AUTHOR QUERY FORM

ELSEVIER	**Journal: YGCEN** **Article Number: 10501**	**Please e-mail or fax your responses and any corrections to:** **E-mail: corrections.essd@elsevier.sps.co.in** **Fax: +31 2048 52799**

Dear Author,

Any queries or remarks that have arisen during the processing of your manuscript are listed below and highlighted by flags in the proof. Please check your proof carefully and mark all corrections at the appropriate place in the proof (e.g., by using on-screen annotation in the PDF file) or compile them in a separate list.

For correction or revision of any artwork, please consult http://www.elsevier.com/artworkinstructions.

Articles in Special Issues: Please ensure that the words 'this issue' are added (in the list and text) to any references to other articles in this Special Issue.

Uncited references: References that occur in the reference list but not in the text – please position each reference in the text or delete it from the list.

Missing references: References listed below were noted in the text but are missing from the reference list – please make the list complete or remove the references from the text.

Location in article	Query / remark Please insert your reply or correction at the corresponding line in the proof
Q1	Batty et al. (1986) is cited in the text but not listed. Please check.
Q2	Nelson (2006) is cited in the text but not listed. Please check.

Electronic file usage
Sometimes we are unable to process the electronic file of your article and/or artwork. If this is the case, we have proceeded by:

☐ Scanning (parts of) your article ☐ Rekeying (parts of) your article ☐ Scanning the artwork

Thank you for your assistance.

General and Comparative Endocrinology xxx (2009) xxx–xxx

Contents lists available at ScienceDirect

General and Comparative Endocrinology

journal homepage: www.elsevier.com/locate/ygcen

Maternal androgen levels during pregnancy are associated with early-life growth in Geoffroy's Marmosets, *Callithrix geoffroyi* ☆

Adam S. Smith [a,*], Andrew K. Birnie [b], Jeffrey A. French [b,c]

[a] Department of Psychology and Program in Neuroscience, Florida State University, Tallahassee, FL, United States
[b] Department of Psychology and Callitrichid Research Center, University of Nebraska at Omaha, Omaha, NE, United States
[c] Department of Biology, University of Nebraska at Omaha, Omaha, NE, United States

ARTICLE INFO

Article history:
Received 17 July 2009
Revised 24 September 2009
Accepted 20 October 2009
Available online xxxx

Keywords:
Testosterone
Maternal androgens
Prenatal development
Postnatal growth
Marmoset
Critical developmental periods
Intrauterine growth retardation

ABSTRACT

Fetal development is a critical period of physical development, and factors in the intrauterine environment can cause lasting effects on the growth and development of offspring. There is little research evaluating organizational effects of early androgen exposure of endogenous maternal origins on the prenatal and postnatal growth of offspring. We evaluated the association between maternal androgen levels during gestation and pre- and postnatal growth of offspring. Maternal androgen levels in marmoset females were measured using enzyme immunoassays of urine samples acquired during 18 pregnancies. Somatic measurements of the resulting 25 viable offspring were taken on postnatal days (PND) 2, 30, 60, 120, 180, 240, and 300. Maternal androgen levels during the first trimester were negatively associated with weight, body length, and several girth measurements (i.e., torso, head, chest, and arm circumference) of offspring on PND 2. First trimester maternal androgen was also negatively associated with physical growth during early and late infancy but seemed to be positively associated with a rebound in juvenile growth. Exposure to maternal androgen during early gestation led to both a reduction in birth weight and postnatal catch-up for both males and females, equally. Fetal growth retardation and the reprogramming of metabolic tissues by exposure to prenatal androgen could be mediating factors of suppressed postnatal growth.

Published by Elsevier Inc.

1. Introduction

Low birth weight followed by catch-up growth during early life seems to be unfavorable and a marker for metabolic deficits (Nobili et al., 2008). Prenatal exposure to androgen excess affects fetal programming and alters postnatal growth and metabolism (Wolf et al., 2002; Bruns et al., 2004; Manikkam et al., 2004; Crespi et al., 2006). Women experiencing hyperandrogenism associated with polycystic ovarian syndrome (PCOS) deliver small-for-gestational age newborns at a higher prevalence (Homburg, 2006; Xita and Tsatsoulis, 2006) and children with metabolic defects such as insulin resistance and compensatory hyperinsulinaemia (Dunaif et al., 1987; Dunaif, 1997). In prenatally androgenized animal models, fetuses exposed to testosterone in early gestational life are born small (Wolf et al., 2002; Bremner and Cumming, 1978) and some have a postnatal catch-up (Manikkam et al., 2004; Crespi et al., 2006). When fetuses are exposed to pharmacological levels of testosterone, insulin-like-growth factor-I (IGF I) availability is reduced in sheep (Manikkam et al., 2004), and there is diminished insulin sensitivity and impaired pancreatic β-cell function in rhesus monkeys (Bruns et al., 2004). Beyond the effects of hyperandrogenism on development, there is little evidence that exposure to biologically relevant levels of androgen early in gestation impairs prenatal growth. In a single study with a human population, elevated maternal androgen concentrations during the first trimester were associated with low birth weights (Carlsen et al., 2006). Additionally, despite the fact that exposure to androgen excess during early gestation can influence fetal growth retardation and the reprogramming of metabolic tissues, there is no knowledge about the organizational effects of exposure to normal fluctuation in androgen during early gestation on the postnatal growth of offspring.

The androgen milieu supplied by pregnant females during early gestational development may modify offspring phenotype via organizational effects on growth and development. Androgen levels rise early in pregnancy in several primate species (Rao and Kotagi, 1983; Hodges et al., 1984; Ellinwood et al., 1989; Castracane et al., 1998) including marmosets (Chambers and Hearn,

☆ This work was supported by grants from NSF (IBN 00-91030), NIH (HD-42882), and UCRCA at UNO.

* Corresponding author. Address: Department of Psychology and Program in Neuroscience, Florida State University, C448 PDB, 1107 W. Call Street, Tallahassee, FL 32306, United States.
E-mail address: asmith@neuro.fsu.edu (A.S. Smith).

0016-6480/$ - see front matter Published by Elsevier Inc.
doi:10.1016/j.ygcen.2009.10.008

1979). The early androgens in maternal androgens cannot be accounted for by steroid hormones from fetal origin (Reyes et al., 1973). Maternal androgen concentrations during early gestation are independent of fetal sex in marmosets (French et al., in press) and humans (Glass and Klein, 1981; Meulenberg and Hofman, 1991). Thus, androgens from maternal origins may be a natural source of prenatal exposure to androgen that can lead to changes in pre- and postnatal growth and metabolic function. If elevated maternal androgens can depress fetal growth and metabolic function, maternal androgens could provide a mechanism for a trade-off in metabolic investment by the pregnant female.

In marmoset and tamarin monkeys, low birth weight offspring have slower growth rates during postnatal development than heavier offspring (Tardif and Bales, 2004) suggesting that intrauterine factors that influence prenatal growth, as reflected in birth weights, may have lasting effects on growth and development throughout life. Like other primate species, there is a surge in androgens during the early gestation of Callitrichid primates (Chambers and Hearn, 1979), a critical window for developmental pathways that are sensitive to androgen (Wolf et al., 2002; Bruns et al., 2004; Manikkam et al., 2004; Crespi et al., 2006). The current study evaluated the activational and organizational effects of normal, maternally-derived androgenic exposure on prenatal and postnatal growth and development in marmoset monkeys, *Callithrix geoffroyi*. We documented the intra- and inter-individual variation in urinary androgens across gestation and tracked the somatic growth and development of offspring from birth through 300 days of life. If androgens disrupt IGF and other metabolic pathways, then maternal androgens during early gestation will be associated with altered offspring growth, reflected in low birth weight and size and leading to a rebound in growth rates during postnatal development.

2. Materials and methods

2.1. Subjects

We studied 18 pregnancies that produced 32 offspring (25 viable, see Table 1) from 5 female Geoffroy's marmosets (*Callithrix geoffroyi*). All animals were socially housed in colony rooms at the University of Nebraska at Omaha (UNO) Callitrichid Research Center (CRC). All animal use procedures were approved by the Institutional Animal Care and Use Committee (Protocol#: 07-033-FC). Colony rooms were maintained at 19.7–22.1 °C and on a 12:12 light–dark cycle. Wire-mesh enclosures varied in size depending on the size of the social group, about 0.8 m^3 per animal and included various enrichment items. All animals were fed once each day at 0800 h. Further details of animal housing and husbandry have been previously reported (Schaffner et al., 1995).

Table 1
Maternal and birth conditions.

Female	Age (yrs)	Litter ratio (males/females)	Length of gestation	Number of previous litters	Litter size at birth	First trimester androgen
Bes	4	1.0	157	0	1	0.350
	6	1.0	148	1	1	0.230
Dar	4	0.2	145	0	2	0.700
	5	1.1	150	1	2	0.326
	5	1.1	144	2	2	0.407
Lor	5	1.1	147	0	2	0.627
	6	0.2	152	1	2	0.407
	6	1.0	152	2	1	0.509
Pop	4	1.1	147	0	2	1.324
	5	2.0	145	1	2	0.766
	6	2.0	149	2	4	0.387
	6	2.1	145	3	3	1.061
	8	1.1	152	4	2	0.436
Swe	4	1.1	147	0	2	0.552
	5	1.0	146	1	1	0.379
	5	1.1	145	2	2	0.369
	6	0.1	157	3	1	0.395
	6	1.1	144	4	2	0.413

Note. Androgen reported in μg/mg Cr.

2.2. Urine collection

First-void urine samples from breeding females were collected during gestation using noninvasive, stress-free collection techniques (Nunes et al., 2002). Specifically, urine samples were collected into pans in exchange for a food reward between 0730 and 0800 h at least twice a week. Samples were centrifuged at 7000 RPM for two minutes to separate the urine, or supernatant, from the sediments. The supernatant was transferred into a test tube and stored at −20 °C until assayed.

2.3. Pregnanediol glucuronide enzyme immunoassay

Conception was determined by enzyme immunoassay (EIA) of urinary progesterone metabolites (pregnanediol glucuronide; PdG) concentrations. Gestation is 148 ± 4 days for *Callithrix* (Lunn et al., 1979; Fite and French, 2000; current study). Maternal levels in 5 female marmosets were measured by EIA during 18 pregnancies. Urinary PdG levels were monitored by EIA following protocol established by Munro et al. (1991) and adapted for marmosets (French et al., 1996). High and low concentration quality control pools were assayed on each plate. Intra- and interassay coefficients of variation were 22.0 and 6.1% (high, $n = 53$) and 28.0 and 6.8% (low, $n = 53$), respectively.

2.4. Androgen enzyme immunoassay

A testosterone (T) EIA adapted for Callitrichid primates (Nunes et al., 2000) was used to measure androgen concentrations for each urine sample at the Endocrine Bioservice Laboratory as a part of the Callitrichid Research Center (CRC). Briefly, urine samples (10 μl) were extracted in 5 ml diethyl ether after enzyme hydrolysis with β-glucuronidase (Sigma Chemical, St. Louis, MO). The ether was evaporated in a warm water bath under a gentle stream of air, and samples were reconstituted in 1.0 ml phosphate buffered saline. Procedural losses of androgen during the extraction procedure were monitored by the recovery of radiolabelled testosterone, and sample concentrations were corrected for loss. Each microtiter plate contained a standard curve (1000—7.8 pg testosterone), high and low concentration quality controls consisting of pooled marmoset urine, and samples. All standards, quality controls, and samples were assayed in duplicate. Cross-reactivity of the T anti-serum with 5-alpha-dihydrotestosterone was 57.3%, and was less than 0.3% with any other androgen (Dloniak et al., 2004), hence the results are described as urinary androgen concentrations, rather than urinary testosterone. In order to control for variable fluid intake and urinary output, all androgen concentrations were corrected by urinary creatinine concentrations using a modified Jaffé endpoint assay (Tietz, 1976) validated for marmosets (French et al., 1996). Two sets of quality control pools were used, and interassay coefficients of variation averaged 17.9% and 7.4% for the high and low concentration pools, respectively. Intra-assay coefficients of variation for the same pools averaged 7.9% and 10.4% for the high and low concentration pools, respectively.

Please cite this article in press as: Smith, A.S., et al. Maternal androgen levels during pregnancy are associated with early-life growth in Geoffroy's Marmosets, *Callithrix geoffroyi*. Gen. Comp. Endocrinol. (2009), doi:10.1016/j.ygcen.2009.10.008

2.5. Neonatal measures and postnatal growth

Somatic measurements of the 25 viable offspring were taken on postnatal days (PNDs) 2, 30, 60, 120, 180, 240, and 300. Offspring were removed from the home enclosure and transported in wire-mesh carriers (0.4 m^3) to a designated measurement station at the CRC. Following the protocols established by Power et al. (2001) and Abbott and Hearn (1978), somatic measurements included body weight, suprasternal-pubic length (SSPL), arm, head, abdominal, chest, and thigh circumference, knee–heel length (KHL), and biparietal diameter of the head (BPD). Measurements were made three times to the nearest 0.1 g or mm and averaged. Animals returned immediately after completing measurements (45 min maximum).

2.6. Statistical analysis

A repeated measure factorial analysis was conducted to determine whether there was a difference in excreted androgen levels during nonpregnant and pregnant females (by trimester). We conducted two analyses of excreted androgen levels during nonpregnant and pregnant periods first separating by trimester and a second analysis separating androgen levels by 10-day blocks. We also nested female identity, litter size, litter order, maternal parity, maternal size, and maternal age to evaluate any differences in the androgen levels by each individual, maternal condition, or litter condition. We assessed the effects of androgen exposure on the growth of offspring during the neonatal, early-infancy, late-infancy, and juvenile periods as defined by Yamamoto (1993). First, we derived the mean androgen excretion concentrations of pregnant females for each trimester of the pregnancies, and a correlational matrix was computed between gestational androgen by trimester, body weight and size of offspring at PND 2, and the sex of the offspring. Second, the relationship between maternal gestational androgen and postnatal growth of offspring during early-infancy (PNDs 2–60), late-infancy (PNDs 60–180), and juvenilehood (PNDs 180–300) was evaluated. These stages represent periods that offspring rely primarily on maternal milk, transition to solid food, and complete dependence upon solid food, respectively. A hierarchical regression was completed to evaluate the effects of first trimester maternal androgen on physical growth of offspring throughout each life stage controlling for body weight and size of each offspring at the beginning of the life stage (PNDs 2, 60, and 180). The sex of offspring was also nested into the analysis to test for sex differences in development. The alpha level for all statistical analyses was $P < 0.05$.

3. Results

3.1. Variation in urinary androgen across gestation

There were significant differences in levels of excreted androgens as females progressed through pregnancy. Fig. 1 indicates mean levels of androgen in nonpregnant females and during trimesters of pregnancy. Androgen excretion varied significantly across reproductive phases ($F_{3, 48} = 25.64$, $P < 0.001$), with mean concentrations during each trimester of pregnancy significantly higher than the nonpregnant value. The highest levels of excreted androgen by pregnant females occurred in the second trimester, where concentrations were almost twofold higher than in the first and third trimesters.

There were also substantial individual differences among females in levels of excreted androgens. Fig. 2a presents androgen excretion rates averaged for 10-day blocks during gestation for all females, and also averaged for each individual female across her two to five pregnancies represented in our sample. As in the trimester analysis, levels of androgen excretion varied significantly across 10-day blocks during gestation ($F_{14, 126} = 13.78$, $P < 0.001$). More importantly, when individual pregnancies were nested within females, there was a significant female by stage of gestation interaction ($F_{56, 126} = 2.32$, $P < 0.001$), indicating that the pattern

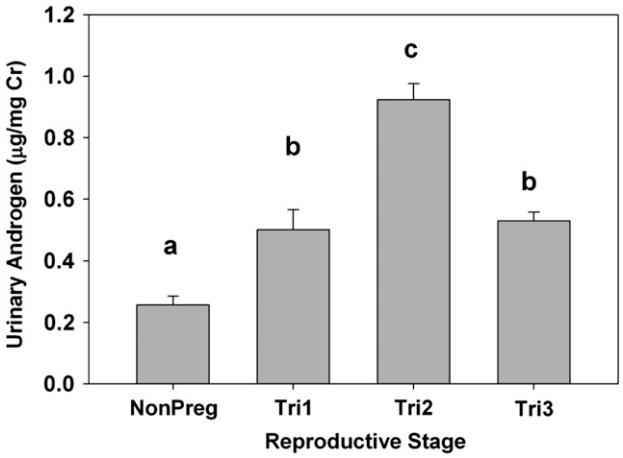

Fig. 1. Mean ± SE levels of urinary androgen excretion by five female marmosets across reproductive phases of 18 total pregnancies. NonPreg = females that are not pregnant; Tri-1, Tri-2, and Tri-3 = first, second, and third trimester of pregnancy, respectively. Bars that have different letters are significantly different from each other ($P < 0.05$).

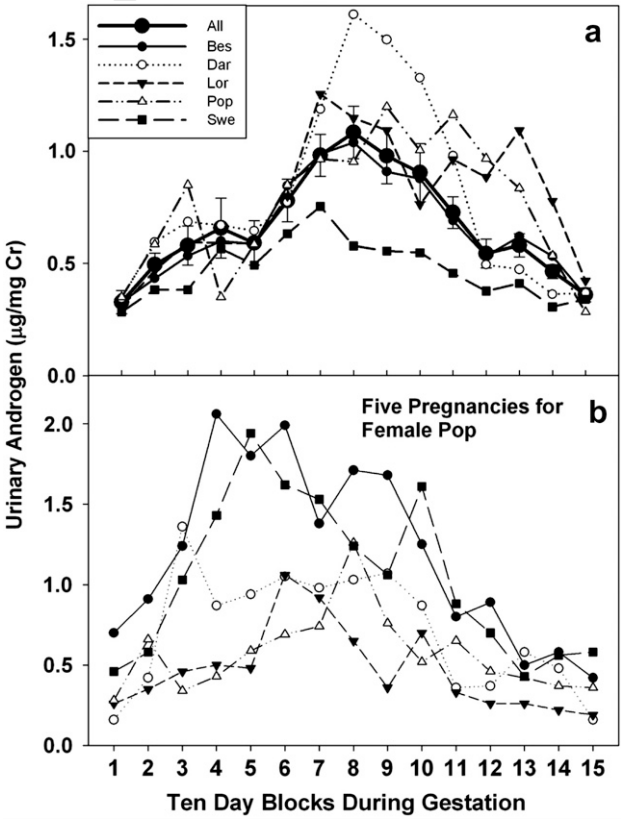

Fig. 2. Levels of androgen excretion across a total of 18 gestations from five females in 10-day blocks. (a) Means for all pregnancies for all females indicated by large solid dot and line, and means for multiple pregnancies (two–five) for each female are also shown. (b) Levels of androgen excretion for five pregnancies from a single female (Pop).

Please cite this article in press as: Smith, A.S., et al. Maternal androgen levels during pregnancy are associated with early-life growth in Geoffroy's Marmosets, *Callithrix geoffroyi*. Gen. Comp. Endocrinol. (2009), doi:10.1016/j.ygcen.2009.10.008

of androgen excretion across gestation differed significantly between females. Although we did no quantitative analysis of within-female differences, Fig. 2b depicts patterns of androgen excretion during five pregnancies for a single female. It is clear that there is also substantial within-female variation in androgen excretion during gestation. Androgen concentrations at any stage of gestation were not associated with litter size (r's$_{17}$ < 0.34, P's > 0.15), and the sex ratio of the litter did not predict maternal androgen excretion levels in any trimester (French et al., in press). In addition, there were no significant relationships between maternal weight (M = 549.43, SD = 58.51), age (M = 5.34, SD = 1.04), or parity (M = 1.56, SD = 1.37) and maternal excreted androgen concentrations during any trimester (r's$_{32}$ < −.27, P's > 0.14).

3.2. Newborn marmoset weight and body measures

Neonatal body size and weights were significantly associated with variation in first trimester maternal androgen. Females with higher excreted androgen concentrations gave birth to smaller infants. Neonatal (PND 2) weights, both body length measurements (SSPL and KHL), and several body girth measurements (head, chest, and torso circumference) were all negatively correlated with first trimester maternal androgen excretion levels (see Table 2). In addition, arm circumference tended to be negatively associated with first trimester levels of excreted maternal androgen (see Table 2). The relationship between first trimester maternal testosterone and body weight and size were identical when litter was the unit of analysis (see Table S.1 in Supplemental data). Androgen concentrations during the second and third trimester did not appear to be associated with variation in offspring size or weight at any life stage (see Table S.2 in Supplemental data). Two of the values of excreted androgen levels (>1.2 μg T/mg Cr) were 0.5 μg T/mg Cr higher than any other value of excreted androgen. Although these values were not statistical outliers, the statistical analyzes were also conducted excluding these values. As a result, all of the measurements of body weight, length, and girth were still negatively associated with first trimester levels of excreted maternal androgen. There was no effect of the sex of the offspring on neonatal body size and weights.

3.3. Early and late infancy and juvenile growth

Changes in five of the body measurements, including body weight, length, and girth, were associated with levels of first trimester maternal androgen excretions. The direction of this association was dependent on the life stage of the offspring (see Fig. 3a-c). During early infancy (PND 2–60), the growth rate of body weight, arm circumference, and thigh circumference were all negatively associated with first trimester maternal androgen excretions (F's$_{1,\ 22}$ > 5.41, P < 0.05). A higher level of excreted maternal androgen during the first trimester was associated with stunted growth during early infancy. However, this negative association became positive in later stages of development (see Fig. 3d–f). The growth rates of chest circumference during late infancy were positively associated with first trimester maternal androgen excretions ($F_{1,\ 21}$ = 4.48, P < 0.05). First trimester maternal androgen excretions were negatively associated with the growth rates of thigh circumference during infancy ($F_{1,\ 21}$ = 4.76, P < 0.05) but positively associated during juvenilehood ($F_{1,\ 20}$ = 6.78, P < 0.05). Again, statistical analyzes were also conducted excluding growth rates of two offspring exposure to androgen levels greater than 1.2 μg T/mg Cr. As a result, all of the measurements of growth by developmental life stage were still associated with first trimester levels of excreted maternal androgen in the same directions. There was no effect of the sex of the offspring on growth during any life stage.

Table 2
Effects of first trimester maternal androgen on neonatal measures.

Body measurements	Neonates	P-values
Weight	−0.41	0.04*
SSPL	−0.55	0.01*
KHL	−0.48	0.02*
BPD	−0.22	0.29
Head circ	−0.45	0.02*
Chest circ	−0.48	0.02*
Abdominal circ	−0.05	0.82
Arm circ	−0.39	0.06†
Thigh circ	−0.13	0.54
Torso circ	−0.49	0.02*

Note. Pearson's r with corresponding P-values for neonate measures. Sample size is 25 *P < 0.05, †P < 0.06, two-tailed.

4. Discussion

The current study suggests that normal variation in maternal androgens can modify fetal programming, thereby altering both prenatal and postnatal growth rates. However, the relationship between androgens and growth was contingent on the life stage in which growth occurred. Infants born to mothers with high levels of first trimester androgen had smaller body weights and girth and slower growth rates during early infancy than infants born to mothers with low levels of first trimester androgen. However, rebounds in growth rates of body girth during late infancy and for juvenile marmosets were positively associated with the levels of excreted maternal androgen concentrations during the first trimester. The fact that prenatal and postnatal growth rates were associated with maternal androgen during gestation suggests that exposure to normal fluctuations of androgens during this critical period of development can have both short-and long-term effects and may achieve similar lasting effects that exposure to androgen excess during gestation has through programming of different developmental pathways.

In normal fetal development, testosterone synthesized from the Leydig cells of the interstitial testicular tissue virilizes male fetuses, leading to the presence of male secondary sex characteristics, masculinized behavior, and spermatogenesis (Forest, 1983; Randall et al., 2002; Parker, 2004). Recently, several studies have explored the effects of pharmacological doses of exogenous testosterone exposure on fetal programming (Wolf et al., 2002; Bruns et al., 2004; Manikkam et al., 2004; Crespi et al., 2006). In different animal models (e.g., sheep, rhesus macaque, and rats), administration of pharmacological doses of androgens have had prolonged effects on growth rates of fetuses, resulting in low birth weights and subsequent rapid weight gain during early development (Manikkam et al., 2004; Crespi et al., 2006). There are also clinical examples of excess androgenic exposure in fetal development. Polycystic ovarian syndrome (PCOS) is a disorder with multiple signs and symptoms that includes hyperandrogenism (Xita and Tsatsoulis, 2006; Abbott et al., 2005). PCOS women give birth to babies who are more likely to be small-for-gestational-age than babies from normal mothers (Homburg, 2006) and have increased abdominal fat in later life (Abbott et al., 2005). Therefore, it is postulated that prenatal exposure to elevated androgen results in low birth weight and late-life manifestation of the PCOS somatic phenotype. The present study has demonstrated that exposure to normal fluctuation of endogenous androgens of maternal origins early in gestation can lead to similar long-term effects on offspring growth throughout development, leading up to puberty.

While there are harmful organizational effects of testosterone on fetal development, there may be several potential benefits for

Please cite this article in press as: Smith, A.S., et al. Maternal androgen levels during pregnancy are associated with early-life growth in Geoffroy's Marmosets, *Callithrix geoffroyi*. Gen. Comp. Endocrinol. (2009), doi:10.1016/j.ygcen.2009.10.008

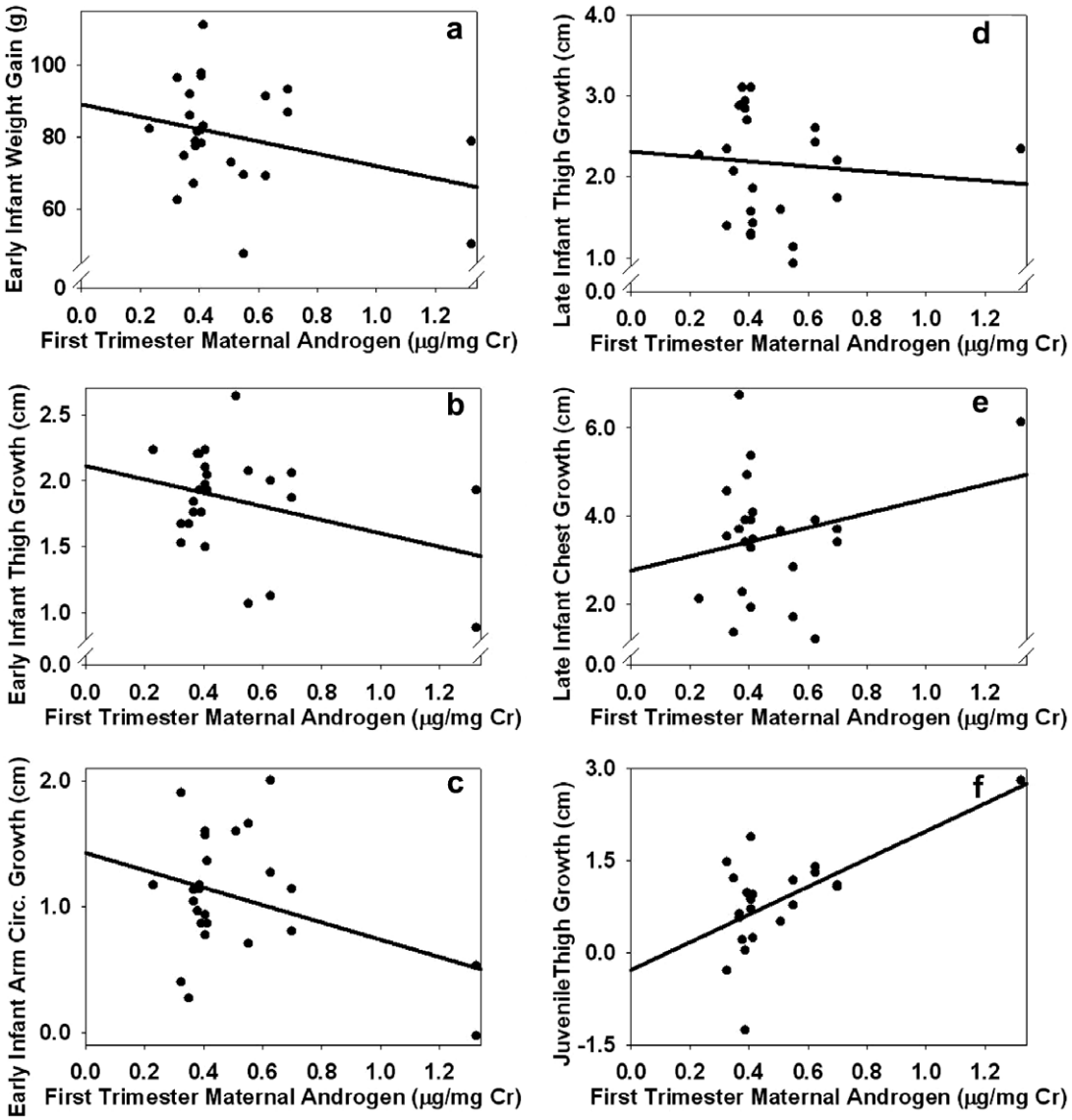

Fig. 3. Relationships between levels first trimester maternal androgen excretions and total postnatal growth of female and male marmosets including (a) early infant weight gain, (b) early infant thigh growth, (c) early infant arm circumference growth, (d) late infant thigh growth, (e) late infant chest growth, and (f) juvenile thigh growth. Life stages included early infants (PND 0–60), late infants (PND 60–180), and juveniles (PND 180–300). Sample sizes are 25 (early infants), 24 (late infants), and 23 (juveniles), *P*'s < 0.05.

both mothers and offspring. Maternal androgen levels may reflect a fundamental trade-off in metabolic effort as described in life history theory (Kaplan and Gangestad, 2005). This perspective emphasizes a trade-off in metabolic effort among growth, maintenance, and reproduction. For fully-grown adult females, the trade-off thus lies between one's own maintenance and the investment in pregnancy and rearing offspring. Given that elevated levels of androgens stifle metabolic rates in fetuses (Manikkam et al., 2004; Crespi et al., 2006; present study), one function of elevated androgen may be to regulate the amount of energetic resources that would be allotted to developing fetuses. This may be required during periods of resource limitation or social instability that pose the potential for increased metabolic efforts through actives of territoriality and aggression. There is evidence that maternal androgens during pregnancy can be altered if the social environment becomes unstable in guinea pigs (Kaiser et al., 2003). In primates, androgen production of female talapoin monkeys varied as a function of social status, an indirect index of social stability (Batty et al., 1986), and at least for male marmosets, aggressive interactions with intruders into a social group are associated with elevations in testosterone productions (Ross et al., 2004). Therefore, the surge in androgens during early gestation may reflect an early conflict of mothers to metabolically invest or allocate time, energy, and resources toward her maintenance versus reproduction, or fetal development.

From the perspective of offspring, while elevated maternal androgen appears to have negative short-term consequences for somatic growth in infant marmosets, there may be longer-term benefits for offspring so exposed. In a number of mammalian species, aggressive bouts between competing conspecifics leads to sequestering territory, resources, and mating opportunities, including mate choice and mating order (reviewed by Nelson, 2006). There is evident that exposure to androgens during gestation is associated with aggression in offspring. Rough-and-tumble play is observed more frequently in juvenile offspring when mothers were treated with testosterone during pregnancy in rats and

Please cite this article in press as: Smith, A.S., et al. Maternal androgen levels during pregnancy are associated with early-life growth in Geoffroy's Marmosets, *Callithrix geoffroyi*. Gen. Comp. Endocrinol. (2009), doi:10.1016/j.ygcen.2009.10.008

rhesus monkeys (Goy et al., 1988; Meany and Stewart, 1981) and a number of other mammalian species (reviewed in Cohen-Bendahan et al., 2005). In spotted hyenas (*Crocuta crocuta*), offspring born to females with elevated gestational androgen in the third trimester show higher rates of sibling play fighting and sexual mounting as cubs, and higher rates of cumulative lifetime aggression than offspring born to low-androgen mothers (Dloniak et al., 2006; Van Meter, 2009). Greater rates of sibling aggression leads to higher social ranking (Wahaj and Holekamp, 2006), and the reproductive success of high-ranking females is significantly greater than low-ranking females, as observed in a number of gregarious mammalian species (Holekamp et al., 1996). Thus, exposure to high levels of androgen may shape advantageous behavioral traits over the lifespan.

Anabolic actions of testosterone include growth of muscle mass, increased bone density, and stimulation of linear growth and bone maturation during pubertal development (Randall et al., 2002). Given the anabolic effects of androgens, a negative relationship between androgens and fetal growth seems counterintuitive. However, androgens as an epigenetic agent seem to have disruptive effects on the metabolic and endocrine pathways. When testosterone is administered to pregnant ewes early in gestation, there is a reduction in IGF-I in developing fetal lambs (Manikkam et al., 2004; Crespi et al., 2006). Furthermore, testosterone excess during early gestation leads to fetal metabolic changes that result in insulin resistance and impaired insulin secretion in adult rhesus monkeys (Bruns et al., 2004; Eisner et al., 2000) and lambs (Recabarren et al., 2005). Therefore, the observed fetal growth retardation and subsequent changes in postnatal growth rates that are associated with maternal androgens may be the product of altered metabolic pathways in the fetus. The reprogramming of metabolic tissues by gestational androgen exposure could be a mediating factor that results in fetal growth retardation and modified postnatal growth patterns.

In summary, our results revealed an association between first trimester maternal androgen levels and fetal and postnatal growth. The results suggest that exposure to maternally-derived androgens during early gestation—a crucial, sensitive period of early development—has organizational effects on fetal growth and development, manifesting in low birth weights and sizes as well as catch-up in postnatal growth rates. In addition, our demonstration of normal androgen exposure on growth patterns suggests that the altered developmental trajectories in fetuses exposed to high levels of exogenous testosterone mirror the natural effects of androgen exposure on fetal development. The fact that prenatal and postnatal growth rates were associated with maternal androgen during gestation suggests that androgen exposure during this critical period of development can have short-term effects and may induce long-term changes in physiology and metabolism.

Acknowledgments

These findings were presented as an oral presentation at the 31st annual meeting of the American Society of Primatologists, June 2008. Jeff Fite initiated the study of lifetime development in Geoffroy's marmosets at UNO, and we thank the numerous students and staff over the last five years at the Callitrichid Research Center for the many hours of contribution in compiling these data. We also thank Heather Jensen, Danny Revers, Liz Gunkelman, and the husbandry staff for their excellent maintenance and care of the marmoset colony. Special thanks to Julienne Rutherford, Stephanie Matthews, Shelton Hendricks, and Claudia Rauter for their comments on earlier versions of our manuscript. This study was reviewed and approved by UNO/UNMC Institutional Animal Care and Use Committee (Protocol#: 07-033-FC).

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.ygcen.2009.10.008.

References

Abbott, D.H., Barnett, D.K., Bruns, C.M., Dumesic, D.A., 2005. Androgen excess fetal programming of female reproduction: a developmental aetiology for polycystic ovary syndrome? Hum. Reprod. Update 11, 357–374.

Abbott, D.H., Hearn, J.P., 1978. Physical, hormonal and behavioural aspects of sexual development in the marmoset monkey, *Callithrix jacchus*. J. Reprod. Fertil. 53, 155–166.

Bremner, W.J., Cumming, I.A., 1978. Inhibition of fetal growth and survival by testosterone administration to pregnant sheep. Metabolism 27, 253–255.

Bruns, C.M., Baum, S.T., Colman, R.J., Eisner, J.R., Kemnitz, J.W., Weindruch, R., Abbott, D.H., 2004. Insulin resistance and impaired insulin secretion in prenatally androgenized male rhesus monkeys. J. Clin. Endocrinol. Metab. 89, 6218–6223.

Carlsen, S.M., Jacobsen, G., Romundstad, P., 2006. Maternal testosterone levels during pregnancy are associated with offspring size at birth. Eur. J. Endocrinol. 155, 365–370.

Castracane, V., Stewart, D., Gimpel, T., Overstreet, J., Lasley, B., 1998. Maternal serum androgens in human pregnancy: early increases within the cycle of conception. Hum. Reprod. 13, 460–464.

Chambers, P.L., Hearn, J.P., 1979. Peripheral plasma levels of progesterone, oestradiol-17 beta, oestrone, testosterone, androstenedione and chorionic gonadotrophin during pregnancy in the marmoset monkey. J. Reprod. Fertil. 56, 23–32.

Cohen-Bendahan, C.C.C., van de Beek, C., Berenbaum, S.A., 2005. Prenatal sex hormone effects on child and adult sex-typed behavior: methods and findings. Neurosci. Biobehav. Rev. 29, 353–384.

Crespi, E.J., Steckler, T.L., MohanKumar, P.S., Padmanabhan, V., 2006. Prenatal exposure to excess testosterone modifies the developmental trajectory of the insulin-like growth factor system in female sheep. J. Physiol. 572, 119–130.

Dloniak, S.M., French, J.A., Holekamp, K.E., 2006. Rank-related maternal effects of androgens on behaviour in wild spotted hyaenas. Nature 440, 1190–1193.

Dloniak, S.M., French, J.A., Place, N.J., Weldele, M.L., Glickman, S.E., Holekamp, K.E., 2004. Non-invasive monitoring of fecal androgens in spotted hyenas (*Crocuta crocuta*). Gen. Comp. Endocrinol. 135, 51–61.

Dunaif, A., 1997. Insulin resistance and the polycystic ovary syndrome: mechanism and implications for pathogenesis. Endocr. Rev. 18, 774–800.

Dunaif, A., Graf, M., Mandeli, J., Laumas, V., Dobrjansky, A., 1987. Characterization of groups of hyperandrogenic women with acanthosis nigricans, impaired glucose tolerance, and/or hyperinsulinemia. J. Clin. Endocrinol. Metab. 65, 499–507.

Eisner, J.R., Dumesic, D.A., Kemnitz, J.W., Abbott, D.H., 2000. Timing of prenatal androgen excess determines differential impairment in insulin secretion and action in adult female rhesus monkeys. J. Clin. Endocrinol. Metab. 85, 1206–1210.

Ellinwood, W.E., Stanczyk, F.Z., Lazur, J.J., Novy, M.J., 1989. Dynamics of steroid biosynthesis during the luteal–placental shift in rhesus monkeys. J. Clin. Endocrinol. Metab. 69, 348–355.

Fite, J.E., French, J.A., 2000. Pre- and postpartum sex steroids in female marmosets (*Callithrix kuhlii*): is there a link with infant survivorship and maternal behavior? Horm. Behav. 38, 1–12.

Forest, M.G., 1983. Role of androgens in fetal and pubertal development. Horm. Res. 18, 69–83.

French, J.A., Brewer, K.J., Schaffner, C.M., Schalley, J., Hightower-Merritt, D., Smith, T.E., Bell, S.M., 1996. Urinary steroid and gonadotropin excretion across the reproductive cycle in female Wied's black tufted-ear marmosets (*Callithrix kuhlii*). Am. J. Primatol. 40, 231–245.

French, J.A., Smith, A.S., Birnie, A.K., in press. Maternal gestational androgen levels in female marmosets (*Callithrix geoffroyi*) vary across trimesters but do not vary with the sex ratio of litters. Gen. Comp. Endocr., doi:10.1016/j.ygcen.2009.1007.1015.

Glass, A.R., Klein, T., 1981. Changes in maternal serum total and free androgen levels in early pregnancy: lack of correlation with fetal sex. Am. J. Obstet. Gynecol. 140, 656–660.

Goy, R.W., Bercovitch, F.B., McBrair, M.C., 1988. Behavioral masculinization is independent of genital masculinization in prenatally androgenized female rhesus macaques. Horm. Behav. 22, 552–571.

Hodges, J.K., Tarara, R., Wangula, C., 1984. Circulating steroids and the relationship between ovarian and placental secretion during early and mid pregnancy in the baboon. Am. J. Primatol. 7, 357–366.

Holekamp, K.E., Smale, L., Szykman, M., 1996. Rank and reproduction in the female spotted hyaena. J. Reprod. Fertil. 108, 229–237.

Homburg, R., 2006. Pregnancy complications in PCOS. Best Pract. Res. Clin. Endocrinol. Metab. 20, 281–292.

Kaiser, S., Heemann, K., Straub, R.H., Sachser, N., 2003. The social environment affects behaviour and androgens, but not cortisol in pregnant female guinea pigs. Psychoneuroendocrinology 28, 67–83.

Kaplan, H.S., Gangestad, S.W., 2005. Life history theory and evolutionary psychology. In: Buss, D.M. (Ed.), The Handbook of Evolutionary Psychology. John Wiley & Sons, Inc., Hoboken, NJ, pp. 68–95.

Lunn, S.F., Hobson, B.M., Hearn, J.P., 1979. Pregnancy diagnosis in the common marmoset (*Callithrix jacchus jacchus*). Folia Primatol. 32, 200–206.

Manikkam, M., Crespi, E.J., Doop, D.D., Herkimer, C., Lee, J.S., Yu, S., Brown, M.B., Foster, D.L., Padmanabhan, V., 2004. Fetal programming prenatal testosterone excess leads to fetal growth retardation and postnatal catch-up growth in sheep. Endocrinology 145, 790–798.

Meany, M.J., Stewart, J., 1981. Neonatal androgens influence the social play of prepubescent rats. Horm. Behav. 15, 197–213.

Meulenberg, P.M.M., Hofman, J.A., 1991. Maternal testosterone and fetal sex. J. Steroid Biochem. Mol. Biol. 39, 51–54.

Munro, C., Stabenfeldt, G., Cragun, J., Addiego, L., Overstreet, J., Lasley, B., 1991. Relationship of serum estradiol and progesterone concentrations to the excretion profiles of their major urinary metabolites as measured by enzyme immunoassay and radioimmunoassay. Clin. Chem. 37, 838–844.

Nobili, V., Alisi, A., Panera, N., Agostoni, C., 2008. Low birth weight and catch-up-growth associated with metabolic syndrome: a ten year systematic review. Pediatr. Endocrinol. Rev. 6, 241–247.

Nunes, S., Brown, C., French, J.A., 2002. Variation in circulating and excreted estradiol associated with testicular activity in male marmosets. Am. J. Primatol. 56, 27–42.

Nunes, S., Fite, J.E., French, J.A., 2000. Variation in steroid hormones associated with infant care behaviour and experience in male marmosets (*Callithrix kuhlii*). Anim. Behav. 60, 857–865.

Parker, K.L., 2004. Sexual differentiation. In: Griffin, J.E., Ojeda, S.R. (Eds.), Textbook of Endocrine Physiology. Oxford University Press, New York, pp. 167–185.

Power, R.A., Power, M.L., Layne, D.G., Jaquish, C.E., Oftedal, O.T., Tardif, S.D., 2001. Relations among measures of body composition, age, and sex in the common marmoset monkey (*Callithrix jacchus*). Comp. Med. 51, 218–223.

Randall, D.J., Burggren, W., French, K., 2002. Eckert Animal Physiology. W.H. Freeman & Company, New York.

Rao, A.J., Kotagi, S.G., 1983. Serum testosterone levels during the menstrual cycle and early pregnancy in the bonnet monkey (*Macaca radiata*). Endocrinol. Jpn. 29, 271–275.

Recabarren, S.E., Padmanabhan, V., Codner, E., Lobos, A., Duran, C., Vidal, M., Foster, D.L., Sir-Petermann, T., 2005. Postnatal developmental consequences of altered insulin sensitivity in female sheep treated prenatally with testosterone. Am. J. Physiol. Endocrinol. Metab. 289, E801–E806.

Reyes, F.I., Winter, J.S.D., Faiman, C., 1973. Studies on human sexual development: I. Fetal gonadal and adrenal sex steroids. J. Clin. Endocrinol. Metab. 37, 74–78.

Ross, C.N., French, J.A., Patera, K.J., 2004. Intensity of aggressive interactions modulates testosterone in male marmosets. Physiol. Behav. 83, 437–445.

Schaffner, C.M., Shepherd, R.E., Santos, C.V., French, J.A., 1995. Development of heterosexual relationships in Wied's black tufted-ear marmosets (*Callithrix kuhlii*). Am. J. Primatol. 36, 185–200.

Tardif, S.D., Bales, K.L., 2004. Relations among birth condition, maternal condition, and postnatal growth in captive common marmoset monkeys (*Callithrix jacchus*). Am. J. Primatol. 62, 83–94.

Tietz, N.W., 1976. Fundamentals of Clinical Chemistry. W. B. Saunders, Philadelphia.

Van Meter, P.E., 2009. Hormones, stress and aggression in the spotted hyena (*Crocuta crocuta*). Zoology. Michigan State University, East Lansing, MI.

Wahaj, S.A., Holekamp, K.E., 2006. Functions of sibling aggression in the spotted hyaena, *Crocuta crocuta*. Anim. Behav. 71, 1401–1409.

Wolf, C.J., Hotchkiss, A., Ostby, J.S., LeBlanc, G.A., Gray, L.E., 2002. Effects of prenatal testosterone propionate on the sexual development of male and female rats: a dose-response study. Toxicol. Sci. 65, 71–86.

Xita, N., Tsatsoulis, A., 2006. Fetal programming of polycystic ovary syndrome by androgen excess: evidence from experimental, clinical, and genetic association studies. J. Clin. Endocrinol. Metab. 91, 1660–1666.

Yamamoto, M.E., 1993. From dependence to sexual maturity: the behavioural ontogeny of Callitrichidae. In: Rylands, A.B. (Ed.), Marmosets and Tamarins: Systematics, Behaviour, and Ecology. Oxford University Press, New York, pp. 235–254.

Please cite this article in press as: Smith, A.S., et al. Maternal androgen levels during pregnancy are associated with early-life growth in Geoffroy's Marmosets, *Callithrix geoffroyi*. Gen. Comp. Endocrinol. (2009), doi:10.1016/j.ygcen.2009.10.008