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Sex-dependent associations of maternal androgen levels with offspring BMI and weight trajectory from birth to early childhood

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Abstract

Context: In preclinical studies, high androgen levels during pregnancy are associated with low birth weight and rapid postnatal weight gain in the offspring. However, human data linking prenatal androgens with birth weight and early life weight gain in the offspring are scarce.

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Design: We evaluated 516 mother-child pairs enrolled in the New England birth cohorts of the Collaborative Perinatal Project (1959-1966). We assayed androgen bioactivity in maternal sera during third-trimester using a receptor-mediated luciferase expression bioassay. Age and sex-specific BMI Z-scores (BMIz), defined using established standards, were assessed at birth, 4-months, 1-year, 4-years and 7-years. We used linear mixed models to evaluate the relation of maternal androgens with childhood BMIz overall and by sex. We examined the association of maternal androgens with fetal growth restriction. The association of weight trajectories with maternal androgens was examined using multinomial logistic regression.

Results: Higher maternal androgen levels associated with lower BMIz at birth (β=−0.39, 95%CI: −0.73, −0.06);this relation was sex-dependent such that maternal androgens significantly associated with BMIz at birth in girls alone (β=−0.72, 95%CI: −1.40, −0.04). The relation of maternal androgens with fetal growth restriction revealed dose threshold effects that differed by sex. There was no significant association between maternal androgens and weight trajectory overall. However, we found a significant sex interaction (p=0.01); higher maternal androgen levels associated with accelerated catch-up growth in boys (aOR=2.14, 95%CI: 1.14, 4.03).

Conclusion: Our findings provide evidence that maternal androgens may have differential effects on programming of intrauterine growth and postnatal weight gain depending on fetal sex.

Keywords

Prenatal; Androgens; Body Mass Index; Weight Growth Trajectory; Childhood; Sex

INTRODUCTION

There is growing evidence that an adverse intrauterine environment during critical periods of fetal development can cause long-term epigenetic effects in the developing fetus, increasing susceptibility to cardiovascular, metabolic, psychiatric, and other chronic diseases in later life [1–3]. Specifically, early prenatal exposure to sex steroids have been well recognized to modulate susceptibility to diseases in adulthood. Recent attention has focused on the role of the maternal androgen milieu because of increase in environmental endocrine-disruptors that interact with the androgen receptor and its signaling [4]. Elevated levels of maternal testosterone during pregnancy have been associated with higher rates of infant morbidity and mortality [5]. Along with fetal and infant adverse effects, several studies in prenatally androgenized animal and non-human primate models also demonstrate cardiometabolic abnormalities including obesity, hypertension and type 2 diabetes in adult life [6,7]. In a prospective birth cohort study of offspring followed for up to 50 years, we recently showed that higher maternal androgen levels were associated with an increased risk of metabolic syndrome in the adult offspring [8], supporting the prenatal programming of cardiovascular disease risk by androgens in a general human population. Despite evidence that the androgen milieu during pregnancy can influence programming of adult metabolic diseases, few studies have examined the underlying mediating factors.

Low birth weight (a surrogate marker for intrauterine growth restriction) is strongly associated with increased risk for future adult CVD [9], underscoring the developmental nature of the disease. In addition, strong inverse relationships have been reported between

birth weight and several CVD risk factors, including obesity [10], high systolic blood pressure [11], dyslipidemia [12], insulin resistance [13,14] and type 2 diabetes[15]. Furthermore, the link between birthweight and cardiovascular risk is most apparent when low birth weight is followed by rapid postnatal weight gain (catch-up growth) and childhood obesity [14–16].

There is strong evidence from animal and non-human primate models that elevated testosterone levels during pregnancy are associated with low birth weight and postnatal weight gain [17–20]. Further, the reductions in birth weight of fetuses exposed to testosterone have also been shown to be dose-dependent [21]. Women with hyperandrogenism associated with polycystic ovarian syndrome (PCOS) and preeclampsia have higher-than-normal prevalence of small-for-gestational age deliveries [22,23]. In addition, children born to mothers with PCOS, who are exposed to higher testosterone levels during pregnancy, tend to exhibit higher body weight and body mass index Z-scores (BMIz) than those born to healthy mothers [24,25]. Some [26–28], but not all [29,30], studies in pregnant mothers with adrenal hyperandrogenism associated with congenital adrenal hyperplasia (CAH) have also reported higher than expected rates of children born small-for-gestational age. However, it is unclear if this is due to the direct effect of maternal adrenal androgen excess *in utero* or differences in glucocorticoid treatment during pregnancy [31]. As an example of a fetal origin of adrenal androgen excess *in utero,* one small study in children with CAH, followed from birth to age 10 years, found that lower birthweight was associated with early childhood adiposity independent of disease severity and glucocorticoid treatment regimen [32]. Despite evidence for sex differences in emotional and behavioral outcomes among CAH children from high levels of androgens during fetal development [33], this study did not detect significant sex effects on growth trajectory, though was limited by small sample size. Taken together, these studies suggest that androgen excess during pregnancy may affect fetal growth and postnatal weight gain, although less is known about the differential impact by sex of the offspring.

The similarity of adult cardiometabolic consequences of prenatally growth-retarded and prenatally hyperandrogenic models suggests that shared metabolic pathways may be involved. Thus, it is possible that many of the adverse adult cardiometabolic effects of prenatal androgen exposure may be mediated by abnormalities in fetal and/or early childhood growth. However, beyond these animal studies and specific patient populations described above, prenatal programming of early childhood growth patterns by androgens in the human population has not been well studied, owing largely to a paucity of longitudinal datasets that can adequately address these developmental changes over time. The small number of population studies examining the association of maternal androgens with offspring growth outcomes have been limited to the birth and infancy time periods, with no studies expanding outcome assessments into the early childhood years. Further, there is lack of research on elucidating the potential sex differences in the effects of maternal androgens on early childhood growth patterns.

Thus, the objective of the current study is to prospectively examine the sex differences in the associations between maternal androgens during pregnancy on BMIz and weight trajectories from birth to early childhood in the offspring of mothers participating in the New England

Family Study. We used data from a community-based population of mothers, who, during and after their pregnancy, were part of the National Collaborative Perinatal Project (CPP) which followed individuals *in utero* (born from 1959-1966), and whose children have been evaluated from birth to 7-years of age. We used a highly sensitive bioassay to measure total circulating androgen bioactivity in maternal serum, which measures the relative bioactivity of all androgen subtypes. We hypothesized that higher levels of prenatal bioactive androgens assessed in maternal serum during the beginning of third-trimester (a critical period for the organizational effects of sex steroids on brain development and maturation of fetal physiological organs) would be associated with lower BMIz at birth and an accelerated postnatal weight gain trajectory through early childhood. Given the sex-dependent regulation of androgen production and signaling in the placenta [34], we also investigated whether the impact of prenatal androgens on early life weight outcomes in the offspring differs by fetal sex.

METHODS

Study Sample

Study participants were from the New England cohorts of the National Collaborative Perinatal Project (CPP). The CPP prospectively followed participating mothers during their pregnancies, collected and stored their sera, and followed their offspring from delivery up to age 7 years [35,36]. There were 17,921 offspring born between 1959 and 1966 at the Providence, Rhode Island and Boston, Massachusetts (MA) sites of the CPP, also known as the New England Family Study (NEFS). Selection and sampling for these NEFS participants has been previously described [35,37]. Among the total pool of offspring (n=17,921), we identified 516 mother-child pairs for the present study who had participated in one of three previous NEFS adult follow-up studies and who had available bioactive androgen data from assayed maternal prenatal serum as well as data on BMI at one or more timepoints assessed during childhood (NIMH-NHLBI R01MH074679: JMG, PI; NIA RC2AG036666: EL and SB, MPIs; NIA R01AG023397: SB, PI). These identified mother-child pairs were from live, singleton births. All three NEFS studies used similar strategies to locate, recruit, and evaluate subjects who were all from the same original CPP cohort. All participants provided written informed consent. The study protocol was approved by the institutional review boards at Partners HealthCare system and Brown University.

Bioactive Androgen Measurement

From 1959 to 1966, maternal blood samples were collected approximately every two months during pregnancy and stored at the National Institutes of Health (NIH) repositories at −20°C. Maternal serum samples for this study were obtained from the CPP central repository at the NIH and assayed for androgen bioactivity at the beginning of the third trimester. Sera were drawn from the beginning of third trimester given that it encompasses a critical period for organizational effects of gonadal steroids on brain development as well as maturation of other fetal physiological organ systems [38].

We measured serum concentrations of bioactive androgens using an androgen receptor-mediated assay developed from HEK 293 cells stably co-transfected with a human androgen

receptor (hAR) plasmid and a luciferase reporter gene under the control of MMTV promoter (2933Y)[39,40]. Luciferase activity was measured by luminometer in cell lysates and expressed as relative light units (RLUs). The amount of bioactive androgen in the sample was directly related to the RLUs detected. Dihydrotestosterone (DHT) was the most potent steroid tested, followed by total testosterone (T), 4-androstene-3, 17β-diol (4-diol), 4-androstene-3, 17-dione (A4), 5α-androstane-3, 17β-diol (5α-diol), and 5-androstene-3, 17β-diol (A5). No significant androgenic activity was induced by DHEA up to a concentration of 10^{-6} M. The assay was not responsive to Estradiol (E2) or Progesterone (P4) up to 10^{-9} M and to Cortisol up to 10^{-8} M. No significant androgenic activity was detected for DHEA sulfate at 10^{-4} M. The bioassay has demonstrated a linear response to T up to 1.0 nM. The analytical sensitivity of the bioactive androgen assay was 7.8 pM/L. The intra- and interassay coefficients of variation were 7.4 and 7.5% at a T level of 0.25 nM and 4.9 and 6.4% at a T level of 0.03 nM, respectively. This androgen bioassay has been previously validated in premenopausal women in whom androgen concentrations are near the lower limit of sensitivity in many clinical immunoassays [40]. Although there are no established reference ranges for pregnant women, we have previously reported levels of circulating bioactive androgens with this bioassay in a smaller sample of pregnant women (range 0.31-126.63 nM) and have shown that these levels differ by fetal sex [8].

Child Growth Outcomes

BMI Z-score: In the CPP, children were assessed at birth, 4-months, 8-months, 1-year, 3-years, 4-years and 7-years of age in standard health exams. Weight and height were measured in the children by trained research assistants with a calibrated stadiometer and weighting scale wearing light clothing and no shoes. Weight and height were used to calculate body mass index (BMI). We calculated age-and sex-specific BMI Z-scores using the World Health Organization (WHO) Child Growth Standards for assessments at birth to 2-years of age [41] and using the Centers for Disease Control Prevention (CDC) 2000 growth references for assessments from ages 2 to 7-years [42]. We excluded the 8-month and 3-year assessments from our analyses given the very limited data available at these timepoints.

Fetal Growth Restriction: Given that low birth weight is a surrogate marker for fetal growth restriction, we performed a sensitivity analysis to further evaluate the association between prenatal androgens on fetal growth restriction. Fetal growth restriction was defined as birth weight less than the 20^{th} percentile for sex and gestational age based on the United States Natality datasets [43].

Growth Trajectory: Sex- and age-specific weight percentiles for the study population were calculated from birth through age 7 according to the WHO and CDC growth references described above. Group-based trajectory modeling was used to estimate a small set of flexible growth trajectories that summarize the observed, individual-level trajectories. This technique is a form of finite mixture modeling that uses maximum likelihood estimation to define growth as a mixture of polynomial regressions [44]. A parsimonious number of trajectories and appropriate smoothness were selected using the Bayesian information criterion (BIC) and posterior probabilities were used to determine which trajectory a child

was most likely to be a member of [45]. The overall uncertainty of trajectory membership was assessed using entropy (defined from 0 to 1), where values approaching 0 indicate an equal posterior probability of membership among trajectories and values approaching 1 indicate a strong posterior probability of belonging to a single trajectory [46]. Trajectory modeling was performed using the TRAJ procedure in SAS 9.4 [47].

Statistical Analyses

Demographic characteristics and clinical measures of the analytic sample were assessed and compared by sex using Pearson's chi-squared and two-independent samples t-tests for categorical and continuous data, respectively. Given the positively skewed distribution of bioactive androgen levels, these data were normalized using a natural logarithm transformation. We used linear mixed models to examine the association between maternal bioactive androgen concentrations (log-transformed) and child BMI Z-scores at birth, 4-months, 1-year, 4-years and 7-years of age, with a separate model for each age. A random intercept was included to account for intrafamilial correlation of siblings and continuous predictors were evaluated for nonlinear relationships with the response. Using generalized estimating equations (GEE) to account for intrafamilial correlation, we examined the association of maternal bioactive androgen levels as a continuous response variable with fetal growth restriction as a binary predictor. Using GEE with a logit link and Bernoulli response, we also estimated the odds ratio (OR) and 95% confidence intervals of having fetal growth restriction by categorizing the bioactive androgen levels into quartiles using the lowest quartile as the reference level to identify potential threshold effects. When using GEE modeling, an independence correlation structure was assumed and robust standard errors were applied to any inference; as with the linear mixed model, predictors were assessed for non-linearity. Multinomial logistic regression fit using GEE was used to calculate the odds ratio (OR) and 95% confidence interval (CI) for the association between maternal bioactive androgens (log-transformed) and child growth trajectory patterns generated from trajectory modeling described above.

Using an iterative process, we identified potential confounders among demographic and clinical factors associated with the outcome (Wald p-value <0.2 in final model) and exposure of interest (accounting for ≥10% change in the exposure estimate when added to the model). Confounders included maternal age, education, pre-pregnancy BMI and weight gain during pregnancy. Due to missing data for covariates, the total number of participants included in each of the fully-adjusted models were variable. However, baseline demographic and clinical characteristics of the participants with complete covariate data did not significantly differ from those with missing covariates. All models were fit for the overall sample and stratified by sex. Effect modification was examined using interaction terms for maternal bioactive androgens and offspring sex. Tests of our hypotheses were two-sided with $\alpha = 0.05$. All analyses were performed using SAS, version 9.4 (SAS Inc., Cary, NC).

RESULTS

Baseline Characteristics

Table 1 summarizes the demographic and clinical characteristics of the mothers and their children. The mothers were predominately non-Hispanic White (87.6%) and mean age at enrollment during pregnancy was 24.7 ± 5.5 years with a pre-pregnancy BMI of 23.1 ± 4.2 kg/m^2. Mothers of girls versus boys were similar in terms of maternal age, race/ethnicity, trimester at registration, socioeconomic index, smoking status, education, pre-pregnancy BMI and parity. Mothers who delivered girls had greater weight gain during their pregnancy compared to those with boys (boys: 18.4 ± 8.8 lb, girls: 20.3 ± 9.9 lb, p = 0.03). Mothers of boys had significantly higher levels of bioactive androgen levels during pregnancy compared to mothers of girls (males: 3.3 ± 10.5 nM, females: 2.2 ± 2.8 nM, p = 0.02). Thirty-seven percent of the offspring were from fetal growth-restricted pregnancies. The mean birth weight of the offspring was 3220 ± 518 grams with boys born heavier than girls. Based on 2000 CDC growth charts as reference [48], the mean prevalence of childhood obesity at age 7 was 7.5% and did not significantly differ by sex.

Maternal Bioactive Androgen Levels and Child BMI Z-score

Higher serum concentrations of maternal bioactive androgens were associated with lower BMIz in the offspring at birth (β=−0.39, 95% CI: −0.73, −0.06). We found suggestive evidence of effect modification by sex for the association between maternal androgen levels and offspring BMIz at birth (p-value for interaction=0.10). When stratified by sex, the inverse association between concentration of bioactive androgens and BMIz at birth was found to be stronger among girls (β=−0.72, 95% CI: −1.40, −0.04) than among boys (β= −0.24, 95% CI: −0.58, 0.11). No statistically significant associations were found at later ages (Table 2).

The adjusted odds ratio for the association of maternal bioactive androgens (as a continuous variable) and fetal growth restriction are presented in Table 3A. In the overall sample, maternal bioactive androgen levels (continuous variable) were significantly associated with fetal growth restriction and did not significantly differ by sex (p-value for interaction=0.38). Table 3B shows the adjusted odds ratios (aOR) for the association between maternal bioactive androgen quartile and fetal growth restriction. When categorizing the bioactive androgen levels into quartiles, the association with fetal growth restriction was only significant at the highest quartile (Q4) of maternal bioactive androgens. Specifically, the newborns exposed to the highest level of maternal bioactive androgen levels had 2.30 times the odds of being classified as growth restricted compared to offspring of mothers in the lowest quartile [Q4 vs Q1 aOR: 2.30 (1.22-4.32)].

Our results of maternal bioactive androgens categorized in quartiles on fetal growth restriction also revealed a threshold effect that differed by sex. In boys, the association with fetal growth restriction was significant only at the highest (4^{th}) quartile of maternal bioactive androgens, while the association among girls was significant starting at a lower threshold level (2^{nd} quartile) of maternal bioactive androgens. Specifically, the boys of mothers in the 4^{th} quartile of maternal bioactive androgen levels had 2.62 times the odds of developing fetal

growth restriction compared to those of mothers in the lowest quartile [Q4 vs Q1 aOR: 2.62 (1.05-6.51)]. This was similar in magnitude to the association among the girls starting at the 2nd quartile of maternal bioactive androgen exposure [Q2 vs Q1; aOR: 2.52 (1.07-5.90)] and persisting through the 3rd [Q3 vs Q1; aOR: 2.05 (0.92-4.58)] and 4th [Q4 vs Q1; 2.68 (1.12-6.39)] androgen quartiles. Test for overall interaction of maternal bioactive androgen quartile (p-value for interaction=0.43) with sex was non-significant to due limited power.

Maternal Prenatal Bioactive Androgen Levels and Weight Growth Trajectory

Based on the group-based trajectory analysis of weight percentiles by age, four growth trajectories were identified: no catch-up growth (A) (24%), regression after 12 months (B) (22%), accelerated catch-up growth (C) (22%) and consistent growth (D) (32%) (Figure 1). The entropy of the associated model was 0.80, indicating high confidence in the classification of children into distinct trajectories. Table 4 shows the distribution of maternal bioactive androgens levels by weight growth trajectory overall and by sex in the offspring. The mothers of offspring categorized into the *accelerated catch-up growth* and *regression after 12-months* growth trajectories had relatively higher levels of bioactive androgens during their pregnancy relative to the other trajectories.

For the multinomial logistic regression analysis, we chose the consistent growth trajectory (D) as our reference group given that all the participants had normal birth weight and their weight trajectory pattern remained relatively stable and consistent through early childhood relative to the other growth curves. The adjusted odds ratios (aOR) for the association between maternal bioactive androgens (measured continuously) and the individual weight growth trajectories are displayed in Table 5. In comparison to the reference group of offspring with the consistent growth trajectory curve, we did not find statistically significant associations in the overall sample between maternal bioactive androgens and any of the individual growth trajectories in the offspring. However, we found a significant interaction between maternal bioactive androgens and offspring sex (p=0.01) and weight trajectory. When stratified by offspring sex, higher maternal bioactive androgens were found to be significantly associated with *accelerated catch-up growth* among boys (aOR=2.14, 95% CI: 1.14, 4.03), but not among girls (aOR: 0.91, 95% CI: 0.45, 1.83).

DISCUSSION

In this prospective prenatal cohort of mothers and their offspring born between 1959-1966, we found that higher levels of maternal bioactive androgens during pregnancy were associated with lower BMIz at birth. Further sex-stratified analyses showed that this association was sex-dependent with the impact being stronger in female than in male offspring. In sensitivity analyses, we further demonstrated that the risk of intrauterine growth restriction occurred at lower thresholds of prenatal androgen exposure in mothers carrying a female versus male fetus, explaining the higher vulnerability of female offspring to lower birth weight. No significant associations were found between maternal androgens and BMIz from infancy through early childhood, over and above low birthweight. Among the male offspring only, higher prenatal levels of maternal bioactive androgens were positively associated with an accelerated catch-up growth trajectory from birth to age 7.

Our study uniquely demonstrated in a longitudinal cohort, sex differences in the association of circulating maternal androgen levels on offspring birth weight and postnatal weight gain through early childhood. These population-level findings support preclinical studies showing that fetal growth restriction (and subsequent low birth weight) and postnatal catch-up growth can be programmed by prenatal exposure to excess androgens. Our results also provide novel evidence that maternal androgens may have differential effects on the programming of growth retardation and postnatal weight gain depending on fetal sex.

Androgens play an important role in the regulation of growth and differentiation during fetal development [49]. During pregnancy, maternal serum testosterone levels increase throughout gestation [50]. There is strong evidence drawn from animal and non-human primate literature demonstrating that excess maternal androgens during pregnancy is associated with intrauterine growth restriction and low birth weight [20,18,21,17,51]. Compared to healthy mothers, pregnant women with elevated androgen levels, such as PCOS and preeclampsia, are at higher risk of delivering small-for-gestational age infants [23,52,53,22]. Aside from these high-risk patient populations, only two prospective studies have examined the association of maternal androgen levels with early life growth patterns in a general population. One study of 147 pregnancies found that elevated maternal testosterone levels in early and late gestation were associated with lower birth weight in the offspring [54], while another smaller study of 49 healthy pregnant women showed elevated maternal testosterone levels associated with lower birth weight Z-scores [55].

In our cohort of 516 mother-child pairs, we showed similar relationships with higher maternal androgen levels associating with lower BMIz in the offspring at birth. In addition, the observed association with maternal androgens on BMIz at birth was sex-dependent, i.e., stronger in female offspring than in males. Although previous animal studies of female offspring have shown similar impact of prenatal androgens on low birth weight [18,56], the sex differences have not been extensively explored. Given that low birthweight is a surrogate marker for intrauterine growth retardation, we also demonstrated that higher prenatal androgen levels associated with fetal growth restriction in both sexes in our study. However, sex-dependent threshold effects were observed with females being impacted at relatively lower levels of maternal androgens, and males only affected at the highest levels.

Our results suggest that the female fetus is more vulnerable to lower perturbations of maternal androgens on intrauterine growth relative to the male fetus. The fact that we demonstrated sex-dependent effects was not surprising, given that the timing of the sera acquisition, i.e. early third trimester, is a critical period of the sexual differentiation of the brain and body. Second trimester begins the secretion of testosterone in the male fetus to masculinize and this continues into third trimester. We previously demonstrated sex-dependent impacts on offspring outcomes (psychiatric, metabolic, and cardiovascular) in previous studies of other markers of maternal prenatal stress-immune perturbations at the same time period as the study presented here, with females demonstrating more adverse outcomes [35,8,57]. This study extends that work to demonstrate the critical importance of maternal androgens in the programming of early fetal growth and their differential impact on male versus female offspring.

A common sequelae to intrauterine fetal growth restriction (i.e. low birth weight) is postnatal catch-up growth, a known independent risk factor for future obesity and adult cardiovascular disorders [58,59,16]. Elevated levels of maternal testosterone have been associated with accelerated postnatal weight gain (i.e. catch-up growth) in mammals [20,56]. In our study, higher maternal bioactive androgen levels in late pregnancy was associated with accelerated postnatal weight gain between birth and 7-years, an association only observed among males. Among the children experiencing an accelerated catch-up growth trajectory, the boys were exposed to 2.5 times higher average maternal prenatal bioactive androgen levels compared to the girls. Our results are similar to animal models exhibiting excessive catch-up growth during postnatal life after prenatal testosterone treatment [20,56]. However, these preclinical studies did not examine sex differences. To our knowledge, only one human study of pregnant mothers and their offspring evaluated sex differences in the relationships of prenatal androgens and weight gain in the postnatal period. Findings, based on a small sample of 49 pregnant women, showed that elevated maternal testosterone levels associated with greater postnatal weight gain from birth to 6-months in male but not female infants [55]. Our sex-dependent findings are consistent with this, but extend beyond infancy and into early childhood (age 7). Interestingly, despite the stronger relationship of maternal androgens with low birth weight in female versus male offspring, this association did not translate into accelerated postnatal weight gain in girls and was only observed among boys. Thus, our findings suggest that the fetal programming effects of prenatal androgen exposure on postnatal weight gain may be sexually dimorphic and not entirely dependent on birthweight. Future studies are needed to replicate these results and elucidate physiological mechanisms underlying sex differences in the impact of maternal androgen exposure on postnatal weight regulation.

The underlying mechanisms of how prenatal androgens program intrauterine growth retardation and postnatal catch-up growth in humans are not well understood. Although several explanatory mechanisms have been proposed, accumulating evidence from the literature suggests that alterations in the growth hormone (GH)-insulin-like growth factor (IGF) system in response to prenatal androgen excess as well the effects of prenatal androgens on placental function may play significant roles. The GH-IGF axis is a key endocrine mechanism that regulates fetal growth, and it has been proposed that an adverse intrauterine environment (i.e. maternal undernutrition, fetal hypoxia) can affect the expression and development of the fetal GH-IGF system and permanently alter the growth trajectory of the offspring throughout life [60]. In one study conducted on female sheep, prenatal exposure to excess testosterone was found to reduce IGF bioavailability resulting in intrauterine growth restriction. In contrast, increases in IGF bioavailability were seen during the postnatal catch-up growth period as a result of testosterone exposure [61]. However, these studies pooled results from both male and female offspring and did not specifically examine for sex differences. In a prenatal stress model, we previously showed that exposure to synthetic glucocorticoids during late gestation in pregnant rats (akin to ~2nd trimester in humans) resulted in reduced body weight in both sexes of the offspring. However, this finding was associated with a decrease in plasma IGF-1 concentration and decreased hypothalamic expression of growth hormone-releasing hormone only among female offspring [62]. Thus, these results provide evidence that intrauterine growth restriction in

offspring exposed to prenatal steroids may be mediated by reduced GH-IGF-1 signaling in a sex-specific fashion.

Given that androgen receptors are expressed in the human placenta [34,63], it has been proposed that prenatal androgens may play a role in placental function. Sathishkumar et al. demonstrated that prenatal testosterone treatment to pregnant rats resulted in decreased expression of a specific placental amino acid transporter, resulting in altered nutrient delivery to the fetus, and subsequent fetal growth restriction and low birth weight in the offspring [18]. In addition, prenatal testosterone treatment in pregnant rats has been shown to reduce placental vascularization (via decreased uterine blood flow) resulting in placenta-fetal hypoxia and reduced fetal growth at term [64]. Thus, the ability of prenatal androgens to directly influence placental nutrient transfer and placental vascularity may contribute to some of the adverse effects of androgens on fetal growth outcomes. However, whether sex differences in fetal growth are mediated by sexually dimorphic differences in placental function is unclear and needs further investigation. Future research is needed to establish the androgen-mediated mechanisms and epigenetic markers involved in the regulation of fetal growth and underlying sex differences.

There is uncertainty in the literature about the mechanistic role of the gonads in androgen synthesis as distinct from the adrenal cortex in impacting placental function and fetal development. Although studies in women with PCOS suggest that fetal outcomes from elevated maternal androgen production may be driven primarily by the ovary [22,17], the adrenal glands have also been hypothesized to be an important source *in utero* given the presence of LH/hCG receptors in both the maternal and fetal adrenal cortex which may contribute to adrenal androgen hypersecretion during pregnancy and impact offspring development [65,66]. Information on diagnosis of female androgen excess disorders, such as PCOS and congenital adrenal hyperplasia, although not available in the CPP women, could contribute to understanding potential pathologic sources of higher levels of maternal androgen exposure. Thus, further investigation is needed to identify the maternal and fetal origins of increased androgen production during pregnancy and their differential contributions to human fetal growth and development.

The findings presented here, that higher prenatal androgens program low birth weight, in conjunction with epidemiological studies linking low birth weight and catch-up growth to adult CVD risk, underscore the adverse consequences of maternal androgen exposure on long-term human health. In addition to low birth weight among girls, we previously showed in this birth cohort that higher androgen levels during pregnancy associated with increased risk for development of the metabolic syndrome in adulthood, an effect that was observed in female offspring and not in males [8]. Thus, it is conceivable that the adult metabolic consequences of high prenatal androgen exposure may be mediated via intrauterine growth restriction (i.e. low birth weight), particularly in mothers carrying a female fetus, while catch-up growth may be a more significant mediator among mothers carrying a male fetus. Based on these findings, maternal androgens could serve as an early biomarker for intrauterine growth retardation, postnatal catch up growth and subsequent cardiometabolic dysregulation in adult life.

Our study has notable strengths and some limitations. Fetal programming of childhood growth patterns by prenatal sex steroids has not been well studied in humans due to lack of longitudinal data that can address these developmental pathways. While prior human studies only examined single time periods within the first two postnatal years (birth and infancy) [55,54], we had the ability to examine the impact of prenatal androgen levels *in utero* on offspring growth at multiple timepoints from birth through early childhood (age 7). We also utilized a highly sensitive reporter gene bioassay for the measurement of maternal bioactive androgens. Bioactive androgen assays have the advantage of detecting the activity of all known and chemically unidentified androgenic compounds. The long-term stability of sex hormones in maternal prenatal serum from the CPP after over 40 years of storage has been validated [67]. Although maternal serum is an indirect biomarker for fetal androgen exposure, studies have shown that neonates born to mothers with elevated serum testosterone levels have higher morbidity and mortality rates [68], suggesting that maternal androgens may be an early predictor of adverse postnatal health outcomes. Our analyses were limited to early third trimester maternal androgen levels and do not rule out the possibility of programming effects on pre- and postnatal growth occurring during early gestation. However, beginning of third trimester has been demonstrated to be a critical developmental period for the organizational effects of gonadal hormones as well as maturation of fetal physiological organ systems [38].

It should be noted that the mother-child participants in our birth cohort were enrolled between 1959 to 1966, before the significant rise in US prevalence of childhood obesity starting in the 1980s [69,70]. In addition to differences in prenatal care practices, the 1960s was also a time when low socioeconomic status was associated with higher risk for being underweight rather than overweight or obese as it is today [71], which could certainly influence the developmental growth patterns of the offspring. Therefore, we cannot establish whether our findings can generalize to more recent birth cohorts.

In summary, we showed that higher maternal androgens associated with lower BMIz at birth, an effect that was observed in girls and not in boys. Although higher prenatal androgen levels were associated with intrauterine growth restriction in both sexes, our results revealed significant sex-dependent threshold effects with female fetuses being more vulnerable than male fetuses. Postnatal growth acceleration, an important predictor of cardiometabolic diseases in later life associated with higher maternal androgens in boys but not in girls, suggesting sexually dimorphic effects on postnatal weight regulation independent of birth weight. Thus, our results highlight the need for better powered studies to further investigate the sex differences in the interaction of fetal growth restriction and/or postnatal weight gain with circulating maternal androgens and the shared pathways involved in the cardiometabolic reprogramming of adult offspring. An understanding of these mechanisms will aid in the development of interventions to improve the health of mothers during pregnancy and potentially prevent conditions that lead to low birth weight and its associated sex-dependent health consequences to the offspring across the lifespan.

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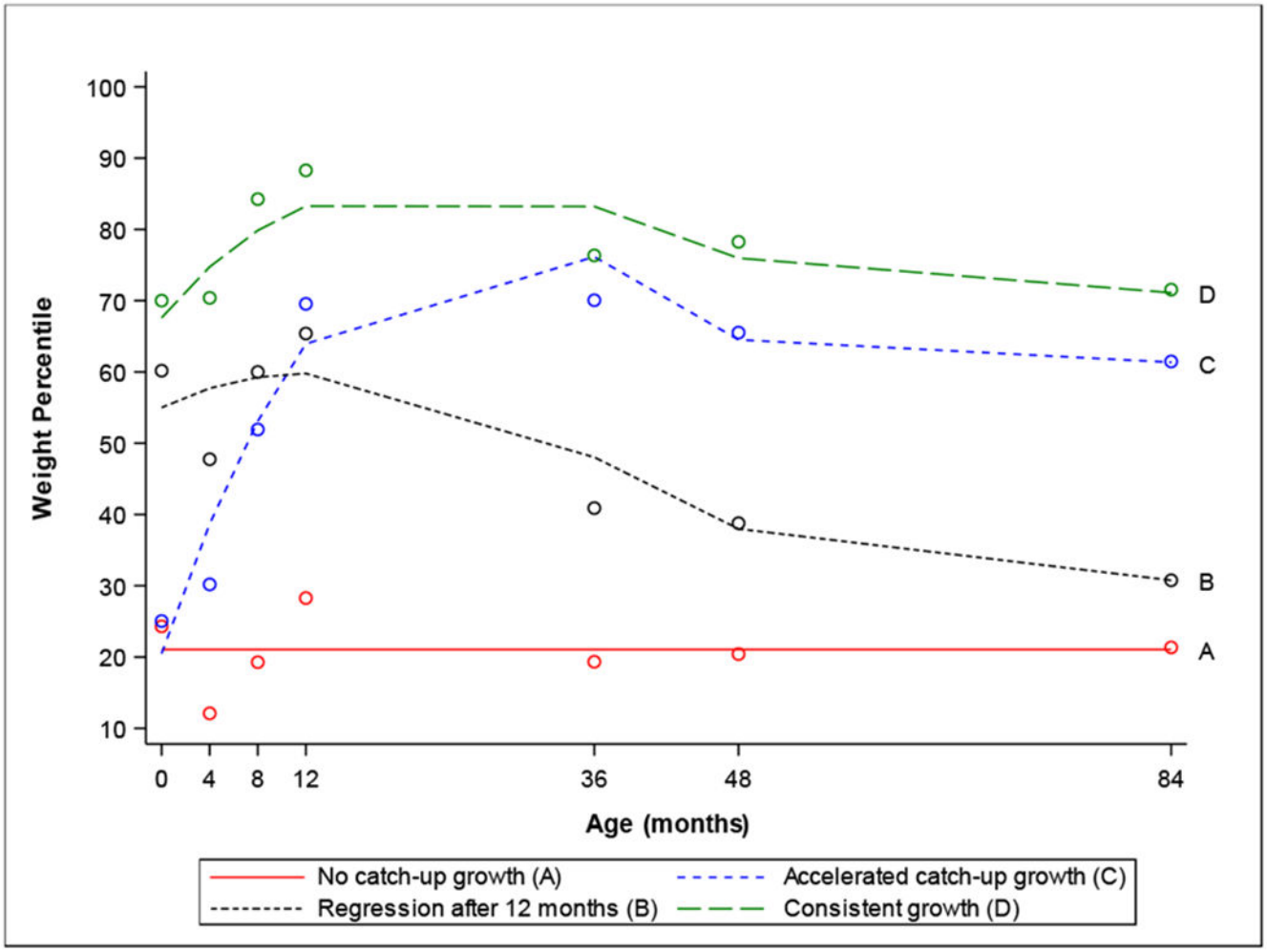

Figure 1.

Weight growth trajectory in child offspring from birth to age 7 years classified by group-based trajectory modeling. Circles represent the observed mean weight percentile of all individuals assigned to a trajectory at each timepoint.

TABLE 1.

Participant Characteristics

	Total Sample (n = 516)	Boys (n = 209)	Girls (n = 307)	p*
Maternal Characteristics				
Maternal age, (years)	24.7 (5.5)	25.0 (5.5)	24.5 (5.5)	0.27
Trimester at registration	1.8 (0.7)	1.8 (0.7)	1.9 (0.7)	0.09
White Race, (N/%)	452 (87.6)	184 (88.0)	268 (87.3)	0.70
Parental socioeconomic index	5.5 (1.9)	5.5 (2.0)	5.5 (1.9)	0.75
Maternal smoking, (N/%)	280 (54.0)	113 (54.6)	167 (55.1)	0.91
Maternal education, (years)	11.1 (2.2)	11.1 (2.5)	11.1 (2.0)	0.98
Pre-pregnancy BMI, (kg/m^2)	23.1 (4.2)	23.3 (4.3)	23.0 (4.2)	0.52
Number of Prior Pregnancies	2.1 (2.1)	2.2 (2.2)	2.2 (2.0)	0.93
Pregnancy Factors				
Offspring weeks gestation, (wks)	39.5 (2.0)	39.5 (2.1)	39.6 (1.9)	0.54
Weeks gestation at serum draw, (wks)	32.8 (3.2)	32.3 (3.3)	32.7 (3.1)	0.32
Bioactive androgens, (nM)	2.6 (7.0)	3.3 (10.5)	2.2 (2.8)	0.02
Pregnancy weight gain, (kg)	8.8 (4.3)	8.3 (4.0)	9.2 (4.5)	0.03
Fetal Growth Restriction (N/%)	190 (37.0)	74 (35.6)	116 (38.0)	0.57
Preeclampsia, (N/%)	135 (28.7)	59 (31.2)	76 (27.1)	0.33
Offspring Characteristics				
Birthweight, (grams)	3220 (518)	3287 (546)	3175 (495)	0.02
Childhood obesity at age 7, (N/%)	32 (7.5%)	15 (8.5%)	17 (6.7%)	0.48

Values are expressed as mean ± sd and/or percentages (%).

*Differences by adult offspring sex compared using Pearson's chi squared test (categorical variables) or two-independent-samples t-test (continuous variables).

Parental socioeconomic index constructed as a composite index using education, occupation and income (range: 0-9.3).

Table 2.

Association between prenatal maternal bioactive androgens and offspring childhood BMI-for-age Z-scores

β estimate (95% CI) for BMI Z-scores									
Adjusted Model	**Total Sample**			**Boys**			**Girls**		
Age	**n**	**β (95% CI)**	**p**	**n**	**β (95% CI)**	**p**	**n**	**β (95% CI)**	**p**
Birth	414	−0.39 (−0.73, −0.06)	0.02	168	−0.24 (−0.58, 0.11)	0.16	246	−0.72 (−1.40, −0.04)	0.04
4-months	399	−0.07 (−0.31, 0.17)	0.55	162	−0.18 (−0.48, 0.19)	0.36	237	−0.02 (−0.43, 0.38)	0.90
1-year	385	−0.17 (−0.40, 0.06)	0.15	157	−0.15 (−0.67, 0.14)	0.17	228	−0.21 (−0.61, 0.20)	0.29
4-year	308	−0.32 (−1.55, 0.90)	0.59	123	0.43 (−7.18, 8.05)	0.83	185	−0.22 (−0.77, 0.34)	0.41
7-year	412	−0.01 (−0.19, 0.17)	0.92	166	−0.03 (−0.28, 0.22)	0.81	246	−0.04 (−0.36, 0.28)	0.80

Analyses by linear mixed model regression including a random intercept to account for intrafamilial correlation and adjusted for maternal age, maternal education, pre-pregnancy BMI, pregnancy weight gain. Bioactive androgen levels were log-transformed.

Table 3

A: Association between prenatal maternal bioactive androgens and fetal growth restriction								
Odds Ratio (95% CI) for Fetal Growth Restriction								
n	Overall	p	n	Males	p	n	Females	p
415	1.66 (1.05, 2.61)	0.03	169	2.01 (0.99, 4.08)	0.05	246	1.74 (0.95, 3.19)	0.07
B. Association between prenatal maternal bioactive androgen quartile and fetal growth restriction								
Odds Ratio (95% CI) for Fetal growth restriction								
n	Quartile	Overall	p	n	Boys	p	n	Girls	p
105	**Q1**	Ref.	-	46	Ref	-	59	Ref.	-
108	**Q2**	1.76 (0.99, 3.14)	0.05	44	1.00 (0.45, 2.69)	0.83	63	2.52 (1.07, 5.90)	0.03
102	**Q3**	1.61 (0.90-2.91)	0.11	38	1.42 (0.52, 3.89)	0.49	66	2.05 (0.92, 4.58)	0.08
100	**Q4**	2.30 (1.22-4.32)	0.01	41	2.62 (1.05, 6.51)	<0.01	58	2.68 (1.12, 6.39)	0.03

Analyses by generalized estimating equations with a logit link and Bernoulli distribution adjusted for maternal age, maternal education, pre-pregnancy BMI, pregnancy weight gain and intrafamilial correlation. In Table 3A, bioactive androgen levels were log-transformed and analyzed as a continuous variable. In Table 3B, p-values represent comparisons between the 2nd, 3rd and 4th quartiles vs. the 1st quartile (reference) of bioactive androgens at the 0.05 significance level. Fetal Growth Restriction was defined as birthweight less than the 20th percentile for sex and gestational age based on 1999-2000 US Natality Data.

Table 4.

Maternal Bioactive Androgen Levels By Offspring Growth Trajectory and Sex

	Overall		Boys		Girls	
Weight Growth Trajectory	**N (%)**	**Mean (SD)**	**N (%)**	**Mean (SD)**	**N (%)**	**Mean (SD)**
No catch-up growth	125 (24%)	2.18 (1.1)	55 (26%)	2.07 (0.7)	70 (23%)	2.26 (1.2)
Accelerated catch-up growth	114 (22%)	3.34 (12)	43 (21%)	5.37 (19)	71 (23%)	2.12 (1.4)
Regression after 12 months	115 (22%)	3.14 (9.1)	45 (21%)	4.20 (13)	70 (23%)	2.46 (5.6)
Consistent growth	165 (32%)	2.10 (0.9)	67 (32%)	2.23 (1.0)	98 (32%)	2.00 (0.7)

Values are expressed as mean ± sd (nM).

Table 5.

Association between prenatal maternal bioactive androgens and offspring weight growth trajectory

Odds Ratio (95% CI) for weight growth trajectory									
n	**Trajectory**	**Overall**	**p**	**n**	**Boys**	**p**	**n**	**Girls**	**p**
99	Consistent growth	Ref.	-	43	Ref.	-	56	Ref.	-
95	No catch-up growth	1.39 (0.99, 1.95)	0.06	37	1.59 (0.84, 3.01)	0.15	58	1.67 (0.87, 3.18)	0.13
92	Accelerated catch-up growth	1.43 (0.97, 2.13)	0.07	35	2.14 (1.14, 4.03)	0.02	57	0.91 (0.45, 1.83)	0.78
131	Regression after 12-months	1.02 (0.59, 1.78)	0.94	54	1.71 (0.85, 3.46)	0.14	77	0.55 (0.30, 1.05)	0.07

Analyses by generalized estimating equations with a logit link and multinomial response adjusted for maternal age, maternal education, pre-pregnancy BMI, pregnancy weight gain and intrafamilial correlation. Bioactive androgen levels were log-transformed.