Published in final edited form as:

J Dev Orig Health Dis. 2013 August ; 4(4): 280–284. doi:10.1017/S2040174413000135.

Sex-specific associations of maternal prenatal testosterone levels with birth weight and weight gain in infancy

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Abstract

Associations between maternal salivary testosterone at 36 weeks' gestation with birth weight and infant weight gain through 6 months of age were examined in a group of 49 healthy, pregnant women and their offspring. The diurnal decline of maternal testosterone was conserved in late pregnancy, and levels showed significant day-to-day stability. Elevated maternal morning testosterone level was associated with lower birth weight *Z*-scores adjusted for gestational age and sex, and greater infant weight gain between birth and 6 months. Although maternal testosterone levels did not differ by fetal sex, relations were sex-specific such that maternal testosterone had a significant impact on weight for male infants; among female infants associations were nonsignificant. Results highlight the opposing influence of maternal androgens during pregnancy on decreased growth *in utero* and accelerated postnatal weight gain.

Keywords

birth weight; pregnancy; sex differences; testosterone; weight gain

The organizing role of the intrauterine environment on developing neural, endocrine, metabolic and immune systems of the fetus, and the developmental origins of health and disease, has attracted significant recent attention.¹ Prominent among these is the maternal endocrine milieu. Over the course of gestation, marked alterations in the maternal endocrine state serve to maintain the pregnancy and facilitate maturation and individuation of the central nervous system, directly reaching the fetus via the placenta and indirectly affecting changes to placental function. Non-physiological concentrations of maternal prenatal

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Conflicts of Interest

None.

Ethical Standards

The authors assert that all procedures contributing to this work comply with the ethical standards of the relevant national guidelines on human experimentation for women and children and with the Helsinki Declaration of 1975, as revised in 2008, and has been approved by the institutional committees review board.

hormones may exert maladaptive effects on the fetus and as such have been termed ‘endogenous functional teratogens’.²

With respect to growth patterns in early life, there is accumulating evidence drawn from animal literature to suggest that high maternal testosterone concentration in pregnancy is associated with intrauterine growth restriction, resulting in low birth weight.^{3–6} To our knowledge, exclusive of studies focused on high-risk pregnant populations with endocrine pathology (i.e. polycystic ovary syndrome, congenital adrenal hyperplasia); only one study has examined maternal androgen levels during human pregnancy as a predictor of birth outcome. Findings, based on a sample of 147 pregnant women, indicated that elevated maternal testosterone measured in serum at 17 and 33 weeks’ gestation was associated with decreased infant birth weight and length.⁷ No associations were found for dehydroepiandrosterone sulfate (DHEAS), androstenedione or sex hormone-binding globulin (SHBG).⁷

The goal of the current study is to add to the small literature examining maternal testosterone in human pregnancy in relation to birth weight, and to extend past studies by exploring the association of prenatal maternal salivary testosterone and postnatal weight regulation, as indicated by infant weight gain between birth and 6 months. Maternal testosterone was indexed in late pregnancy, at 36 weeks’ gestation, marking a time when endogenous levels of circulating testosterone have peaked⁸ and of maximum fat deposition in the fetus.⁹ Given male vulnerability to prenatal exposures¹⁰ and sex-specific adaptation of the placenta,¹¹ the potential moderating role of fetal sex was examined.

Method

Participants

Eligibility was restricted to normotensive, nonsmoking pregnant women older than 18 years of age carrying normally developing, singleton fetuses. Women with existing or developing medical conditions that complicate pregnancy or jeopardize fetal growth (e.g. type I diabetes, primary hypertension) were excluded from participation. There were no cases of endocrine disorders known to affect sex steroid levels (e.g. polycystic ovary syndrome). Pregnancy dating was based on last menstrual period and early confirmation by ultrasound (M gestational age at pregnancy detection = 4.9 weeks, $s.d.$ = 1.9 weeks). Of the 55 self-referred women enrolled, 49 women were retained in the current analysis. The remainder withdrew from the study at an earlier time point owing to preterm delivery or moving out of the area ($n = 5$) or had missing salivary data ($n = 1$). The sample represents a population of well-educated (M years education = 16.9 years, $s.d.$ = 1.7), mature (M age = 32.2 years, $s.d.$ = 4.0) and married (94%) women, approximately half expecting their first child (53%). Most were non-Hispanic white (78%); the remainder was Asian (20%) or African-American (2%). Fifty-seven percent ($n = 28$) of the infants were male. Infants were born at appropriate size ($M = 3465$ g, $s.d.$ = 505 g) for gestational age ($M = 39.4$ weeks, $s.d.$ = 1.1 weeks). Forty mother–infant dyads participated in a laboratory follow-up visit when infants were 6 months of age (M age = 6.4 months, $s.d.$ = 0.3). The study was approved by the local Institutional Review Board and participants provided written informed consent.

Procedure

Participants were part of a prospective, longitudinal study that included diurnal saliva collection in the 36th week of gestation. Women collected saliva at home via oral swab in the morning (30min after waking), midday (1200 h) and late afternoon (1600 h) on 2 consecutive days, and were instructed to refrain from eating, drinking or brushing teeth for 30min before collection. Following saturation (held in the mouth 2 min), the swabs were returned to storage tubes and frozen at -70°C until assay. Women completed a saliva collection record form, noting awakening and collection times. Following delivery, nurses completed a labor and delivery record for the study, abstracted from medical records. Participants returned for a laboratory-based postnatal visit when their infants were 6 months of age.

Measures

Maternal characteristics—Maternal age, parity and pre-pregnancy weight and height were extracted from demographic questionnaires completed at the first prenatal study visit. Pre-pregnancy body mass index (BMI) was indexed by weight in kilograms divided by the square of height in meters.

Testosterone assay—Samples were expressed by centrifugation and assayed for salivary testosterone at the Center for Interdisciplinary Salivary Bioscience Research at Johns Hopkins University, using commercially available enzyme immunoassays (Salimetrics, PA, USA) without modification to the manufacturer's recommended protocols. The testosterone assay uses 25 μl of saliva for a singlet determination, has a range of sensitivity from 1 to 600 pg/ml and shows high specificity, indicated by negligible cross-reactivity (1.2%) with other androgens (e.g. androstenedione, DHEA). Performance is robust for samples with pHs ranging from 4.0 to 9.0. The concentration of Salimetrics testosterone standards and controls are traceable to NIST standards in accordance with ISO 17025 and ISO Guide 34. The purity of the neat material is characterized by HPLC/PDA and calibrated against LC/MS and $^1\text{H-NMR}$. Unbound testosterone in serum enters the primary secretory fluid by passive diffusion through the epithelial membrane¹² and is correlated with salivary levels.¹³ Salivary assays do not discriminate between total and free testosterone, but best approximate the free concentration.¹² Samples were assayed in duplicate, and the criterion for repeat testing was variation between duplicates $>20\%$. Average intra- and inter-assay coefficients of variation were $<5\%$ and 10% , respectively. The mean maternal salivary testosterone level of the duplicate assay was used in analysis.

Fetal and infant growth—Birth weight, gestational age and sex of the child were obtained from the labor and delivery record. At the follow-up visit, mothers reported on infant weight and length as measured at their infants' 6-month pediatric examination (M age = 6.3 months, $\text{s.d.} = 0.3$), with 75% of pediatric exams occurring before the visit ($M = 8$ days prior). In the event the pediatric exam was scheduled for a future date mothers were subsequently contacted via phone or email to obtain growth measurements and the date of the visit. Infant age was calculated by subtracting the birth date from the exam date. Birth weights were standardized to gestational age and sex-specific Z -scores based on World Health Organization (WHO) standards.¹⁴ Infant weight, length and weight-for-length at 6

months were also standardized by age and sex using WHO standards (weight-for-age Z = WAZ; length-for-age Z = LAZ; weight-for-length Z = WLZ). Weight gain was calculated as the difference between weight Z -scores at 6 months and birth.

Data analysis plan

Maternal testosterone values were not significantly skewed and did not require transformation. The SAS MIXED procedure, using maximum likelihood estimation for repeated measures data, was used to model the testosterone diurnal decline. Variation in collection timing within each sampling occasion was not associated with testosterone. Mean testosterone level across the two sampling days at each occasion was used in the analysis. Failure to collect as directed or insufficient sample volume resulted in six missing samples; in those cases, the value from one sampling day was used in place of the mean. General linear models in SAS were used to examine the prediction of birth weight Z -scores adjusted for gestational age and sex by maternal testosterone; maternal characteristics including age, parity and pre-pregnancy BMI were tested as covariates. Separate models examined whether maternal prenatal testosterone predicted weight gain from birth to 6 months (Z -score difference) and infant size at 6 months of age (WAZ, LAZ, WLZ). Sex was included as a moderator in all models.

Results

Mean maternal salivary testosterone levels (s.d.) in pg/ml at each time of day were: 97.0 (21.5), 78.1 (18.3) and 76.6 (16.6). There was considerable stability in the overall day mean maternal testosterone level, $r(49) = 0.84$, $P < 0.001$, and within each sampling window (morning: $r(47) = 0.64$, $P < 0.001$; midday: $r(46) = 0.58$, $P < 0.001$; late afternoon: $r(48) = 0.55$, $P < 0.001$). Mixed modeling revealed a significant overall decline in testosterone levels from morning to late afternoon ($t(48) = -7.36$, $P < 0.01$), driven by the reduction from morning to midday ($t(48) = -7.54$, $P < 0.01$); there was no evidence for further decline from midday to late afternoon. Maternal testosterone did not differ by fetal sex at any point across the day or when averaged over time (range $ts = 0.66$ – 1.00).

Maternal age, pre-pregnancy BMI and parity were explored as potential covariates but were unrelated to infant birth weight and as such, were not included in the final model to maximize degrees of freedom. In the final model predicting gestational age and sex-specific birth weight Z -score, there were significant main effects for morning maternal salivary testosterone level ($\beta = -0.04$, s.e. = 0.02, $t = -2.14$, $P < 0.05$) and fetal sex ($\beta = -3.32$, s.e. = 1.50, $t = -2.22$, $P < 0.05$), mitigated by a significant interaction ($\beta = 0.04$, s.e. = 0.02, $t = 2.47$, $P < 0.05$) such that with higher levels of maternal prenatal testosterone, male infants were born at lower birth weights relative to female infants. Neither variation in midday and late afternoon maternal salivary testosterone nor the diurnal decline significantly predicted size at birth.

As depicted with raw values in Figs 1a and 1b, the interaction between morning maternal salivary testosterone and sex also significantly predicted weight gain Z -scores from birth to 6 months ($\beta = -0.055$, s.e. = 0.023, $t = -2.42$, $P < 0.05$). Male infants of women with high levels of maternal testosterone *in utero* presented a more accelerated rate of weight gain

across the first 6 months, relative to female infants and to male infants of women with low levels of testosterone. Only morning maternal testosterone was predictive, testosterone measured from saliva collected at time points later in the day and the diurnal decline were nonsignificant. In addition, greater infant weight-for-age Z ($\beta = 0.02$, s.e. = 0.01, $t = 3.18$, $P < 0.01$) and weight-for-length Z ($\beta = 0.02$, s.e. = 0.01, $t = 2.58$, $P < 0.05$) at 6 months were predicted by higher morning maternal salivary testosterone level; no sex differences were found. The model predicting length-for-age Z was nonsignificant.

Discussion

These data contribute to the sparse normative literature on testosterone in women in general and its contribution to the hormonal milieu during pregnancy. The detected association between elevated prenatal maternal salivary testosterone at 36 weeks gestation and lower infant birth weight corroborates previous animal studies^{3–6} and the single existing study of uncomplicated human pregnancy.⁷ Although several explanatory mechanisms for the association between testosterone and birth weight have been proposed including testosterone-induced changes in maternal energy regulation, direct effects of testosterone reaching the fetal compartment and indirect effects of testosterone on placental function,⁷ the latter is emerging as most compelling. In a rodent sample, compared with the negligible magnitude of direct effects, testosterone administration was shown to modify placental function, restricting nutrient delivery to the fetus.¹⁵ If by this mechanism metabolic set points are altered *in utero* as a means of adaptive phenotypic plasticity, it would be expected that these organizational effects would extend to the postnatal period.

This study is the first in human pregnancy to generate preliminary support for effects of testosterone exposure *in utero* extending to postnatal weight regulation. Higher maternal testosterone in late pregnancy was associated with more accelerated weight gain between birth and 6 months, and ultimately greater infant weight-for-age and weight-for-length at 6 months of age. These findings suggest if maternal testosterone acts on the placenta to restrict nutrient delivery during the prenatal period¹⁵ yet the offspring is exposed to a discordant nutrient-rich postnatal environment, disruption of metabolic regulation and accelerated weight gain may result. Independent of birth weight, accelerated weight gain in infancy is a risk factor for subsequent obesity and morbidity.^{16–18}

The observed associations with testosterone were sex-specific to male infants; the impact of elevated testosterone on weight in female infants was nonsignificant. Although animal studies have focused on the impact of excess exogenous testosterone treatment on female offspring,^{4,6} or found no evidence of sex differences,⁵ our finding adds to the accumulating evidence that the male fetus is more vulnerable to endogenous maternal factors relative to the female.¹⁰ This may be mediated by sexually dimorphic placental adaptations to a sub-optimal fetal environment.¹¹ In the current study, the association between testosterone and weight in male fetuses was not explained by sex differences in maternal testosterone levels, which did not differ based on fetal sex. This supports other reports,^{19–21} with the exception of one study that reported higher testosterone in women carrying male fetus.⁸ Moreover, fetal testosterone levels would not be expected to reach the maternal compartment, as male-

bearing placentae have higher aromatase expression, which converts testosterone to estrogens, relative to female fetus.²²

Interpretation of these results is limited by reliance on endogenous testosterone sampling at a single point in late gestation. Although considerable stability in maternal testosterone levels on contiguous days was observed in the current study, these data do not provide information on the degree to which endogenous testosterone levels reflect a stable characteristic of the maternal hormonal milieu over the course of gestation. Thus, we are unable to evaluate whether the observed associations are based on contemporaneous mechanisms related to peak testosterone exposure during the late, catabolic state of pregnancy, which corresponds to maximum fetal fat deposition,⁹ or reflect more persistent variation in testosterone levels at earlier points in gestation. Animal studies have shown that excess exogenous testosterone treatment of non-physiological concentration early in gestation has profound effects on fetal growth, yielding accumulating disruption of metabolic tissues (i.e. insulin sensitivity) across gestation.^{3–6}

Although the circadian rhythm of maternal testosterone was maintained in late pregnancy and levels exhibited considerable day-to-day stability, significant associations with fetal and infant weight were found only for morning levels. The biological relevance of the morning peak in testosterone remains unclear. However, it may be independently regulated outside of pulsatile secretion ongoing throughout the day and preparatory for daily metabolic demands. Thus morning testosterone may be a unique and discrete measure of maternal androgen activity.

In summary, elevated maternal testosterone during late pregnancy affects prenatal and postnatal growth, exerting opposing influences on weight regulation. Lower birth weight, in combination with observed postnatal growth acceleration, increases risk for subsequent obesity and associated morbidity.^{16–18} Further study warrants a larger sample and more intense sampling design of the maternal androgen milieu over the course of gestation, along with morphologic measures that correspond to fetal testosterone exposure, such as the 2D–4D ratio²³ or anogenital distance.²⁴ Finally, future work is needed to elucidate physiological mechanisms of action of maternal testosterone, in particular, placental function and sex-specific placental adaptations to testosterone exposure.

Acknowledgments

We are grateful for the diligent and generous participation of our study families, without whom this research would not be possible.

Financial Support

This research was supported by National Institute of Child Health and Human Development grant 2R01 HD27592 awarded to J. A. DiPietro.

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Fig. 1.

(a, b) Unadjusted trajectories of infant weight gain between birth and 6 months of age by maternal testosterone levels at 36 weeks gestation and infant sex relative to World Health Organization standard growth curve percentiles. Male infants exposed to high levels of maternal testosterone in late pregnancy show accelerated postnatal weight gain (b).