who completed both research visits and had a Tanner Stage of 1-3 (early puberty) at the first research visit and a Tanner Stage of 4 or 5 (late puberty) at the second research visit. We tested four separate linear regression models. Model 1 assessed the effect of exposure to maternal diabetes on WHR in late puberty, while models 2-4 assessed associations along the hypothesized pathway throughout puberty—the effect of exposure to maternal diabetes on WHR in early puberty; the association between WHR in early puberty and APHV (pubertal timing); and the effect of APHV on WHR in late puberty. The conceptual model for this aim is shown in Figure 2. Age at peak height velocity estimated in aim 1 was used as the measure of pubertal timing.

Statistical approach

Hypothesis 3.1

We hypothesized that youth exposed to diabetes would have a larger waist-to-height ratio in late puberty. We used a linear regression model to examine the association between exposure to maternal diabetes and WHR in late puberty. We used WHR as it has been previously shown to be a better predictor of adiposity in children and adolescents than BMI[59]. We controlled for child's sex, race/ethnicity, age at research visit, and household income at birth. The potential effect modification by child's sex was evaluated using a cross-product term (exposure status*child's sex).

Hypothesis 3.2

We hypothesized that youth exposed to diabetes would have a larger waist-to-height ratio in early puberty. We used a linear regression model to examine the association between exposure to maternal diabetes and WHR in early puberty. We controlled for child's sex, race/ethnicity, age at

research visit, and household income at birth. The potential effect modification by child's sex was evaluated using a cross-product term (exposure status*child's sex).

Hypothesis 3.3

We hypothesized that youth with a larger waist-to-height ratio in early puberty would have earlier pubertal timing. We used a linear regression model to examine the association between WHR in early puberty and APHV, used to define pubertal timing. Age at peak height velocity was estimated using the SITAR method detailed in aim 1. We controlled for child's sex, race/ethnicity, and household income at birth. The potential effect modification by child's sex was evaluated using a cross-product term (early puberty WHR*child's sex).

Hypothesis 3.4

We hypothesized that youth with earlier pubertal timing, defined by APHV, would have a larger waist-to-height ratio in late puberty. We used a linear regression model to examine the association between pubertal timing and WHR in late puberty. We controlled for child's sex, race/ethnicity, age at research visit, and household income at birth. The potential effect modification by child's sex was evaluated using a cross-product term (late puberty WHR*child's sex).

Tables

Table 1. Characteristics of exposed and unexposed EPOCH participants at each research visit

	First Research Visit							Second Research Visit						
	Exposed			Unexposed			p-value	Exposed			Unexposed			p-value
Maternal characteristics	N	%	Mean (SD)	N	%	Mean (SD)		N	%	Mean (SD)	N	%	Mean (SD)	
Exposed to maternal diabetes	99	16.4		505	83.6			77	18.4		341	81.6		
Age at first research visit (years)			33.0 (5.5)			29.9 (5.6)	<0.001			33.0 (5.5)			30.3 (5.4)	<0.001
Self-reported household income at birth							0.15							0.52
≤$49,999	39	39.4		238	47.2			32	41.6		155	45.6		
≥$50,000	60	60.6		266	52.78			45	58.4		183	54.4		
Self-reported education at birth							0.57							0.83
≤ High School	19	19.2		110	21.8			13	16.9		61	17.9		
> High School	80	80.8		395	78.2			64	83.1		280	82.1		
Smoking status at birth (yes)	16	16.2		37	7.3		0.005	11	14.3		22	6.5		0.02
Insulin Use (yes)	25	25.5					<0.001							
Pre-pregnacy BMI (kg/m²)			27.4 (6.2)			25.3 (5.9)	0.004			26.9 (5.7)			25.6 (6.0)	0.13
Offspring characteristics														
Age at first research visit (years)			9.6 (1.6)			10.7 (1.4)	<0.001			15.9 (1.0)			16.8 (1.2)	<0.001
Sex (female)	46	46.5		257	50.9		0.42	34	44.2		175	51.3		0.26
Race Ethnicity							0.05							0.17
Non-Hispanic White	60	60.6		229	45.4			48	62.3		165	48.4		
Hispanic	30	30.3		202	40.0			22	28.6		127	37.2		
Non-Hispanic Black	5	5.1		43	8.5			4	5.2		29	8.5		
Other	4	4.0		31	6.1			3	3.9		20	5.9		
Self reported Tanner Stage*							0.02							0.87
Pre-pubertal (TS-1)	62	62.6		216	42.8			0	0.0		0	0.0		
Pubertal (TS-2)	25	25.3		172	34.1			0	0.0		3	0.9		
Pubertal (TS-3)	9	9.1		88	17.4			4	5.2		17	5.0		
Pubertal (TS-4)	3	3.0		27	5.4			32	41.6		134	39.5		
Pubertal (TS-5)	0	0.0		1	0.2			41	53.2		185	54.6		
Child's birthweight (g)			3337.5 (526.6)			3177.8 (575.0)	0.01			3392.0 (498.0)			3188.8 (612.9)	
Body Mass Index (kg/m²)			19.0 (4.6)			18.9 (4.5)	0.86			23.6 (5.2)			23.6 (5.7)	0.96

*Self-reported Tanner staging based on pubic hair for males and breast development for females

Table 2. Summary of data collected on EPOCH participants

	KPCO database	First research visit	Second research visit
Maternal Data			
GDM	X		
Pre-pregnant weight	X		
OGTT results	X		
GDM treatment	X		
Pregnancy complications	X		
Current height & weight		X	
Current blood pressure		X	
Demographics: Race/Ethnicity, SES, Parity		X	X
Offspring Data			
Birth weight	X		
Gestational age	X		
Height & Weight trajectories	Well-Child Clinic Visits		
Current height & weight		X	X
Waist Circumference		X	X
Subcutanious Adipose Tissue (SAT)		X	X
Visceral Adipose Tissue (VAT)		X	X
Skinfolds		X	X
Blood pressure		X	X
Fasting glucose, insulin		X	X
ALT, OGTT (glucose, insulin)			X
Puberty: Tanner Stage (self-report)		X	X
Puberty: Sex hormones		X	X

Figures

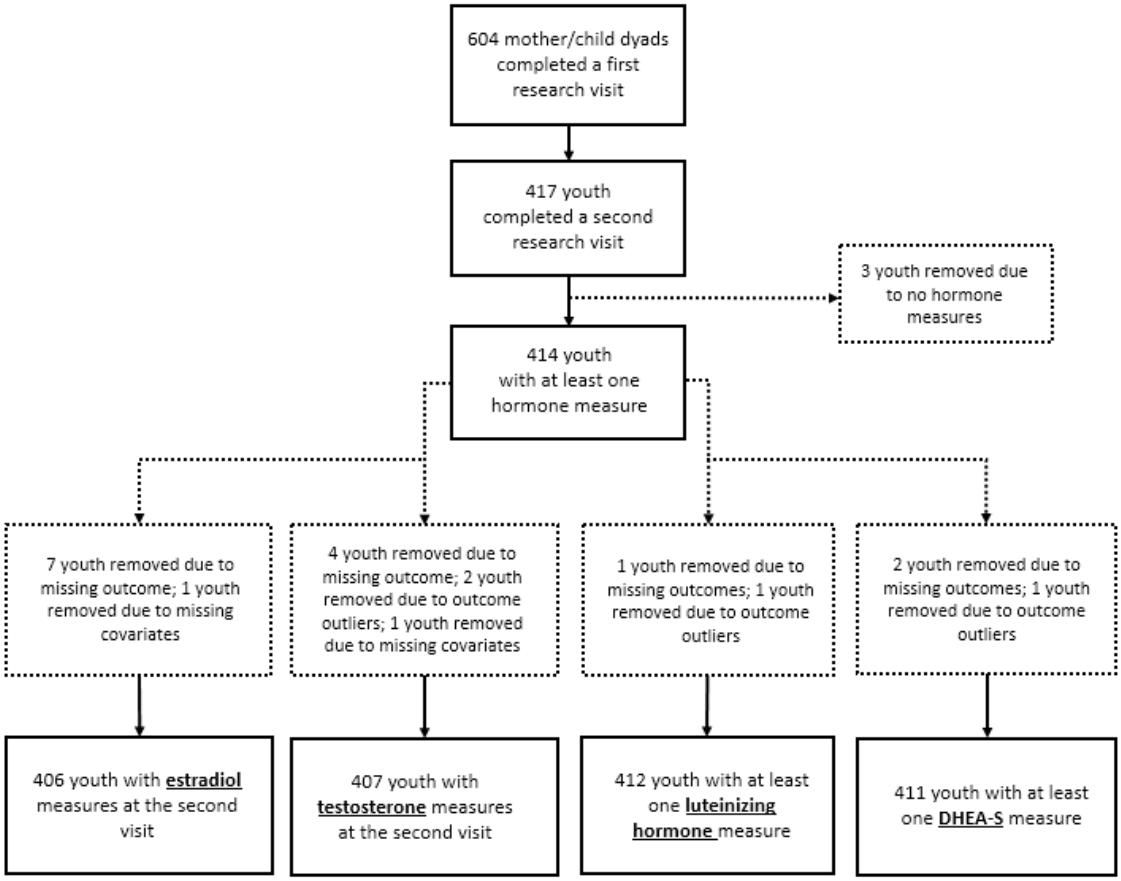


Figure 1. Analytic cohort for specific aim 2: To investigate the relationship between exposure to maternal diabetes and hormone concentrations of puberty, specifically, estradiol, total testosterone, luteinizing hormone and DHEA-S.

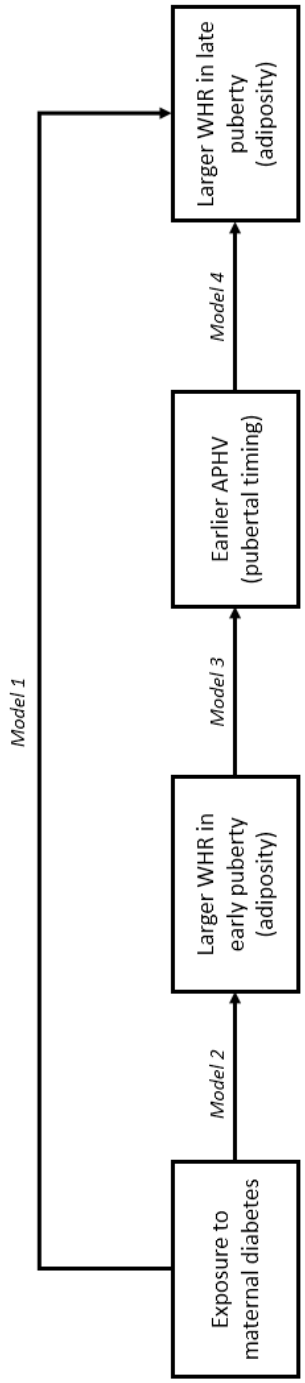


Figure 2. Conceptual model for specific aim 3: to further understand the effect of maternal diabetes on puberty and offspring adiposity, we studied a hypothesized pathway from exposure to maternal diabetes to adolescent adiposity, and the contributions of early pubertal adiposity and pubertal timing along this pathway.

CHAPTER IV

EXPOSURE TO DIABETES *IN UTERO* IS ASSOCIATED WITH EARLIER PUBERTAL TIMING AND FASTER PUBERTAL GROWTH IN THE OFFSPRING: THE EPOCH STUDY

Abstract

Objective: We examined the associations of *in utero* exposure to maternal diabetes with surrogate measures of offspring pubertal timing (age at peak height velocity [APHV]) and speed of pubertal growth (peak height velocity [PHV]). Study Design: Data from 77 exposed and 340 unexposed youth followed from age 2 to 19 years (51% non-Hispanic White, 50% females) were analyzed. Maternal diabetes status was collected from obstetric records and child heights from 2 years to current age from pediatric records. Other covariates (e.g., race/ethnicity) were collected during research visits. The SuperImposition by Translation and Rotation (SITAR) method, using height measurements (4-52 per participant), modeled APHV and PHV. Accelerated failure time analyses were used to test whether exposure to maternal diabetes was associated with younger APHV and faster PHV. Results: Males reached APHV later than females ($p<0.001$). Hispanic and non-Hispanic Black youth reached APHV significantly earlier than non-Hispanic White youth (both, $p<0.001$). Adjusting for sex and race/ethnicity, median APHV was reached ~3 months earlier in youth exposed to maternal diabetes compared to unexposed youth ($p=0.047$). Youth exposed to maternal diabetes had a faster PHV than unexposed youth (sex-by-exposure interaction, $p<0.001$): exposed females had 10.4% greater median PHV compared to unexposed females and exposed males had a 4.0% greater median PHV compared to unexposed males. Conclusion: Our findings provide evidence that exposure to maternal diabetes *in utero* is associated with earlier pubertal timing and faster pubertal growth. Whether earlier puberty or faster speed of growth mediates the association between maternal diabetes exposure and later chronic disease risk remains to be studied.

Introduction

Puberty is a complex physiological process defined by a period of intense hormonal changes and rapid physical growth, leading to psychological and physical maturation. A population-level trend towards earlier pubertal onset has been reported over the last several decades in both males and females[7,60]. Numerous studies have investigated the long-term effects of earlier pubertal timing on chronic diseases in adulthood and have found an association with type 2 diabetes (T2D)[8], cardiovascular disease[61], breast cancer[62], and all-cause mortality[63], especially in females. In males, earlier pubertal timing has been associated with higher adiposity[64], increased blood pressure[65], and a higher risk of cardio-metabolic diseases in adulthood[66].

Exposure to maternal diabetes during the intrauterine life has been shown to result in fetal over-nutrition and endocrine dysfunction[1,14]. Exposure to maternal hyperglycemia causes the developing fetal pancreas to respond by producing additional anabolic hormones and growth factors, which promote growth[14,27]. Furthermore, this exposure has also been linked to increased obesity and cardio-metabolic outcomes in the offspring later in life through fetal programming[1,11,16,67–69]. It is possible that such exposure also plays a role in the programming of puberty onset and maturation. Given the population-level increase in the prevalence of diabetes during pregnancy and the decrease in the age of pubertal onset[60,70–73], understanding if exposure to maternal diabetes influences puberty in the offspring is important. However, few researchers have investigated the impact of maternal diabetes during pregnancy on offspring pubertal timing and velocity[17,18].

When measuring pubertal timing and speed of puberty, the gold standard is assessment of Tanner stages by a pediatric endocrinologist[35,36,74]. The initial description of Tanner stages was by visual inspection alone, but since obesity has been significantly increasing in pre-pubertal children, palpation for thelarche (breast development) and gonadarche (testicular growth) is now considered the best method of assessment. Nonetheless, this type of assessment is difficult to obtain on a large longitudinal cohort. An additional measure of pubertal timing in females is self-reported age of menarche, which is accurate when reported prospectively and in detail. However, there is no measure

of pubertal development in males analogous to menarche in females, which leads to males being studied less often than females. Another hallmark of puberty is a period of rapid growth[37]. As puberty approaches, the growth velocity slows ("preadolescent dip") before it suddenly accelerates ("peak height velocity [PHV]") during mid-puberty, resulting in each individual experiencing a peak height velocity. For females, the age at peak height velocity usually occurs earlier than males, typically during Tanner stage 3; while males usually reach peak height velocity during Tanner stage 4[38,75,76].

We aimed to examine the association of exposure to maternal diabetes during pregnancy with pubertal timing, estimated using age at peak height velocity (APHV), and speed of pubertal growth, estimated using peak height velocity (PHV), using data from an ethnically diverse observational cohort study in Colorado.

Methods

Study population

The Exploring Perinatal Outcomes among Children (EPOCH) study is a historical prospective multiethnic cohort study that recruited 604 mother/child dyads in Colorado. Eligible participants were children exposed to maternal diabetes and a random sample of children not exposed. Participants were offspring of singleton pregnancies, born at a single hospital in Denver between 1992 and 2002, whose biological mothers were members of the Kaiser Permanente of Colorado Health Plan (KPCO). Children and their mothers were invited to participate in two research visits, at average ages of 10.5 years and 16.5 years. Longitudinal heights were obtained from the child's medical records from birth to their current age. For this report, we used data from 417 youth who completed both research visits and had at least 4 or more longitudinal height measurements from 2 years of age to their current age. One particpant was removed from the dataset due to an extereme unrealistic outcome measure. The study was approved by the appropriate Institutional Review Boards. All participants provided written informed consent and the youth provided written assent.

Exposure definition

Exposure to maternal diabetes was defined as a presence of pre-existent diabetes or a physician diagnosis of GDM during the index pregnancy and was ascertained from the KPCO perinatal database, an electronic database that links prenatal and neonatal medical records. All pregnant women at KPCO were routinely screened for gestational diabetes (GDM) at 24-28 weeks using the 2-step standard protocol[53]. GDM was diagnosed if glucose values exceeded $\geq$2 thresholds set by the National Diabetes Data Group on the 3-hour, 100g oral glucose tolerance test[54]. Seventy mothers were diagnosed with gestational diabetes mellitus (GDM) and seven mothers were diagnosed with type 1 diabetes prior to pregnancy. Since our research question focused on the effects of hyperglycemia during pregnancy on puberty we included all diabetes types.

Youth height and weight measurements

Longitudinal heights and weights were obtained from the child's medical records from birth to their current age. For youth with a KPCO enrollment gap or who were no longer a KPCO member, standing heights and weights were obtained from their non-KPCO providers. Longitudinal heights from birth to 2 years of age were excluded from these analyses to focus on the pubertal growth spurt rather than the postnatal growth spurt that occurs during this time. The median number of height measurements for participants from 2 years of age to current age was 19 (range: 4-52 height measurements) and did not differ by exposure status.

In addition, standard anthropometric measures were recorded at each research visit. Height and weight were measured in light clothing and without shoes. Weight was measured to the nearest 0.1 kg, using a portable electronic SECA scale. Height was measured to the nearest 0.1 cm, using a portable SECA stadiometer. Height measured to the nearest 0.1 cm using a stadiometer, if the first two recorded measures were more than 0.5 cm apart then a third measure was taken, the measurements were then averaged. Weight was measured to the nearest 0.1 kg using an electronic, calibrated scale, if the first two recorded measures were more than 0.3 kg apart then a third measure

was taken, the measurements were then averaged. Scales and stadiometers were calibrated every 2 months using standard weights for scales and an aluminum measuring rod for the stadiometer.

Pubertal timing and speed of pubertal growth

Pubertal timing and speed of pubertal growth were defined by APHV and PHV, respectively, and were estimated using longitudinal height records and Superimposition by Translation and Rotation (SITAR) growth curve analysis[56]. The SITAR method uses a shape invariant spline curve and a nonlinear random-effects model to estimate an average growth curve for the entire sample and each individual's deviation from this average curve. There are three subject-specific parameters termed size (cm), velocity (PHV), and timing (APHV); conceptually, these subject-specific parameters define how much bigger or smaller each child is compared to the population average (size), how much faster and slower the child's growth velocity is compared to the population average (velocity), and how much earlier or later the child experienced peak velocity compared to the population average (timing). The models were fitted with the SITAR package in R[57]. Since SITAR estimates individual parameters using the underlying growth curve of the population, four separate models were fitted by sex and maternal diabetes exposure status (exposed females, unexposed females, exposed males, unexposed males). The SITAR model allows for the flexibility in fitting the spline curves by adjusting the degrees of freedom, in doing so, the optimal models are selected by evaluating the BIC score. The lowest BIC score indicates the best fit model. Due to the variability of the longitudinal height measurements, measurements with a residual greater than 4 were excluded from the analysis (n=42), this accounted for less than 0.7% of the total measurements used. The SITAR models of height by age were fitted for each group with 4 degrees of freedom (DF) for exposed females, 5 DF for unexposed females, 6 DF for exposed males, 6 DF for unexposed males. Subject-specific parameters size, velocity, and timing were estimated as random effects. Mean height growth curves from 2 years to current age are shown in Figure 3 by sex and exposure status.

Other measurements

Offspring age at each research visit was calculated from the date of delivery. Race/ethnicity was self-reported at each research visit using the 2000 US Census base questions and categorized as Hispanic (any race), non-Hispanic White, non-Hispanic Black, or non-Hispanic other. Maternal pre-pregnancy BMI (kg/m^2) was calculated from the KPCO measured maternal weight before the last menstrual cycle preceding pregnancy and measured maternal height that was collected at the first research visit. Maternal level of education and total household income at the time of birth were self-reported during the first research visit. Mother's insulin use during pregnancy, delivery method, and child's birth weight were collected from the KPCO perinatal database. Pubertal development at the time of each research visit, including Tanner stage and age of menarche (females only), was ascertained by child's self-report. Youth were categorized as prepubertal (Tanner stage < 2) and pubertal (Tanner stage ≥ 2) using a diagrammatic representation of Tanner staging adapted from Marshall and Tanner[35,36]. Tanner stage based on breast development was used for females and Tanner stage based on pubic hair was used for males. Age of menarche was reported by the child to the half year for females.

Statistical analyses

Accelerated failure time (AFT) models with a log-logistic distribution were used to evaluate the association between exposure to maternal diabetes and pubertal timing (APHV) and speed of pubertal growth (PHV). There was no right censoring of any individuals since APHV and PHV were estimated and achieved by all participants. A time ratio was estimated using the AFT beta coefficients, which can be interpreted as the ratio of the median time-to-event for a given level of a covariant to the referent level. The associations between exposure to maternal diabetes and APHV and PHV were examined separately using two main models: an initial model including adjustment for child's sex and race/ethnicity; and a fully adjusted model including additional adjustment for potential socio-economic confounders (mother's education, total household income at birth). A potential effect modification by child's sex was evaluated using a cross-product term (child's sex*exposure status) in

both models. As a secondary analysis in a subset of data, we explored the effect of the mother's pre-pregnancy BMI on the relationships of maternal diabetes with pubertal timing and speed of pubertal growth. SAS statistical software, version 9.4 (SAS Institute, Inc., Cary, North Carolina) was used for all AFT analyses.

Results

Longitudinal heights (range: 4-52 height measurements per participant, total: 6363 height measurements) were collected on 417 children (77 exposed and 340 unexposed to maternal diabetes) and included in the analytic cohort. There were no significant differences in exposure status, child's race or sex between the analytical cohort and the larger EPOCH cohort of 604 participants. Anthropometric and demographic characteristics are summarized in Table 3. Mean (± SD) current ages of exposed and unexposed youth were 15.8 ± 1.0 and 16.8 ± 1.2 years respectively ($p<0.001$). Of the 77 exposed youth, 44.2% were female and of the 340 unexposed youth 51.2% were female. Youth exposed to maternal diabetes were heavier at birth than youth unexposed (3,392 ± 498 vs. 3,191 ± 616 g, respectively, $p=0.008$). A total of 29% of mothers with diabetes were treated with insulin during their pregnancy. The mean ages at menarche were 11.9 ± 1.4 and 12.3 ± 1.3 years for exposed and unexposed females, respectively ($p = 0.20$). Mean unadjusted APHV (pubertal timing) for exposed and unexposed youth were 12.1 ± 1.4 and 12.2 ± 1.3 years, respectively ($p=0.62$). Mean unadjusted PHV (speed of pubertal growth) for exposed and unexposed youth were 9.3 ± 0.4 and 8.6 ± 0.6 cm/year, respectively ($p<0.001$).

Pubertal timing and exposure to maternal diabetes

After adjustment for the child's sex and race/ethnicity, the median age of pubertal timing was 2% younger, or ~3 month age difference, between exposed youth compared to unexposed youth ($\beta= -0.02$, $p= 0.03$). Table 4 shows the association between exposure to maternal diabetes and pubertal timing adjusting for child's sex and race/ethnicity (model 1), as well as additional covariates (model 2). The interaction between exposure status and child's sex on APHV was not significant, therefore the interaction was excluded from the model. Males reached APHV significantly later than females

(β= 0.15, p<0.001) and Hispanics and non-Hispanic Blacks reached APHV significantly earlier than non-Hispanic Whites (β= -0.03, p< 0.001; β= -0.06, p<0.001, respectively) (Table 4). Further adjustment for socio-economic characteristics (model 2), as well as, additional adjustment for diabetes treatment and delivery method (data not shown) did not influence our findings.

Speed of pubertal growth and exposure to maternal diabetes.

Figure 4 shows the effect modification of child's sex on the association between exposure to maternal diabetes and PHV, adjusting for race/ethnicity (p<0.001). Exposed females had a 10.4% greater PHV than unexposed females (β= 0.10, p<0.001), while exposed males had a 4.0% greater PHV compared to unexposed males (β= 0.04, p<0.001). Further adjustment for socio-economic characteristics, as well as, additional adjustment for diabetes treatment and delivery method (data not shown) did not influence our findings. In a post-hoc analysis, we evaluated the independent effect of exposure on PHV, while controlling for APHV. We found that the effect of exposure to maternal diabetes on PHV remained significant (p<0.001).

Sub-set analyses including maternal pre-pregnancy BMI

Two hundred and ninety-eight mother/child dyads had available pre-pregnancy BMI data (78.9% were unexposed; 46% were females). In the subset with available data, when maternal pre-pregnancy BMI was included the effect of maternal diabetes on APHV become non-significant (β= -0.01, p=0.37) and maternal pre-pregnancy BMI was inversely associated with APHV (β= -0.003, p=0.0004) (Table 5). For speed of pubertal growth, maternal pre-pregnancy BMI was positively associated with PHV (β= 0.003, p=0.01), however, it had no effect on the relationship between maternal diabetes and PHV. The interaction between exposure to maternal diabetes and child's sex was still significant, where exposed females had a 10.6% greater PHV compared to unexposed females (β= 0.10, p<0.001) and exposed males had a 3.5% greater PHV compared to unexposed males (β= 0.03, p<0.001), even after adjustment for pre-pregnancy BMI.

Discussion

We found that youth exposed to maternal diabetes during the intrauterine life had an earlier pubertal timing and a faster speed of pubertal growth than youth who were not exposed, independent of child's sex, race/ethnicity and socio-economic factors. The difference in the speed of pubertal growth between exposed and unexposed was greater in female offspring than in male offspring, and was independent of APHV.

Consistent with previous literature, our study results support the difference in pubertal timing between males and females[77] and among race/ethnicity groups[73]. Our finding of a relationship between exposure to maternal diabetes and earlier pubertal timing is consistent with the limited number of other studies that have explored this relationship[17,18]. Kubo et al. (2016) found that girls of mothers who were obese prior to pregnancy, based on pre-pregnancy BMI, and had GDM during their pregnancy, had an earlier pubertal onset compared to girls of mothers who had neither perinatal condition. Monteilh et al. (2010) found that GDM was associated with a 2 month earlier pubertal onset of pubic hair among males. Although both of these studies used Tanner stages focused on pubic hair, which is specific to androgens, and our study focused on pubertal timing and velocity related to growth, which is predominately driven by estrogens, the effects on pubertal timing were similar to our findings of about a 3-month difference[17,18]. The median difference of 3 months in the pubertal timing shifts the distribution of the population to the left increasing the number of adolescents who experience earlier pubertal timing, which has been shown in previous studies to be linked to an increase in chronic disease risk later in life.

In our study, when using age of menarche instead of APHV for females only, we found a non-significant effect of exposure to maternal diabetes during pregnancy on pubertal timing (results not shown). However, this variable was collected to the half-year (i.e. 12.0 years at age of menarche or 12.5 years at age of menarche) and may have not been a sensitive enough to assess menarche.

To our knowledge, no studies have reported on the effect of maternal diabetes on the speed of pubertal growth. Researchers have shown that pubertal timing is related to physical growth, such as

PHV[78,79], so our results of exposure to maternal diabetes on PHV may be explained by the earlier APHV. However, in a post-hoc analysis, we evaluated the independent effect of exposure on PHV, while controlling for APHV. We found that the effect of exposure to maternal diabetes on PHV remained significant ($p<0.001$) and only changed the beta coefficients of exposure by about 2% in girls and 5% in boys. Therefore, exposure to maternal diabetes had a direct effect on PHV, not explained by APHV. During normal pubertal development, maturation of the hypothalamic-pituitary-gonadal (HPG) axis, along with increased adrenal androgen and growth hormone secretion, drive achievement of pubertal milestones. Previous animal and human studies have shown that maternal diabetes and obesity are associated with offspring adiposity and metabolic dysfunction[1,11,16,67–69]. Thus, the hyperinsulinemia seen in exposed offspring may permanently alter the HPG axis and subsequent sex hormone secretion that is the foundation of pubertal development[34]. These altered sex hormones may explain the sex differences we observed in the association between exposure to maternal diabetes and speed of pubertal growth. Understanding these potential mechanisms are important and should be further studied, as linear growth is an important biomarkers of a child's development and overall health.

The difference in the size of the association between maternal diabetes and pubertal timing vs. pubertal growth observed in our study may be related to the multiple hormonal pathways associated with pubertal onset and maturation. Pubertal timing is primarily dependent on the reemergence of gonadotropin-releasing hormone (GnRH) secretion, which is the initial step in the HPG axis, while pubertal growth is not only dependent on the HPG axis, but also the GH-IGF-1 axis and the interaction of the GH-IGF-1 and HPG axes[80,81]. It is likely that exposure to maternal diabetes influences these axes and their associated hormones differently, resulting in the somewhat larger effects of maternal diabetes exposure on pubertal speed vs pubertal timing. Another explanation could be that these differences are due to possible variability in the sensitivity of the APHV and PHV measurements.

We also explored the effect of additional adjustment for maternal pre-pregnancy BMI on the relationship between maternal diabetes exposure and markers of pubertal status in the offspring. Although only in a subset, we found that adjustment for maternal BMI attenuated the relationship. Since pre-pregnancy BMI is highly related to maternal diabetes, it may be part of the fetal overnutrition pathway[12], in which case this modeling may result in over adjustment. Alternatively, such adjustment may partially control for competing mechanisms, such as genes associated with both obesity and timing of puberty. As for speed of pubertal growth, maternal pre-pregnancy BMI had no effect on the relationship between exposure to maternal diabetes and speed of pubertal growth.

While our findings represent an important addition to the existing evidence regarding risk factors for earlier puberty, further investigation into potentially responsible mediators, specifically hormones related to the GH-IGF-1 and HPG axes, is needed. Additionally, further study is required to understand the role that puberty may play on the relationship between exposure to diabetes *in utero* and subsequent disease risk in the offspring. It is possible that puberty may be another critical or sensitive period in the lifecycle when the effects of *in utero* exposure to maternal diabetes may be enhanced via biological programming.

This prospective study is one of the largest cohorts to investigate the relationships between maternal diabetes and offspring outcomes. A potential limitation of our study was the inability to control for genetic factors associated with pubertal timing and growth. However, this would not bias our results, only decrease our precision, unless the genes in question were also associated with maternal diabetes, which has not been shown to date. Additionally, the height measurements used in calculating APHV and PHV were not obtained in a standardized fashion, which introduces variability and decreases the precision of the APHV and PHV estimates. Further research should examine whether the relationship between exposure to maternal diabetes and pubertal development mediates, at least in part, the increased risk of obesity and cardio-metabolic outcomes associated with this exposure. Such findings may then provide evidence for life-stage targeted interventions aimed at reducing or halting the transgenerational vicious cycle of diabetes and obesity.

In summary, these novel findings provide evidence that exposure to diabetes during the intrauterine life may affect pubertal development and growth in the offspring, and support the hypothesis that perinatal exposures are among the multiple contributors to the general trend of earlier puberty seen over the last couple of decades.

Tables

Table 3. Characteristics of youth and their mothers by maternal diabetes exposure

Variable	Unexposed to DM (n=340)			Exposed to DM (n=77)			p-value
	N	%	Mean (SD)	N	%	Mean (SD)	
Current age (years)			16.8 (1.2)	77		15.8 (1.0)	<0.0001
Sex (female)	174	51.2		34	44.2	34 (44.2)	0.27
Race/Ethnicity							0.18
Non-Hispanic White	165	48.5		48	62.3		
Hispanic	126	37.1		22	28.6		
Non-Hispanic Black	29	8.5		4	5.2		
Non-Hispanic Other	20	5.9		3	3.9		
Self-reported Tanner Stage at current age^							0.71
Pre-pubertal (TS-1)	0	0.0		0	0.0		
Pubertal (TS-2)	3	0.9		0	0.0		
Pubertal (TS-3)	17	5.0		4	5.2		
Pubertal (TS-4)	133	39.4		32	41.6		
Pubertal (TS-5)	185	54.7		41	53.3		
Child's Age at Menarche (female only)			12.3 (1.3)			11.9 (1.4)	0.20
Child's birthweight (g)			3190.5 (613.0)			3392.0 (498.0)	0.008
Mother's pre-pregnancy BMI (kg/m2)	235		25.6 (6.0)	63		26.9 (5.7)	0.12
Mother's self-reported income at birth							0.51
≤$49,999	155	45.7	155 (45.7)	32	41.6		
≥$50,000	184	54.3	184 (54.3)	45	58.4		
Mother's self-reported education at birth							0.83
≤ High School	61	17.9		13	16.9		
> High School	279	82.1		64	82.1		
Mother's Insulin Use (Yes)				22	29.0		

Abbreviations: BMI, body mass index; DM, maternal diabetes; TS, Tanner stage

^ Females breast TS, males pubic hair TS

Table 4. The association between exposure to maternal diabetes and age at peak height velocity

Variable	Model 1				Model 2			
	TR	ß	95% CI	p-value	TR	ß	95% CI	p-value
Race/Ethnicity								
Non-Hispanic Whites	1.00	Referent	Referent	Referent	1.00	Referent	Referent	Referent
Non-Hispanic Black	0.94	-0.06	(-0.09, -0.04)	<0.001	0.98	-0.06	(-0.09, -0.03)	<0.001
Hispanics	0.97	-0.03	(-0.05, -0.01)	<0.001	0.96	-0.03	(-0.04, -0.01)	<0.002
Non-Hispanic Other	0.97	-0.03	(-0.06, 0.005)	0.09	0.99	-0.03	(-0.06, 0.007)	0.12
Sex								
Females	1.00	Referent	Referent	Referent	1.00	Referent	Referent	Referent
Males	1.16	0.15	(0.14, 0.17)	<0.001	1.16	0.15	(0.14, 0.16)	<0.001
Exposure status								
Unexposed to DM	1.00	Referent	Referent	Referent	1.00	Referent	Referent	Referent
Exposed to DM	0.98	-0.02	(-0.04, -0.002)	0.03	0.99	-0.02	(-0.04, -0.003)	0.03
Maternal Income								
<$50,000/year					1.00	Referent	Referent	Referent
≥$50,000/year					1.01	0.01	(-0.006, 0.03)	0.23
Maternal Education								
High School					1.00	Referent	Referent	Referent
Any College					1.00	0.006	(-0.01, 0.03)	0.57

Abbreviations: TR, time ratio AFT, accelerated failure time; APHV, age at peak height velocity; DM, maternal diabetes
Model 1 adjusted for race/ethnicity and child's sex
Model 2 adjusted for child's race/ethnicity, child's sex, maternal income and maternal education

Table 5. The association between exposure to maternal diabetes and age at peak height velocity adjusting for pre-pregnancy BMI (n=298)

Variable	TR	ß	95% CI	p-value
Race/Ethnicity				
Non-Hispanic Whites	1.00	Referent	Referent	Referent
Non-Hispanic Black	0.98	-0.05	(-0.08, -0.01)	0.01
Hispanics	0.96	-0.02	(-0.04, -0.002)	<0.03
Non-Hispanic Other	0.99	-0.006	(-0.048, 0.035)	0.76
Sex				
Females	1.00	Referent	Referent	Referent
Males	1.16	0.16	(0.14, 0.17)	<0.001
Exposure status				
Unexposed to DM	1.00	Referent	Referent	Referent
Exposed to DM	0.99	-0.01	(-0.03, 0.01)	0.28
Pre-pregnancy BMI	1.00	-0.003	(-0.004, -0.001)	<0.001

Abbreviations: TR, time ratio AFT, accelerated failure time; APHV, age at peak height velocity; DM, maternal diabetes
Adjusted for child's race/ethnicity, child's sex, and maternal pre-pregnancy BMI

Figures

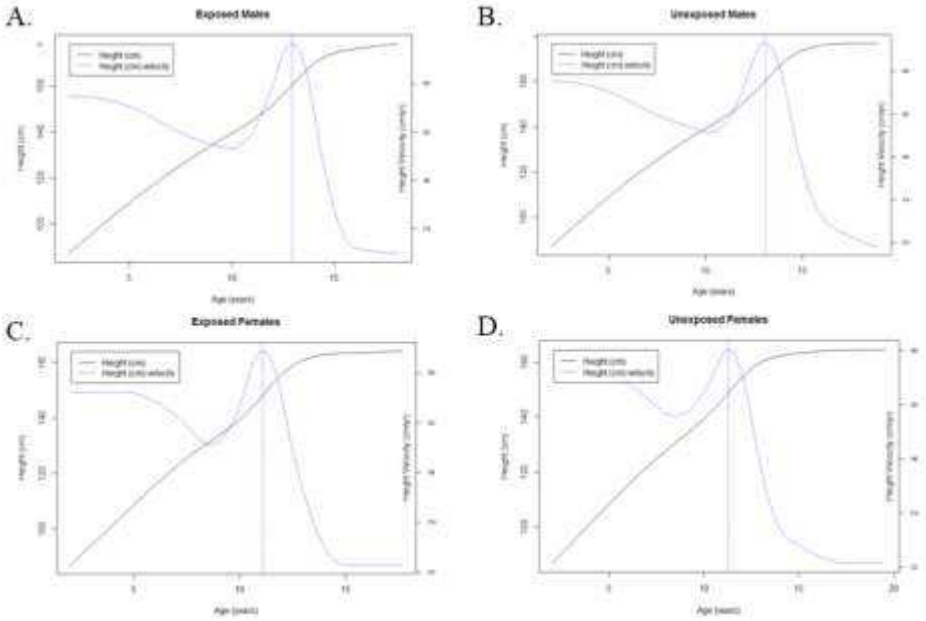


Figure 3. The SITAR output for exposed males (A), unexposed males (B), exposed females (C), unexposed females (D). Within each graph, shows the group mean height trajectory (black solid line), height velocity (blue dashed line), and the age at which the maximum height velocity was achieved (vertical dotted line).

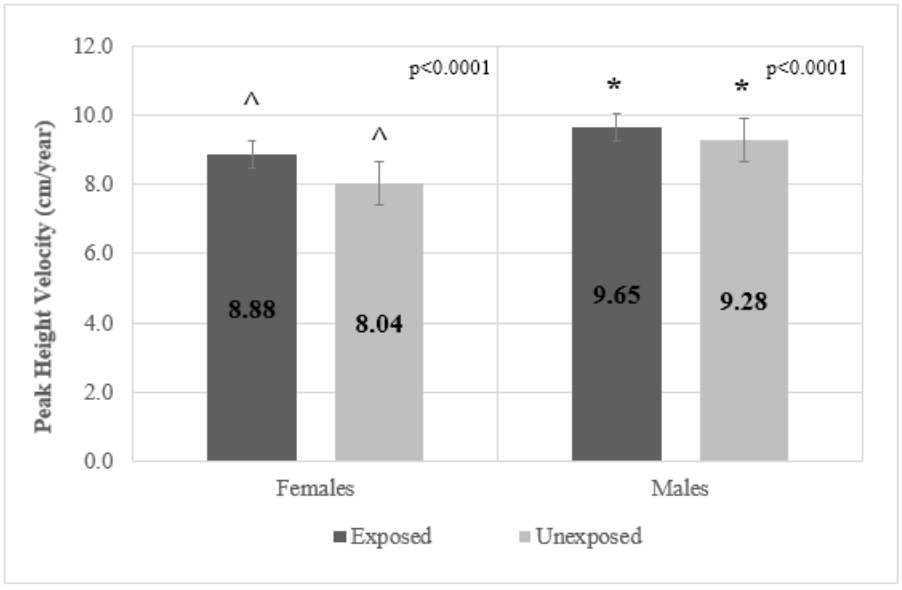


Figure 4. The sex difference in the association of exposure to diabetes *in utero* with offspring peak height velocity using a log-logistic AFT model.

CHAPTER V.

EFFECTS OF INTRAUTERINE EXPOSURE TO MATERNAL DIABETES ON OFFSPRING HORMONES OF PUBERTY: THE EPOCH STUDY

Abstract

Objective(s): We examined the association of exposure to maternal diabetes in utero with offspring hormones of puberty in females and males, specifically luteinizing hormone (LH), dehydroepiandrosterone sulfate (DHEA-S), estradiol (E2), and total testosterone (TT). We also explored the degree to which these associations were accounted for by offspring body size. Study Design: Four hundred fourteen youth who were exposed (n=76) and not exposed (n=338) to maternal diabetes in utero completed two research visits at average ages of 10.5 (SD=1.5) and 16.7 (SD=1.2) years. Pubertal hormones were analyzed from fasting blood samples taken at each visit. We used linear models to evaluate the association of maternal diabetes with offspring hormones, adjusting for age, race/ethnicity, Tanner stage, and household income. Results: Child's sex modified the relationship between exposure to maternal diabetes and luteinizing hormone concentrations (p=0.016): exposed females had 29% higher luteinizing hormone concentrations than unexposed females (2.3 mIU/mL vs. 1.8 mIU/mL, p=0.048), while no such relationship was observed in males p=0.17). Maternal diabetes exposure was not associated with estradiol (p=0.88), total testosterone (p=0.11), and DHEA-S (p=0.45) concentrations and there was no effect modification by offspring sex. Additional adjustment for offspring body mass index (BMI) did not significantly change the associations between maternal diabetes exposure and offspring hormones for females or males. Conclusion: Our results provide some evidence that intrauterine exposure to maternal diabetes may potentially influence hormonal pathways related to puberty, specifically luteinizing hormone, in a sex-specific manner, affecting females but not males. Further research is needed to determine whether such hormones mediate the association between maternal diabetes exposure and later chronic disease risk.

Introduction

Puberty is a complex physiological process defined by a period of intense hormonal changes and rapid physical growth, leading to psychological and physical maturation. Normally, puberty is centrally activated as the hypothalamic-pituitary-gonadal (HPG) axis initiates secretory pulses of gonadotropin releasing hormone (GnRH)[34]. The sustained increase in pulsatile levels of GnRH induce gonadotropin (luteinizing hormone [LH] & follicle stimulating hormone [FSH]) synthesis and secretion from the pituitary gland, which initiate gonadal development, synthesis and secretion of sex steroids and gamete maturation.

A person's sex is a major determinant of hormone concentrations and is determined during the early embryonic phase of intrauterine development. These sex differences in hormone concentrations drive physical differences between males and females that exist throughout the life course. For example, females have higher estrogen production promoting breast and uterine development and contributing to body fat redistribution during and after puberty, while males have higher androgen production promoting changes in the musculoskeletal system and spermatogenesis[34]. Sex hormones also differentially influence the development of adipose tissue: estrogen promotes adiposity, while testosterone and dehydroepiandrosterone sulfate (DHEA-S) inhibit adiposity[82]. Sex hormones themselves can be further influenced by obesity in both childhood[83] and adulthood[84]. An imbalance in the regulation of hormones, specifically estradiol (E2), total testosterone (TT), LH and DHEA-S, have been associated with obesity[82,84,85], polycystic ovarian syndrome (PCOS)[86], cardio-metabolic diseases[87], cancers[88,89], and impaired brain function[90,91].

Exposure to maternal hyperglycemia during the intrauterine life has been shown to lead to fetal over-nutrition and endocrine dysfunction, causing the developing fetal pancreas to respond by producing additional anabolic hormones and growth factors[1,14]. Furthermore, this exposure has been linked to increased obesity and cardio-metabolic outcomes in the offspring later in life through fetal programming[1,11,16,68,69,92]. It is possible that such exposure also plays a role in the programming of pubertal hormones. Given the recent finding that maternal diabetes influences offspring's pubertal

timing[17,18,] and growth velocity[94], it is important to understand if this exposure influences another component of puberty in the offspring. Therefore, the purpose of this analysis was to examine the association of intrauterine exposure to maternal diabetes with hormones of puberty, specifically estradiol, total testosterone, luteinizing hormone and DHEA-S in females and males. We also explore the degree to which these associations are accounted for by offspring body size.

Methods

Study population

The Exploring Perinatal Outcomes among Children (EPOCH) study is a historical prospective multiethnic cohort study that recruited 604 mother/child dyads in Colorado. Eligible participants were children exposed to maternal diabetes and a random sample of children not exposed. Participants were offspring of singleton pregnancies, born at a single hospital in Denver between 1992 and 2002, whose biological mothers were members of the Kaiser Permanente of Colorado Health Plan (KPCO). Children and their mothers were invited to participate in two research visits at average ages of 10.5 (SD=1.5) and 16.7 (SD=1.2) years, at which time demographic, anthropometric, and hormone measures were collected and pubertal staging was assessed. The study was approved by the appropriate Institutional Review Boards. Mothers provided written informed consent and the youth provided written assent.

Exposure definition

Exposure to maternal diabetes was defined as a presence of pre-existent diabetes or a physician diagnosis of GDM during the index pregnancy and was ascertained from the KPCO perinatal database, an electronic database that links prenatal and neonatal medical records. All pregnant women at KPCO were routinely screened for gestational diabetes (GDM) at 24-28 weeks using the 2-step standard protocol[53]. GDM was diagnosed if glucose values exceeded $\geq$2 thresholds set by the National Diabetes Data Group on the 3-hour, 100g oral glucose tolerance test[54]. Sixty-nine mothers were diagnosed with gestational diabetes mellitus (GDM) and seven mothers were diagnosed

with type 1 diabetes prior to pregnancy. Since our research question focused on the effects of hyperglycemia during pregnancy on puberty, we included both types of maternal diabetes.

Outcome measurements

During the first and second research visit, we collected a fasting venous blood draw from the offspring. Sera from the first research visit were frozen and stored at -80^{o}C for an average of 6.2 years. Sera from the second research visit were refrigerated and analyzed within 24 hours of collection. Sera from the first and second research visits were analyzed at the same time. We measured E2, TT, LH, and DHEA-S in all samples. Serum estradiol was measured by using a Beckman Coulter Chemilluminescence with a sensitivity of 10.0 pg/mL. Serum testosterone (total) was measured by using a Beckman Coulter 1-step competative with sensitivity of 17 ng/dL. Serum luteinizing hormone was determined by using a Beckman Coulter Chemilluminescence with a sensitivity of 0.12 mIU/mL. Serum DHEA-S was measured by using a Beckman Coulter Chemilluminescence with a sensitivity of 2 ug/dL. Due to the majority of the offspring being pre-pubertal during the first research visit over 60% of the samples had an undetectable value for estradiol and testosterone. Therefore, we did not include the estradiol and testosterone hormone data from the first research visit in our statistical analyses.

Other measurements

Offspring age at each research visit was calculated from the date of delivery. Race/ethnicity was reported at each research visit using the 2000 US Census base questions and categorized as Hispanic (any race), non-Hispanic White, non-Hispanic Black, or non-Hispanic other. For this analysis, we collapsed race/ethnicity into two race categories, Non-Hispanic White and Other, due to small sample sizes in each category. Maternal level of education, total household income, and smoking status at the time of birth were self-reported during the first research visit. Offspring height and weight were measured in light clothing and without shoes at each research visit. Body Mass Index (BMI) was calculated by weight (kg) divided by height (cm) squared. Pubertal development was self-reported by the offspring at each visit using diagrammatic representations of Tanner staging

adapted from Marshall and Tanner[94]. Tanner stage (1-5) was classified on the basis of pubic hair for males and breast development for females.

Statistical analyses

Analyses were conducted in SAS 9.4 (SAS Institute, Cary, NC). Outcome variables were examined for normality and all required log transformation prior to analysis. Age at each research visit was centered to the mean of the analytic sample as this was a meaningful reference value. Descriptive statistics are presented using percentages or means and standard deviations. Cochran-Mantel-Haenszel tests and t-tests were used to identify bivariate associations between characteristics and exposure to maternal diabetes. Although mean values are shown for untransformed data, all P values are based on log-transformed data. Three outliers that had standardized residuals greater than five were identified in the dataset: one outlier for DHEA and LH concentrations, and two outliers for TT concentrations. These outliers were removed from their relevant model (Figure 5).

The associations of exposure to maternal diabetes with offspring hormone concentrations were evaluated using separate linear models for each hormone. For hormones analyzed for only the second research visit (E2 and TT), general linear models were used for analyses. For hormones analyzed for both research visits (LH and DHEA-S), linear mixed models were used with repeated measures included in each model with time as the repeated effect. The linear mixed models distinguish variability between participants and the variability between repeated measurements over time within participants. Hormone concentrations were modeled using a three-step procedure. First, initial models were constructed predicting each of the offspring's hormone (E2, TT, LH, DHEA-S) concentrations. The covariates included in the initial model were child's sex, race, Tanner stage, and age, which were chosen *a priori* and remained in each model regardless of significance. An exposure-by-sex interaction was included *a priori* due to the known sex difference in hormone concentrations. If the interaction was not significant, it was removed and the main effect of exposure became the variable of interest. As the relationships between hormone concentrations and age are non-linear, the highest significant quadratic term for age (age squared) was used. We also considered the following

two-way interactions and were removed from the model if they were not significant: sex-by-age and Tanner stage-by-age. Secondly, models were constructed using the initial model with the addition of perinatal characteristics (maternal education, household income and maternal smoking status). Lastly, the initial model was further adjusted for offspring current BMI to evaluate whether associations of intrauterine exposure with offspring pubertal hormones were mediated by or independent of offspring body size. An exposure-by-BMI interaction was also tested.

Alpha was set at 0.05 for statistical significance. We report β estimates, 95% confidence intervals (95%CI), and p-values that reflect a status change for exposure to maternal diabetes (exposed versus unexposed). For the log-transformed hormone concentrations, we back-transformed the resulting beta estimates and 95% confidence intervals and interpreted the coefficient estimate as a multiplicative change in the hormone concentrations for those exposed versus those not exposed to maternal diabetes.

Results

Four hundred fourteen youth completed two research visits and had at least one of the four hormone concentrations measured were included (Figure 5). The analytic sample of 414 was similar to the full cohort of 604 in all aspects, except that the analytic sample had a higher percentage of mothers who completed more than a high school education (82.6 vs. 78.6%) and a higher percentage of non-Hispanic Whites (51.0% vs. 47.9%).

Maternal and offspring characteristics by exposure status are summarized in Table 6. Mean (± SD) ages of exposed and unexposed youth were 9.6 ± 1.6 and 10.7 ± 1.4 years for the first research visit, respectively ($p<0.001$), and 15.9 ± 1.0 and 16.8 ± 1.2 years for the second research visit, respectively ($p<0.001$). About 15% percent of mothers with diabetes during pregnancy smoked during the index pregnancy compared to 6% of mothers without diabetes ($p<0.02$). Sixty-two percent of exposed youth were non-Hispanic White compared to 49% in unexposed youth ($p<0.04$). There was a larger proportion of exposed youth who reported Tanner stage 1 and 2 at the first research visit

compared to unexposed youth, however there was no difference in the proportion of youth who reported Tanner stage 4 and 5 at the second research visit (Table 6).

Child's sex modified the relationship between exposure to maternal diabetes and luteinizing hormone concentrations (p=0.016). Table 7 shows that exposed females had 29% higher luteinizing hormone concentrations than unexposed females (β=0.253, p=0.048), while exposed males had no difference in luteinizing hormone concentrations compared to unexposed males (β=-0.159, p=0.17). Inclusion of perinatal characteristics slightly attenuated the association of diabetes exposure with luteinizing hormone concentrations in females, and addition of child's BMI did not have any effect on the association (Table 7). Maternal diabetes exposure was not associated with estradiol (β=0.018, p=0.88), TT (β=0.094, p=0.11), or DHEA-S (β=0.040, p=0.45) concentrations and there was no effect modification by sex on the relationship between exposure to maternal diabetes and estradiol (β=-0.216, p=0.31), total testosterone (β=0.136, p=0.21), or DHEA-S (β=0.093, p=0.37) concentrations. Inclusion of perinatal characteristics or child's BMI did not change any of these associations (Table 8).

Figure 6 shows estimated mean hormone concentrations for exposed and unexposed females and males at a mean age of 11.2 years, adjusting for child's race, Tanner Stage, sex and age. The sex-by-age and sex-by-age^2 interactions were included in the model predicting luteinizing hormone concentrations to account for the hormone differences in sex and age. Exposed females had an average luteinizing hormone concentration of 2.3 mIU/mL at the age of 11.2 years compared to unexposed females with an average of 1.8 mIU/mL at the age of 11.2 years; this is about a 28% increase in luteinizing hormone concentration. No significant differences were found for the other hormones—estradiol, testosterone and DHEA-S.

Discussion

We found that intrauterine exposure to maternal diabetes is associated with higher mean concentrations of luteinizing hormone in females, but not in males, throughout adolescence, and that this association is not attenuated by adjustment for the offspring's concurrent BMI. These data

provide some evidence for the fetal programming effect of maternal diabetes on hormones in female offspring and raise the question of whether alterations in these hormones, luteinizing hormone in particular, are associated with later chronic disease risk.

Animal and human studies have established that maternal diabetes leads to persistent changes in offspring metabolism consistent with abnormal control of energy regulation and obesity[95–99]. Consistent with other studies[100–102], we have recently reported that exposure to maternal diabetes is associated with decreased offspring insulin sensitivity and compensatory hyperinsulinemia in this cohort[100]. This may explain why exposed youth have increased luteinizing hormone concentrations, since insulin acts directly on GnRH signaling leading to increase in luteinizing hormone production[34]. The increased luteinizing hormone concentrations in exposed females and our previous finding of earlier pubertal timing in youth exposed to maternal diabetes[93] supports the notion that exposure to maternal diabetes is associated with alterations to multiple aspects of puberty.

Increased luteinizing hormone and androgen concentrations due to altered GnRH signaling is are hallmarks of polycystic ovary syndrome (PCOS), a complex disease in women with many phenotypes and risk factors, including earlier pubertal onset and being born small-for-gestational age[103,104]. Our results warrant further investigation to determine whether exposure to maternal diabetes is associated with PCOS and if this relationship is driven by the effect of maternal diabetes on pubertal development.

Our finding of different associations between exposure to maternal diabetes and luteinizing hormone between females and males may be explained by the sex-specific hormone regulation of the offspring gonads (testes vs. ovaries); however, this is an area of limited research. There is also some evidence of sex differences in fetal programming effects on cardio-metabolic diseases. For example, in animal studies, maternal undernutrition was associated with increased blood pressure[105] and abnormal vascular function in peripheral arties in males only[106], while exposure to overnutrition during pregnancy was associated with hypertension in female offspring[107]. Epidemiological studies also found sex-specific associations between a variety of perinatal exposures and risk factors for type

2 diabetes, cardiovascular disease, and obesity[39,40]. Our findings of sex differences in the effect of exposure to maternal diabetes on luteinizing hormone concentrations may help explain the sex differences seen by others in later disease risk.

Sex hormones are closely tied to the regulation of adiposity and drive the differences in the sex-specific distribution of body fat, which have important effects on the regulation of energy balance. Our results suggest that current offspring BMI does not mediate the relationship between exposure to maternal diabetes and increased luteinizing hormone concentrations and support a direct effect of exposure to maternal diabetes on luteinizing hormone concentrations.

Kubo et al. found that exposure to maternal obesity and gestational diabetes was associated with earlier onset of pubarche (appearance of pubic hair)[17], which is driven in females predominately by adrenocortical androgens, such as DHEA and DHEA-S. We did not find an association between maternal diabetes and offspring DHEA-S concentrations. We also did not find an association between maternal diabetes and offspring concentrations of estradiol or total testosterone. While there may be no true relationship between maternal diabetes and offspring concentrations of estradiol and total testosterone, there are several limitations that may explain the null finding. For example, the hormone concentrations may have too little variable to detect a significant difference between exposed and unexposed offspring, particularly at the low ranges found at these ages. Moreover, we did not have a measure of biological testosterone action (free testosterone). Due to the sensitivity of the hormone assays and the young ages of our cohort during the first research visit, over 60% of the estradiol and total testosterone concentrations at the first research visit were below the sensitivity level and were not included in data analysis; the analyses of estradiol and total testosterone concentrations were solely based on measurements obtained from the second research visit. An additional limitation was the limited number of hormone markers available. Further studies are needed to expand the number of hormones of interest, such as sex-hormone binding globulin hormone, free testosterone, or insulin-like growth factor-1 and insulin-like growth factor binding

proteins, to better understand how exposure to maternal diabetes may affect the underlying hormone physiology in adolescents.

In summary, our results provide some evidence that exposure to maternal diabetes affects hormonal pathways related to puberty in a sex-specific manner. These data also raise the possibility that the association between maternal diabetes exposure and later chronic disease risk is mediated, at least in part, by alterations in the physiology of puberty and the effects that these alterations may have on adult body composition and metabolism.

Tables

Table 6. Descriptive statistics of exposed (n=76) and unexposed (n=338) youth and their mothers by exposure status.

	Exposed			Unexposed			p-value
Maternal characteristics	N	%	Mean (SD)	N	%	Mean (SD)	
Self-reported household income at birth							0.60
≤$49,999	32	42.1		153	45.4		
≥$50,000	44	57.9		184	54.6		
Self-reported education at birth							0.94
≤ High School	13	17.1		59	17.5		
> High School	63	82.9		279	82.5		
Smoking status at birth (yes)	11	14.5		21	6.2		<0.02
Offspring characteristics							
Age at first research visit (years)			9.6 (1.6)			10.7 (1.4)	<0.001
Age at second research visit (years)			15.9 (1.0)			16.8 (1.2)	<0.001
Sex (female)	33	43.4		174	51.5		0.20
BMI at first research visit (kg/m^2)			18.6 (3.8)			18.8 (4.6)	0.71
BMI at second research visit (kg/m^2)			23.6 (5.3)			23.7 (5.7)	0.97
Race/Ethnicity							<0.04
Non-Hispanic White	47	61.8		164	48.5		
Other	29	38.2		174	51.5		
Self-reported Tanner Stage at first research visit^							<0.01
Pre-pubertal (TS-1)	48	63.2		136	40.4		
Pubertal (TS-2)	19	25.0		122	36.2		
Pubertal (TS-3)	6	7.9		56	16.6		
Pubertal (TS-4)	3	4.0		22	6.5		
Post-pubertal (TS-5)	0	0.0		1	0.3		
Self-reported Tanner Stage at second research visit^							0.87
Pre-pubertal (TS-1)	0	0.0		0	0.0		
Pubertal (TS-2)	0	0.0		3	0.9		
Pubertal (TS-3)	4	5.3		17	5.0		
Pubertal (TS-4)	31	40.8		133	39.5		
Post-pubertal (TS-5)	41	54.0		184	54.6		

^Self-reported Tanner staging based on pubic hair for males and breast development for females.
†Mean values are from untransformed data and the p-values were obtained from t-tests on natural log-transformed data.

Table 7. Effect modification by child's sex of the association between maternal diabetes and offspring luteinizing hormone concentrations.

Outcome ‡	Exposed Females			Exposed Males		
	Relative association	95% CI	p-value	Relative association	95% CI	p-value
Luteinizing Hormone						
Model 1: Initial model	1.29	1.00, 1.66	0.048	0.85	0.68, 1.07	0.17
Model 2: Initial + Maternal Characteristics	1.28	0.99, 1.65	0.057	0.84	0.67, 1.06	0.13
Model 3: Initial + Offspring BMI	1.29	1.00, 1.66	0.049	0.86	0.69, 1.08	0.20

BMI, body mass index; DHEA-S, dehydroepiandrosterone sulfate

Model 1 included the following covariates—exposure-by-sex interaction, child's sex, race/ethnicity, Tanner stage, age, age^2, age-by-sex and age^2-by-sex interactions

Model 2 included the covariates in Model 1 and household income, maternal education at birth and smoking status at birth.

Model 3 included the covariates in Model 1 and offspring BMI

‡ Outcome measures were natural log-transformed for analyses; the relative associations are based on the exponentiation of the coefficient estimates and are interpreted as a multiplicative change in the mean outcome with status change in maternal diabetes exposure.

Table 8. The association between maternal diabetes and offspring estradiol, total testosterone and DHEA-S concentrations.

Outcomes ‡	Relative association	95% CI	p-value
Estradiol			
Model 1: Initial model	1.02	0.81, 1.27	0.88
Model 2: Initial + Maternal Characteristics	1.03	0.82, 1.29	0.81
Model 3: Initial + Offspring BMI	1.02	0.81, 1.27	0.89
Total Testosterone			
Model 1: Initial model	1.10	0.98, 1.23	0.11
Model 2: Initial + Maternal Characteristics	1.10	0.98, 1.24	0.10
Model 3: Initial + Offspring BMI	1.09	0.97, 1.22	0.14
DHEA-S			
Model 1: Initial model	1.04	0.94, 1.15	0.45
Model 2: Initial + Maternal Characteristics	1.04	0.93, 1.15	0.49
Model 3: Initial + Offspring BMI	1.04	0.94, 1.16	0.41

BMI, body mass index; DHEA-S, dehydroepiandrosterone sulfate

Model 1 included the following covariates—exposure status, child's sex, race/ethnicity, Tanner stage, age, and age^2.

Model 2 for each outcome included the covariates in Model 1 and household income, maternal education at birth and smoking status at birth.

Model 3 for each outcome included the covariates in Model 1 and offspring BMI

‡ Outcome measures were natural log-transformed for analyses; the relative associations are based on the exponentiation of the coefficient estimates and are interpreted as a multiplicative change in the mean outcome with status change in maternal diabetes exposure.

Figures

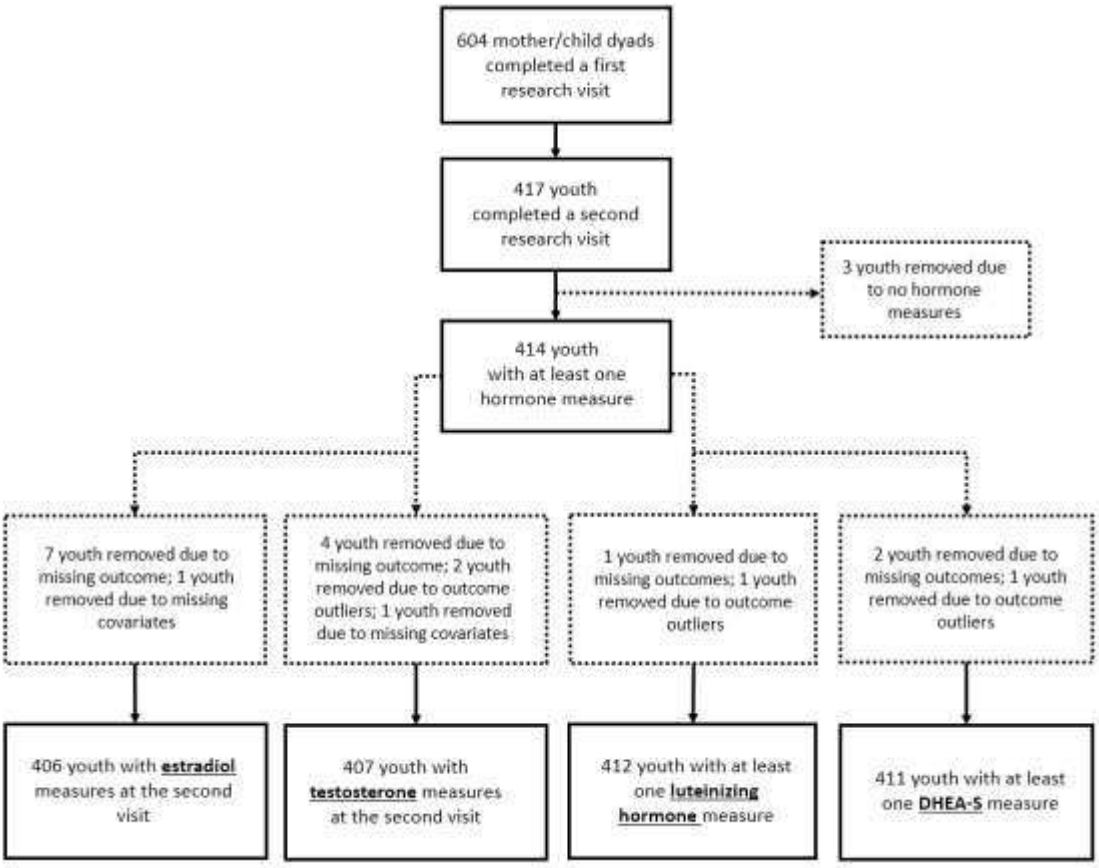


Figure 5. Flow diagram of the analytic sample for each outcome.

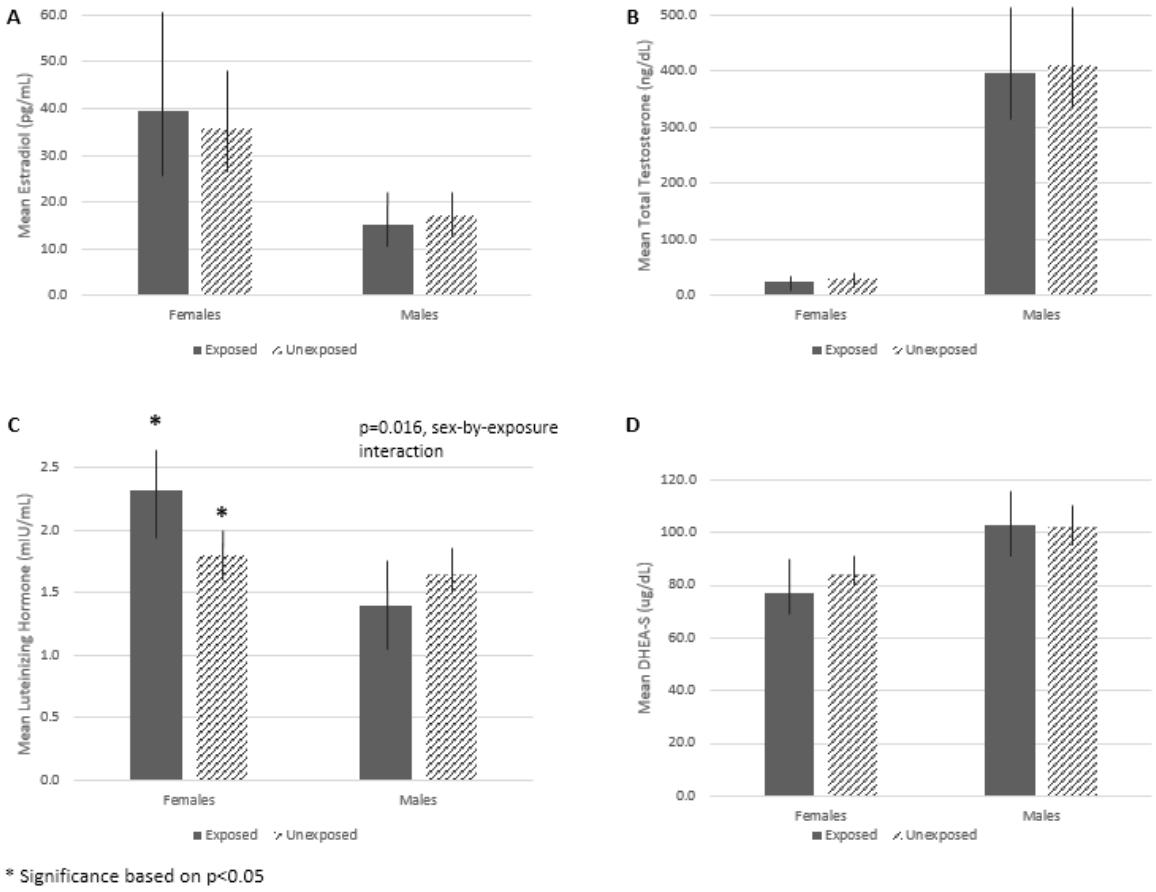


Figure 6. Estimated mean hormone concentrations for exposed and unexposed females and males at a mean age of 11.2 years, adjusting for child's race, Tanner Stage, and age. Two sex-by-age and sex-by-age^2 interactions were included in the model predicting LH concentrations. Graph A represents mean estradiol, graph B represents mean total testosterone, graph C represents mean luteinizing hormone, and graph D represents mean DHEA-S.

CHAPTER VI

EXPOSURE TO MATERNAL DIABETES IN UTERO, PUBERTAL TIMING AND OFFSPRING ADIPOSITY: THE EPOCH STUDY

Abstract

Objective(s): We examined the relationship between intrauterine exposure to maternal diabetes and childhood adiposity, as assessed by waist-to-height ratio (WHR) in early and late puberty, and the contribution of pubertal timing to this relationship, using data from an ethnically diverse observational cohort study in Colorado. Study Design: A total of 364 youth (69 exposed and 295 unexposed to maternal diabetes) completed two research visits in early [Tanner Stages 1-3, mean age 10.1 (SD=1.4) years] and late puberty [Tanner Stage 4-5, mean age 16.5 (SD=1.2) years]. Heights, weights and waist circumference measurements were collected at each visit and were used to calculate waist-to-height ratio (WHR). Age at peak height velocity (APHV), a measure of pubertal timing, was estimated by the SuperImposition by Translation and Rotation (SITAR) method, using longitudinal height measurements collected from pediatric records. Separate multiple linear regression models evaluated the association between exposure to maternal diabetes and WHR in late puberty, as well as the contributions of early-puberty WHR and of pubertal timing along this pathway. Results: Compared to unexposed children, those exposed to maternal diabetes had a 0.27 standard deviation (SD) increase in late-pubertal WHR (R^2=0.055, p=0.06) and a 0.40 SD increase in early-pubertal WHR (R^2=0.105, p<0.02. A 1 SD increase in early-pubertal WHR was associated with a 0.14 SD decrease in APHV (R^2=0.390, p<0.001) and a 1 SD decrease in APHV was associated with a 0.21 SD increase in late-pubertal WHR (R^2=0.074, p<0.001). Conclusion: Our results suggest that the association between exposure to maternal diabetes and childhood adiposity is established in early puberty and tracks throughout puberty, with adiposity being both a possible driver and consequence of earlier pubertal timing. This highlights the need for early life interventions to prevent childhood obesity.

Introduction

The prevalence of obesity in children has significantly increased in the past 30 years. The percentage of obese adolescents in the U.S. aged 12-19 years increased from 5% to nearly 21% from 1980 to 2012[2]. Childhood and adolescent obesity is associated with an increased risk of adult obesity and other chronic cardiovascular and metabolic diseases, such as type 2 diabetes and cardiovascular disease[3–6].

Fetal metabolic programming occurs during the critical period of early development in utero when nutritional stressors or chemical stimuli are hypothesized to induce long-lasting alterations of the offspring's physiology and metabolism[10], the consequences of which are often seen later in life[28]. Maternal diabetes, including gestational diabetes (GDM) and pre-gestational diabetes, is an intrauterine exposure that may result in fetal over-nutrition with excess glucose and other nutrients transferring from mothers to offspring and promoting growth and adiposity[27,28].

The prevalence of diabetes, including GDM, has increased over the recent years[70,71,108] and there is a growing understanding that even mild changes in the intrauterine environment influence offspring outcomes, not only in the perinatal period, but also over the entire life course[10]. Several studies have explored the effects of fetal exposure to maternal diabetes on childhood adiposity[11–16]; however, few have assessed whether these effects persist throughout puberty[17,18]. Previous results from our group showed that exposure to maternal diabetes was associated with increased adiposity, both overall and central, in children aged 6-13. We also found that body mass index (BMI) growth velocity of children exposed to GDM in utero were similar to those not exposed in early childhood; however, they started to diverge around the time of puberty (10-13 years of age) when the BMI growth velocity of GDM exposed children became faster than that of unexposed children[33].

The current literature shows that earlier pubertal timing is associated with higher adult BMI and greater risk of obesity later in life, and some studies show this effect to be partially independent of childhood BMI[61,109]. Recent studies, including from our own cohort, have also shown that exposure to maternal diabetes during pregnancy is associated with earlier pubertal timing[17,18,93] and

faster speed of pubertal growth[93]. However, none have considered how fetal overnutrition may influence childhood adiposity throughout puberty, nor have they explored the role of pubertal timing.

Puberty is a complex physiological process of intense sex-specific hormonal changes and rapid physical growth, leading to psychological and physical maturation. Pubertal development can be detected through the identification of several physical and clinical signs. The characteristic pubertal growth spurt is due to both the indirect increase in sex steroids and the direct increase in growth hormone (GH) stimulation[34] and is marked by a peak in height growth velocity. Age at peak height velocity (APHV) can thus be used as a surrogate measure of pubertal timing[110,111].

The aim of this study was to explore associations along a hypothesized pathway from exposure to maternal diabetes in utero to adolescent adiposity throughout puberty. More specifically, we explored the association between exposure to maternal diabetes and offspring adiposity in late puberty, and the roles of early-puberty adiposity and pubertal timing along this pathway, using data from an ethnically diverse observational cohort study in Colorado.

Methods

Study population

The Exploring Perinatal Outcomes among Children (EPOCH) study is a historical prospective multiethnic cohort study that recruited 604 mother/child dyads in Colorado. Eligible participants were children exposed to maternal diabetes and a random sample of children not exposed. Participants were offspring of singleton pregnancies, born at a single hospital in Denver between 1992 and 2002, whose biological mothers were members of the Kaiser Permanente of Colorado Health Plan (KPCO). Children and their mothers were invited to participate in two research visits at average ages of 10.5 (SD=1.5) and 16.7 (SD=1.2) years, at which time demographic, anthropometric, and adiposity measures were collected and pubertal staging was assessed. For this report, we used data from 364 youth who completed both research visits and had a Tanner Stage of 1-3 (early puberty) at the first research visit and a Tanner Stage of 4 or 5 (late puberty) at the second research

visit. The study was approved by the appropriate Institutional Review Boards. Mothers provided written informed consent and the youth provided written assent.

Exposure definition

Exposure to maternal diabetes was defined as a presence of pre-existent diabetes or a physician diagnosis of GDM during the index pregnancy and was ascertained from the KPCO perinatal database, an electronic database which links the prenatal and neonatal medical records. All pregnant women at KPCO were routinely screened for gestational diabetes at 24-28 weeks using the 2-step standard protocol[53]. GDM was diagnosed if glucose values exceeded $\geq$2 thresholds set by the National Diabetes Data Group on the 3-hour, 100g oral glucose tolerance test[54]. Sixty mothers were diagnosed with gestational diabetes mellitus (GDM) and seven mothers were diagnosed with type 1 diabetes prior to pregnancy. Since our research question focused on the effects of hyperglycemia during pregnancy (i.e., fetal overnutrition) on puberty, we included both types of maternal diabetes.

Childhood adiposity measures: Waist-height ratio

Child's height and weight were measured in light clothing and without shoes at each research visit. Waist circumference was measured according to the National Health and Nutrition Examination Survey protocol[112]. Waist-to-height ratio (WHR) was calculated for each research visit as waist circumference divided by height, both measured in the same units (cm). In this analysis, we used waist-to-height ratio as a measure of central adiposity in youth since it is a practical epidemiologic measure of adiposity and accounts for the growth during puberty, as height and waist circumferences are strongly age dependent[5]. Additionally, WHR has been previously shown to be a better predictor of adiposity in children and adolescents than BMI[59].

Pubertal timing

In this analysis, we estimated pubertal timing using APHV based on longitudinal heights obtained from the pediatric medical records from age two years to their age at the second research visit. We used the Superimposition by Translation and Rotation (SITAR) growth curve analysis, a method that uses a shape invariant spline curve and a nonlinear random-effects model to estimate an

average growth curve for the entire sample and each individual's deviation from the average growth curve[56]. The SITAR method provides subject-specific parameters that define how much bigger or smaller each child is compared to the population average (size), how much faster or slower the child's growth velocity is compared to the population average (velocity), and how much earlier or later the child experienced peak velocity compared to the population average (timing). In this paper we used APHV as the variable of interest defining pubertal timing[76]. Age at peak height velocity is one of several methods that are used to define pubertal timing. Previous studies have shown that age at peak height velocity usually occurs earlier in females than males, typically during Tanner stage 3; while males usually reach peak height velocity during Tanner stage 4[38,75,76].

Other measurements

Race/ethnicity was self-reported at the first research visit using the 2000 US Census base questions and categorized as Hispanic (any race), non-Hispanic White, non-Hispanic Black, or non-Hispanic Other race. For this analysis, we collapsed race/ethnicity into two race categories, Non-Hispanic White and Other, due to small sample sizes in each category. Maternal education and total household income at the time of birth were self-reported by the mother at the first research visit. Pubertal stage at each visit was self-reported by the child using diagrammatic representations of Tanner Staging adapted from Marshall and Tanner[94]. Tanner stage (1-5) was classified on the basis of pubic hair for males and breast development for females.

Statistical analyses

Analyses were conducted in SAS 9.4 (SAS Institute, Cary, NC). Descriptive statistics are presented using percentages or means and standard deviations. Cochran-Mantel-Haenszel tests and t-tests were used to identify bivariate associations between characteristics and exposure to maternal diabetes. Outcome variables, WHR in early puberty, WHR in late puberty, and age at peak height velocity, were standardized to the sample mean and SD.

To address our research questions, we used four separate linear regression models. Model 1 assessed the effect of exposure to maternal diabetes on WHR in late puberty, while models 2-4

assessed associations along the hypothesized pathway throughout puberty: (2) the effect of exposure to maternal diabetes on WHR in early puberty; (3) the association between WHR in early puberty and APHV (pubertal timing); and (4) the effect of APHV on WHR in late puberty. Covariates included in each model were decided *a priori*: child's sex, race/ethnicity, and household income at birth; child's age at the research visit was included in models 1, 2, and 4 to control for the varying ages of youth at each research visit. To explore possible effect modification of sex, we tested the following interactions: exposure-by-sex (model 1 and 2), early-pubertal WHR-by-sex (model 3), and APHV-by-sex interaction (model 4). None of the explored interactions were significant at a p-value of 0.05.

Alpha was set at 0.05 for statistical significance. We report β estimates, 95% confidence intervals (95%CI), and p-values that reflect a change in the variable of interest. For WHR in early and late puberty in models 1 and 2, we interpreted the beta estimate as the standard deviation difference in exposed youth compared to unexposed youth. For model 3 and 4, we interpreted the beta estimate as the standard deviation difference in 1 standard deviation change in the independent variable, WHR in early puberty for model 3 and APHV for model 4.

Results

A total of 364 youth (69 exposed and 295 unexposed to maternal diabetes) were included in these analyses. The analytic sample of 364 was similar to the full EPOCH cohort of 604 youth in all aspects (data not shown). Anthropometric and demographic characteristics according to exposure status are summarized in Table 9. Mean (SD) ages of exposed and unexposed youth were 9.5 (1.6) and 10.6 (1.3) years for the first research visit and 15.8 (1.0) and 16.9 (1.1) years for the second research visit, respectively ($p<0.001$, $p<0.001$). Of the 69 exposed youth, 43.5% were female and of the 295 unexposed youth 48.5% were female ($p=0.46$). Sixty-two percent of the exposed youth were non-Hispanic White, compared to 50.5% in the unexposed youth ($p=0.08$).

The mean WHR in early puberty for exposed and unexposed youth at the mean age of 10.1 years was 0.48 (0.44, 0.52) and 0.46 (0.41, 0.50), respectively, and the mean WHR in late puberty for exposed and unexposed youth at the mean age of 16.5 years was 0.49 (0.47, 0.51) and 0.47 (0.46,

0.48) (Figure 7). Figure 8 presents the results of the multiple regression analysis for each model. Adolescents exposed to maternal diabetes during pregnancy had a 0.27 standard deviation increase in late-pubertal WHR, which was borderline significant (R^2=0.055, p=0.06), and a 0.40 standard deviation increase in early-pubertal WHR (R^2=0.105, p<0.02) compared to unexposed adolescents (models 1 and 2, respectively). Model 3 showed that a 1 standard deviation increase in early-pubertal WHR was associated with a 0.14 standard deviation decrease in APHV (R^2=0.390, p<0.001) and model 4 showed that a 1 standard deviation decrease in APHV was associated with a 0.21 standard deviation increase in late-pubertal WHR (R^2=0.074, p<0.001).

Discussion

We explored the associations along a hypothesized pathway from exposure to maternal diabetes in utero to adolescent adiposity throughout puberty. Compared to unexposed youth, youth exposed to maternal diabetes during the intrauterine life had a larger WHR in early puberty, which in turn was associated with an earlier pubertal timing, and an earlier pubertal timing was associated with a higher WHR in late puberty. Our results suggest that the association between exposure to maternal diabetes and offspring adiposity is established early in life, likely before puberty, and tracks throughout puberty, with adiposity being both a possible driver and consequence of earlier pubertal timing.

Obesity is a complex, multifactorial issue that results from a combination of causes and contributing risk factors, including behavioral, environmental and genetic factors. Our findings agree with several previous studies suggesting that exposure to maternal diabetes during pregnancy increases the risk of offspring adiposity during childhood[1,11,12,16]. However, the novelty of our study is the investigation of whether pubertal timing plays a role in the pathway from exposure to maternal diabetes to increased offspring adiposity. Our results suggest that the relationship between exposure to maternal diabetes and adiposity is present early in life, likely before puberty and persists, though loses strength, as the youth moves throughout puberty. Nevertheless, exposure to maternal diabetes only accounted for about 11% of the variation in adiposity in early puberty, and about 7% of the

variation in adiposity in late puberty, suggesting that other factors (e.g., genetic predisposition or postnatal exposures and behaviors) potentially play more important roles. The decrease in the variance in adiposity explained by maternal diabetes exposure as youth move from early to late puberty also suggest an increasing influence of other competing exposures.

We found that early-pubertal adiposity accounted for about 39% of the association with pubertal timing. Other studies have shown a relationship between childhood obesity and earlier pubertal timing[7,45,113–116], which agrees with our finding that a larger WHR in early puberty is associated with a younger APHV. We also found that earlier pubertal timing was associated with a larger WHR in late puberty, which is consistent with previous literature[8,51]. This suggests that childhood adiposity is likely both a driver and a consequence of early pubertal timing.

Davis et al. looked at how exposure to GDM modified the relationship between pubertal Tanner stages and total body fat and found that offspring exposed to GDM had a larger change in total body fat across Tanner stages compared to offspring not exposed to GDM. However, this was a cross-sectional study, unable to evaluate individual changes over time and was conducted in an overweight Latino population which is not generalizable to normal-weight children, or other ethnic populations[52]. Using our own cohort, Crume et al. showed BMI growth trajectories of children exposed to GDM *in utero* were similar to those not exposed, until around the time of puberty, whereas by pubertal entry, by ~10 years of age, the mean BMI growth velocities of exposed children were significantly higher than those of youth not exposed[33].

While our findings represent important additions to the evidence base, they should be interpreted with caution. First, the effect sizes are small. There is potential misclassification introduced by using a self-reported measure of Tanner Stage at each visit. However, it is likely that this misclassification is non-differential with respect to exposure to maternal diabetes. Also, due to the unstandardized methods used to obtain height measurements during well-child pediatric visits, there may be potential non-differential measurement error in the estimate of APHV, which could contribute to an underestimation of the effects. Nevertheless, the prospective assessment of offspring

adiposity, combined with objective assessment of intrauterine exposures from medical records, allowed us to evaluate the associations of interest without concern for recall bias. As with all observational studies, there is potential for residual confounding by unmeasured variables.

In summary, these findings provide evidence that intrauterine exposure to maternal diabetes influences childhood adiposity throughout puberty, and that the association is already present before or during early puberty. Our findings also show that adiposity is likely both a driver and a consequence of early pubertal timing. Efforts to reduce obesity risk in children prior to puberty are urgently needed to reduce the risk of chronic diseases that have been associated with childhood obesity.

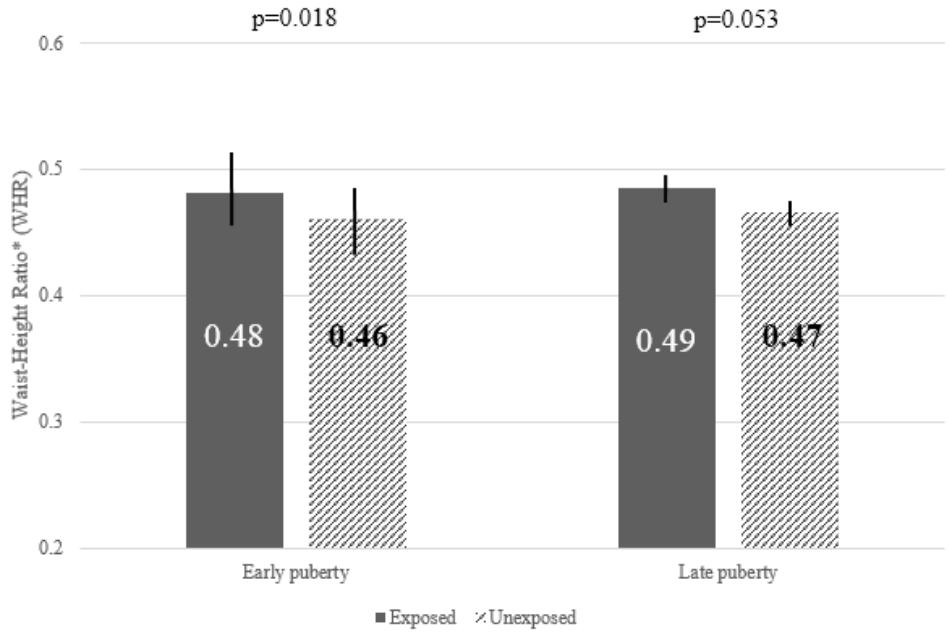
Tables

Table 9. Descriptive statistics of exposed (n=69) and unexposed (n=295) youth according to exposure to diabetes status.

Variables	Exposed to DM			Unexposed to DM			
	N	%	Mean (SD)	N	%	Mean (SD)	p-value
Age at first research visit (years)			9.5 (1.6)			10.6 (1.3)	<0.001
Age at second research visit (years)			15.8 (1.0)			16.9 (1.1)	<0.001
Sex (female)	30	43.5%		143	48.5%		0.46
Race/Ethnicity							0.08
Non-Hispanic White	43	62.3%		149	50.5%		
Other	26	38.8%		121	48.2%		
Tanner Stage at first research visit^							<0.001
Pre-pubertal (TS-1)	45	65.2%		121	41.0%		
Pubertal (TS-2)	19	27.5%		120	40.7%		
Pubertal (TS-3)	5	7.3%		54	18.3%		
Tanner Stage at second research visit^							0.93
Pubertal (TS-4)	30	43.5%		130	44.1%		
Pubertal (TS-5)	39	56.5%		165	55.9%		
Mother's self-reported income at birth							0.77
≤$49,999	30	43.5%		105	45.4%		
≥$50,000	39	56.5%		146	54.6%		
Mother's self-reported education at birth							0.84
≤ High School	11	15.9%		50	17.0%		
> High School	58	84.1%		245	83.0%		

Abbreviations: DM, maternal diabetes; TS, tanner stage
^ Females breast Tanner stage; males pubic hair Tanner stage

Figures



* Back-transformed LS MEANS for WHR at the first research visit (early puberty, mean age 10.1 years) and second research visit (late puberty, mean age 16.5 years), adjusted for child's sex, race, age at research visit and household income at birth. P-values are based on log-transformed data.

Figure 7. Mean waist-height ratios in early and late puberty, at mean ages of 10.1 years and 16.5 years, respectively, for exposed and unexposed youth.

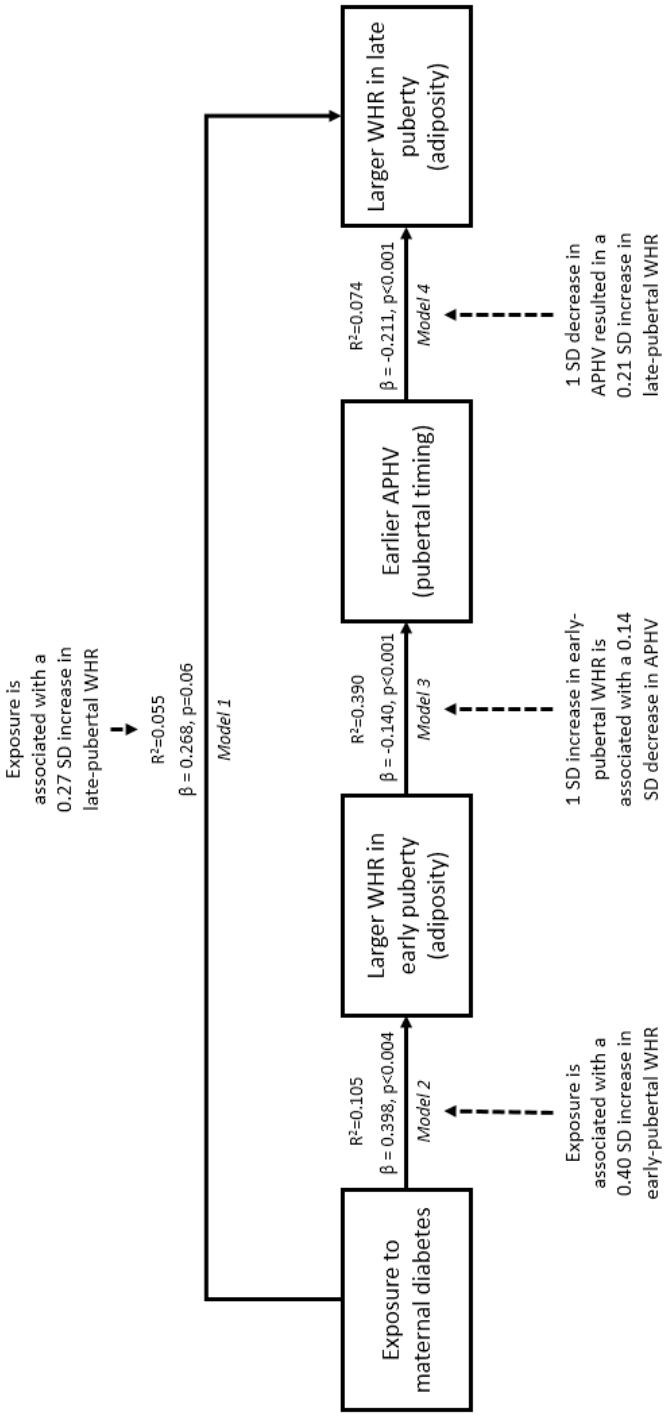


Figure 8. Associations between exposure to maternal diabetes, WHR at two time points and the role of APHV

CHAPTER VII

DISCUSSION

Obesity is a complex, multifactorial issue that results from a combination of causes and contributing factors, including behavioral, environmental and genetic factors. The prevalence of obesity in children has increased significantly in the past 30 years and is a major public health concern. Childhood obesity has been associated with an increased risk of adult obesity[20] and in general, with other chronic diseases such as cardio-metabolic diseases, stroke, cancer, low quality of life, and mental illness[21–24]. The economic impact of obesity and its related health problems is substantial in the United States. In 2008, the medical care costs of obesity were estimated to be $147 billion[25], and childhood obesity alone is responsible for about $14 billion[117]. Unfortunately, as a Nation we have not been successful in significantly reducing the obesity rate at a population level[118]. Given the substantial consequences of obesity and other related-chronic diseases, it is important to find more effective ways to prevent the development of obesity and to curb the progression of chronic disease, particularly in youth.

The intrauterine environment is a key determinant of long-term health outcomes. Maternal diabetes is an intrauterine exposure that results in fetal over-nutrition with excess glucose transfer from the mother to offspring, promoting growth and adiposity[27,28]. Many studies have looked at the effects of exposure to maternal diabetes on offspring adiposity[11–16]; however, few have investigated whether these effects persist throughout puberty[17,18]. Additionally, given the population-level increase in the prevalence of maternal diabetes and the decrease in the age of pubertal onset[60,70–73], understanding if exposure to maternal diabetes influences puberty in the offspring and whether puberty plays a role in the relationship seen between intrauterine exposure and obesity is important. This dissertation used a multi-ethnic cohort based in Colorado to investigate the effects of intrauterine exposure to maternal diabetes on different components of puberty and hypothesized a pathway from intrauterine exposure to maternal diabetes to adolescent adiposity.

In the first aim, we focused on differences in pubertal timing and speed of pubertal growth in the offspring of mothers with and without diabetes. Little is known about the impact of maternal diabetes during pregnancy on offspring pubertal timing and velocity[17,18]. Our results support that exposure to maternal diabetes during intrauterine life leads to an increased risk of earlier pubertal timing and a faster speed of pubertal growth, where the difference in the speed of pubertal growth between exposed and unexposed youth was greater in female offspring than in male offspring. With the population trend of earlier puberty over the last couple of decades, our findings support the hypothesis that perinatal exposures may be among the multiple contributors to this population trend. We are the first to investigate the effect of intrauterine exposure to maternal diabetes on linear growth velocities in youth. Linear growth provides one of the most important biomarkers of a child's development and overall health. Additionally, there was a significantly higher proportion of youth exposed to maternal diabetes in Tanner stage 1 and 2 compared to unexposed youth at the first research visit (88% and 77%, respectively), while at the second research visit there was no discrepancy in Tanner stages between exposed and unexposed (95% and 94%, respectively) (Table 1). This suggests that exposed youth not only had faster speed of pubertal growth (in linear growth terms), but also faster transition from pre-pubertal to post-pubertal Tanner stages.

While these findings represent an important addition to the existing evidence regarding risk factors for earlier puberty and altered linear growth, there were some limitations. We were unable to assess how exposure to maternal diabetes and subsequent faster growth ultimately affects the final adult height, as most of the EPOCH cohort was still growing at the time of the second research visit. Also, since we did not have longitudinal pubertal assessments throughout puberty, we used longitudinal heights and the SITAR method to estimate APHV, a measure of pubertal timing, and PHV, a measure of speed of pubertal growth. Due to the unstandardized methods used to obtain height measurements during well-child pediatric visits, which are used to estimate APHV, there may be potential of increased variability and decreased precision of the pubertal estimates (APHV and PHV). As some of these findings are novel, replication studies are needed to confirm that our results

are not spurious. Additionally, further investigation into potentially responsible mediators, specifically hormones related to the GH-IGF-1 and HPG axes, are needed.

Puberty is comprised of many different aspects that drive physical and hormonal changes that occur during puberty. For the second aim, we investigated whether exposure to maternal diabetes altered hormones of puberty. We found that exposure to maternal diabetes was associated with increased concentrations of luteinizing hormone in exposed females, but not in males; exposure to maternal diabetes did not impact estradiol, total testosterone or DHEA-S. As hormones are closely tied to the regulation of adiposity and drive the differences in the sex-specific distribution of body fat, which have important effects on the regulation of energy balance, we tested whether current BMI mediated the associations between exposure and luteinizing hormone concentrations and found that current BMI does not mediate the relationship. This supports a direct effect of exposure to maternal diabetes on luteinizing hormone concentrations. Insulin is known to act on GnRH signaling that is responsible for the regulation of luteinizing hormone and follicle stimulating hormone. Previously, Sauder et al.[100] showed that exposure to maternal diabetes is associated with decreased insulin sensitivity in the offspring in this cohort, which is consistent with other studies[101,102]. Additionally, they showed that females exposed to maternal diabetes and obesity during pregnancy had lower insulin sensitivity compared to males, independent of child's BMI. This decrease in insulin sensitivity causes compensatory hyperinsulinemia, while resulting in an increase in luteinizing hormone production, supporting our finding of increased luteinizing hormone in exposed females. This type of altered GnRH signaling is similar to what is seen in PCOS patients. While the positive association between in utero exposure to maternal diabetes and luteinizing hormone seen in our results could be spurious, these findings are concerning as it suggests a possible relationship between exposure to maternal diabetes and future PCOS risk. Better understanding of this relationship is important, and further research is needed. Additionally, our results of increased luteinizing hormone concentrations in females exposed to maternal diabetes, as well as our previous findings of earlier pubertal timing and faster speed of pubertal growth in youth exposed to maternal diabetes[64] supports

the idea that exposure is associated with different components of puberty. It is possible that puberty may be a critical or sensitive period in the lifecycle when the effects of *in utero* exposure to maternal diabetes may be enhanced via biological programming.

This study has some important limitations as the primary aims of EPOCH were not focused on assessing the effect of perinatal exposure on hormones of puberty. We did not have longitudinal measures of hormones throughout puberty and the study protocol was not specific in the collection of specimen samples for hormone assays, limiting the ability to capture the highest concentrations of the pulsatile hormones, which may explain the lack of significant findings for estradiol, testosterone and DHEA-S. Also, due to the sensitivity level of the hormone assays and the young ages of our cohort, over 60% of the estradiol and total testosterone concentrations at the first research visit were below the limit of detection and, thus, unable to be included in the data analysis. Therefore, the analyses of estradiol and total testosterone concentrations were solely based on measurements obtained from the second research visit; while, the analyses of luteinizing hormone and DHEA-S concentrations were based on measurements taken from the first and second research visit. Another important limitation was the limited number of hormone markers that were available. Without measures of sex binding globulin hormone or free testosterone itself, we were not able to determine to the bioavailability of testosterone, which is one of the diagnostic criteria for PCOS. Further studies are needed to expand the analysis of hormones of interest, such as sex binding globulin hormone or IGF-1, to better understand how exposure to maternal diabetes may affect the underlying hormone function in adolescents.

The third aim built on the first, along with previous findings of increased adiposity in exposed youth in this cohort[1] and explored a hypothesized pathway from exposure to maternal diabetes in utero to adolescent adiposity, measured by waist-to-height ratio. More specifically, we explored the association between exposure to maternal diabetes and offspring WHR in late puberty, and the contribution of early-puberty WHR and pubertal timing along this pathway. We found that compared to unexposed youth, youth exposed to maternal diabetes during intrauterine life had a larger

WHR in early puberty, which in turn was associated with earlier pubertal timing, and earlier pubertal timing led to a higher WHR in late puberty. Our results suggest that the association between exposure to maternal diabetes and offspring adiposity is established early in life, likely before puberty, and tracks through puberty, with adiposity being both a possible driver and consequence of earlier pubertal timing. This agrees with previous studies that have looked at the potential paths independently—exposure to childhood adiposity, adiposity to pubertal timing, or pubertal timing to adolescent adiposity, but not in the same cohort. The novelty of our study is the investigation of whether pubertal timing plays a role in the pathway from exposure to maternal diabetes to increased offspring adiposity.

While our findings represent important additions to the evidence base, they should be interpreted with caution. One of the important limitations in these analyses was the potential misclassification introduced by the unstandardized methods used to obtain height measurements during well-child pediatric visits. This potential non-differential measurement error of the height measurements used to estimate APHV, similarly seen in aim 2, could have moved the effect estimate towards the null, underestimating the effect of exposure. Nevertheless, our findings support the hypothesis that the effect of fetal over-nutrition on adiposity occurs relatively early in life and tracks from childhood through adolescents, with pubertal timing likely being on the causal pathway.

A person's sex is a major determinant of hormone concentrations and these concentrations begin to differ between males and females in the early embryonic phase of intrauterine development. Sex differences in hormone concentrations drive both the mental and physical differences seen between males and females that exist throughout the life course. Although some of these differences wane during childhood, they reappear around the start of puberty. Some of these sex differences were observed in our results: females had earlier pubertal timing than males, exposure to maternal diabetes influenced the speed of pubertal growth more in females than in males, and exposed females had higher concentrations of luteinizing hormone than unexposed females, while no differences were observed in males. Sex hormones also differentially influence the development of adipose tissue:

estrogen promotes adiposity, while testosterone and DHEA-S inhibit adiposity[82]; and sex hormones themselves can be further influenced by obesity in both childhood[83] and adulthood[84]. Taken together, our findings suggest that exposure to maternal diabetes has a larger impact on female offspring than male offspring, however the mechanisms to explain this sex difference is unknown.

Much of the current epidemiological literature examining timing of puberty utilize different pubertal measures in females and males making it difficult, or impossible, to compare them. This results in many of the studies focusing on either males or females, or the inclusion of both in the study but not being able to compare the results. For example, Day et al. included both males and females in their study, but used age of menarche for females and voice breaking for males not allowing for the comparison of the effect of pubertal timing between males and females[66]. Additionally, with the increase in childhood obesity, Tanner stages are becoming more unreliable in epidemiological studies, specifically self-reported Tanner stages, as the current population is heavier than it was 50 years ago when Tanner stages were developed and now require the assistance of a trained resource to assess Tanner staging correctly. Advanced statistical methods, such as SITAR, allow epidemiologists to estimate the timing and progression of puberty using observational data and provides an objective measure for both males and females allowing for comparisons between the two sexes. Further research is needed to investigate the correlations between different pubertal measures, such as age at peak height velocity, Tanner stages and pubertal hormone concentrations in a contemporary cohort in order to identify potential alternative standardized methods of pubertal assessment for large observational studies. As the mechanisms underlying the effects of maternal diabetes on puberty and subsequent adiposity are not fully understood, further research is also needed to better understand the possible sex-specific effects of fetal programming and the involvement of puberty. As previously mentioned, the results of this dissertation are novel, however the need for replicating these results in other cohorts is important and could provide stronger evidence of true relationship.

Puberty is a critical period in the life course and may be a window of susceptibility that magnifies the effects of fetal programming. The concept of windows of susceptibility over the life course provides an important conceptual framework for understanding how intrauterine exposures may influence the health of the offspring and how the vicious cycle may continue over generations. This dissertation suggests that exposures during the intrauterine period affect different components of puberty and adiposity in the offspring, which subsequently shape health outcomes later in life, especially in females. Because of this it is imperative that research on childhood obesity move more upstream, focusing on young children, infants, pregnant women or even women who are planning to become pregnant in order to improve their health over the life course and that of their offspring[119]. Ongoing intervention studies targeting pregnant or preconceptual women[120] have promising clinical and public health implications to slow the ever-increasing rate of obesity and diabetes in the United States. The White House Task Force on Childhood Obesity[121] and the Surgeon General[122] have recommended promoting effective perinatal interventions in an effort to interrupt the transgenerational cycle of obesity.

The long-term public health goal of the current research are two-fold: to prevent obesity and related adverse health outcomes in youth and to stop the vicious transgenerational cycle of obesity. Currently, the primary paths toward these goals would be through early prevention using obesity and lifestyle interventions in women of child-bearing age so they can achieve and maintain a healthy weight before entering into a pregnancy. Early prevention is critical because once established, reversal of obesity is often inefficient, ineffective, and costly[119,123]. The second path is toward further understanding how intrauterine exposures affect puberty and its relationship with increased risk of obesity later in life or throughout the lifecourse. As described above, there are still many unanswered research questions about how maternal diabetes effects puberty and what the consequences are on obesity and other chronic diseases, such as PCOS, in the offspring. Understanding these types of population-level patterns is an important role for epidemiologists, who are trained to investigate these

types of complex relationships. Despite the challenges and the need for further studies, I believe that interventions in women of child-bearing age, will help disrupt the intergenerational cycle of obesity.

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