Experimentally induced gestational androgen excess disrupts glucoregulation in rhesus monkey dams and their female offspring

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Submitted 22 January 2010; accepted in final form 29 July 2010

Abbott DH, Bruns CR, Barnett DK, Dunai A, Goodfriend TL, Dumesic DA, Tarantal AF. Experimentally induced gestational androgen excess disrupts glucoregulation in rhesus monkey dams and their female offspring. Am J Physiol Endocrinol Metab 299: E741–E751, 2010. First published August 3, 2010; doi:10.1152/ajpendo.00058.2010.—Discrete fetal androgen excess during early gestation in rhesus monkeys (Macaca mulatta) promotes endocrine antecedents of adult polycystic ovary syndrome (PCOS)-like traits in female offspring. Because developmental changes promoting such PCOS-like metabolic dysfunction remain unclear, the present study examined time-mated, gravid rhesus monkeys with female fetuses, of which nine gravid females received 15 mg of testosterone propionate (TP) subcutaneously daily from 40 to 80 days (first to second trimesters) of gestation [term, mean (range): 165 (155–175) days], whereas an additional six such females received oil vehicle injections over the same time interval. During gestation, ultrasonography quantified fetal growth measures and was used as an adjunct for fetal blood collections. At term, all fetuses were delivered by cesarean section for postnatal studies. Blood samples were collected from dams and infants for glucose, insulin, and total free fatty acid (FFA) determinations. TP injections transiently accelerated maternal weight gain in dams, very modestly increased head diameter of prenatal androgenized (PA) fetuses, and modestly increased weight gain in infancy compared with concurrent controls. Mild to moderate glucose intolerance, with increased area-under-the-curve circulating insulin values, occurred in TP-injected dams during an intravenous glucose tolerance test in the early second trimester. Moreover, reduced circulating FFA levels occurred in PA fetuses during a third trimester intravenous glucagon-tolbutamide challenge (140 days gestation), whereas excessive insulin sensitivity and increased insulin secretion relative to insulin sensitivity occurred in PA infants during an intravenous glucagon-tolbutamide test at ~1.5 mo postnatal age. Data from these studies suggest that experimentally induced fetal androgen excess may result in transient hyperglycemic episodes in the intrauterine environment that are sufficient to induce relative increases in pancreatic function in PA infants, suggesting in this nonhuman primate model that differential programming of insulin action and secretion may precede adult metabolic dysfunction.

fetal programming; polycystic ovary syndrome; hyperglycemic pregnancy; metabolic syndrome

REPRODUCTIVE AND METABOLIC traits reminiscent of polycystic ovary syndrome (PCOS) are prevalent in a wide variety of female mammals exposed to testosterone excess during gestation (5, 28). Reproductive PCOS-like traits found in prenatally androgenized (PA) nonhuman females include combinations of hyperandrogenism, intermittent or absent ovulatory cycles, and polycystic ovaries, traits that comprise the diagnostic criteria required for PCOS in women (4). Metabolic PCOS-like traits in animal models of fetal androgen excess, moreover, are diverse and can include hyperinsulinemia, insulin resistance, pancreatic β-cell defects, increased incidence of type 2 diabetes, enhanced adiposity, and hyperlipidemia [monkeys (1), sheep (58), and rats (25)]. Such heterogeneity of PCOS-like trait expression in PA females resembles that found in women with PCOS (4, 5), providing a novel perspective into potential developmental origins of PCOS, particularly with regard to metabolic phenotype (33).

Experimentally induced PCOS-like traits in PA monkeys and sheep allow investigation of fetal origins (3, 32), providing insight into how altered developmental trajectories and mechanistic derangements can precede adult PCOS-like traits in these animal models. Understanding prepubertal expression of PCOS-like traits is important, since daughters of women with PCOS manifest PCOS-like antecedents, such as ovarian hormone hyperscretion, as early as 2 mo of age (63) and increased ovarian volume and hyperinsulinemia before or during pubertal progression (24, 45, 64). In this regard, endocrine and ovarian antecedents of adult PCOS-like traits exist in PA female offspring in both monkeys and sheep beginning in late fetal/early neonatal development (2, 72), presumably because female fetuses exposed to fetal male levels of testosterone (or its androgenic or estrogenic metabolites) develop permanent epigenetic and pathophysiological changes in endocrine and reproductive target tissues. Although little attention has focused on the physiological responses of pregnant dams to exogenous testosterone administration and their effects on female fetal development, maternal metabolic dysfunction, particularly from hyperglycemia (18–19, 35–37), may program fetal and subsequent postnatal metabolic traits of offspring from PA females.

Insulin resistance can be induced in humans by administrating testosterone to regularly menstruating women for 10–12 days, (27), to reproductive-aged female and male transsexuals for 4 mo (56), or to postmenopausal women for 3 mo (74). In the latter treated group of women, androgen rather than estrogen receptor-mediated action has been implicated in insulin resistance, since estrogen administration does not impair insulin action (74). Such androgen specificity also occurs in a fetal
androgen excess sheep model for PCOS in which gestational treatment of dams with the nonaromatizable androgen, dihydrotestosterone, induces comparable insulin resistance in exposed female offspring to that found in females exposed to testosterone excess during gestation (51). In vitro androgen specificity also exists as androgen receptor blockade prevents testosterone-induced insulin resistance in human subcutaneous adipocytes (21).

Testosterone treatment of gravid rhesus monkeys, therefore, may induce maternal insulin resistance in addition to elevating circulating androgen levels in both maternal and fetal compartments (2). Because pregnancy-induced insulin resistance in primates [female rhesus monkeys (43) and women (66)] may be exacerbated by androgen excess, as evident by hyperandrogenic PCOS women being at risk for gestational diabetes (13), testosterone treatment of rhesus monkey dams may expose their female offspring to hyperglycemia (4, 25, 51). Understanding the development of metabolic PCOS-like traits in PA female monkeys, therefore, is crucial since maternal hyperglycemia is readily conveyed to the fetus via transplacental transport, causing phenotypic changes in both the exposed human fetus (hyperinsulinemia and macrosomia) and subsequent neonate (hypoglycemia and increased adiposity) (35, 36).

The present study, therefore, examines whether discrete, early gestation testosterone treatment of rhesus monkey dams causes predictable metabolic antecedents of PCOS-like traits in PA female offspring, such as insulin resistance [as found in young PA female sheep and rats (25, 58)] and failure of pancreatic β-cells to appropriately compensate for insulin resistance (β-cell defect). This study also examines whether gestational testosterone treatment disrupts maternal glucoregulation and whether preconception maternal body weight exaggerates any glucoregulatory perturbation. Evidence for testosterone inducing any form of hyperglycemia during pregnancy, with maternal metabolic dysfunction linked to a postnatal metabolic phenotype in PA female offspring, would support the proposed “second hit” on the female fetus (3, 4), in which relative hyperinsulinemia induced by fetal exposure programs postnatal metabolic dysfunction (26). This metabolic second hit, coexisting with the direct effects of testosterone (or its metabolites) on fetal and postnatal development (i.e., “first hit”), could cause insulin-amplified reproductive dysfunction, as found in PA adult female monkeys (76) and sheep (67). Such implications for fetal origins of metabolic (and reproductive) phenotypes in adult female PA monkeys also may apply to the pathogenesis of PCOS in women (26).

**MATERIALS AND METHODS**

*Animals and Fetal Programming*

Animal procedures conformed to the requirements of the Animal Welfare Act, and animal protocols were approved before implementation by the Institutional Animal Care and Use Committee at the University of California, Davis. Fifteen adult, nonprimiparous, pregnant female rhesus monkeys, housed at the California National Primate Research Center, were selected for study, based upon the absence of Y-chromosomal DNA in their peripheral circulation (39) at 30 days of gestation (range: 28–32 days), confirming a female fetus. Between 40 and 80 days of gestation (late first to midsecond trimester), gravid animals received daily subcutaneous injections of either 15 mg testosterone propionate (TP, n = 9) or vehicle (100–200 µl sesame oil; control, n = 7; Fig. 1). Such maternal TP treatment has been shown to induce fetal androgen excess in PA females equivalent to that found in fetal males and programs for a PCOS phenotype in adulthood (1, 2, 60).

As previously reported (2), before conception, adult females in TP and control groups had similar ages [TP: 11.2 ± 1.5 (SE) yr; control: 8.4 ± 2.0 (SE) yr] and body weights (TP: 7.0 ± 0.4 kg; control: 6.8 ± 0.5 kg). At term, newborns were delivered by cesarean section from dams of comparable term body weights (TP: 8.5 ± 0.4 kg; control: 8.1 ± 0.5 kg). Routine newborn assessments, including body weights and crown-rump lengths (see Fig. 3), and gross appearance of the placenta, were unremarkable and similar between the groups. Infants were nursery reared under standard operating procedures for housing conditions and diet for animals in this age group (71). One control dam and the associated offspring were omitted from the data analyses because they were identified as outliers [by extreme studentized deviate test] for elevated maternal/fetal (P < 0.05/P < 0.01) basal serum glucose levels in the third trimester and elevated (138 mg/dl) infant (P < 0.05) basal serum glucose levels at 2 mo postnatal age. The birth weight of this latter infant (546 g) was ~23% greater than the mean of remaining controls (445 ± 19 g), although within the normative weight range for newborn rhesus monkeys.

*Morphometrics*

Crown-rump and femur lengths were assessed at birth and at the time of tissue harvest (~2 mo postnatal age) using standardized methods, and placental weights were assessed at delivery (71). Infants were weighed daily or weekly during the study period until the endpoint of ~2 mo postnatal age. Organ weights were assessed at tissue harvest (71).

*Glucoregulatory Assessment*

**Dams.** After an overnight fast, maternal blood was collected from a peripheral vessel under telazol (5–8 mg/kg im) or ketamine (10 mg/kg im) approximately every 3 wk from 40 days of gestation (first trimester) through term. A modified intravenous glucose tolerance test (ivGTT) was also performed at 80 days of gestation. Glucose (300 mg/kg at 0 min) was administered intravenously, and blood samples were collected at select time points via a peripheral vessel. Insulin sensitivity (SI) was determined using the modified minimal model method (9). Further measures obtained from the ivGTT were basal (~5 min relative to glucose infusion) circulating levels of insulin (basal insulin), glucose (basal glucose), and total free (nonesterified) fatty acids (basal FFA); acute insulin responses to glucose (elevation of posthepatic plasma insulin concentration above baseline at 5 min); glucose disappearance rate (slope of the log linear regression of plasma glucose concentration between 10 and 20 min); and disposition index (β-cell compensation index; product of SI and acute insulin responses to glucose) (42). Area under the curve for glucose (AUCglucose).
insulin (AUCinsulin), and FFA (AUCFFA) were determined using the trapezoidal rule. The validity of using such limited numbers of time points to determine dam (and infant monkey, see below) S1 and the derived measure of disposition index, were validated by correlating S1 and disposition index values calculated from a 180-min ivGTT performed on 12 normal adult female rhesus monkeys with S1 and disposition index values calculated from the same adult ivGTTs, but only including the time points used in the current study. S1 values derived from the reduced number of time points, −5 to 60 min (dam) or 45 min (infant), were positively correlated with those S1 values obtained using all time points across the 180-min ivGTT (r² values, 0.47–0.55; P values, 0.01–0.006, respectively; slopes and y-axis intercepts did not differ from 1 and 0, respectively, P values >0.10). Disposition index comparisons were similarly correlated (r² values, 0.52–0.71; P values, 0.005–0.001, respectively; slopes and y-axis intercepts did not differ from 1 and 0, respectively, P values >0.40).

**Fetuses.** Fetal blood samples for basal glucose and insulin determinations were collected when the dams were sedated for ultrasound-guided fetal cardiocentesis at 80, 100, 120, and 140 days of gestation using established techniques (69). Fetal blood samples were also obtained at −5, 5, 15, 25, 35, and 45 min after a direct injection of 20 mg glucagon in the fetal circulation at 0 min at 140 days gestation (third trimester). Tolbutamide (20 mg/kg) was also injected directly in the fetal circulation 20 min following the glucagon injection (portal vein). The following measures were derived from this glucagon-tolbutamide test: basal insulin, glucose, and FFA; acute fetal insulin responses to glucagon (fetal insulin concentration above baseline at 5 min) and to tolbutamide (fetal insulin concentration at 25 min above 15 min value); as well as AUCglucose, AUCinsulin, and AUCFFA.

**Infants.** A modified ivGTT was performed in overnight-fasted infants on postnatal day 45 (0.5 g glucose/kg iv at 0 min, tolbutamide 20 mg/kg iv at 20 min; blood samples taken at −5, 5, 15, 25, 35, and 45 min). S1 was determined using the modified minimal model method (9). Further measures derived from the ivGTT were basal insulin, glucose, and FFA; acute insulin responses to both glucose and tolbutamide; disposition index; AUCglucose, AUCinsulin, and AUCFFA.

**Statistical Analyses**

Glucoregulatory parameters not normally distributed were log transformed to achieve homogeneity of variance and to increase linearity. Treatment group comparisons were conducted using Student’s t-test or repeated-measures-design ANOVA (version 7.0; Systat, Evanston, IL). Log-transformed glucoregulatory or morphometric parameters not normally distributed were analyzed using nonparametric statistics. A P value of <0.05 was considered significant. All log-transformed parameters are expressed as the antilog of the transformed means (95% confidence limit), nonnormal data are expressed as the median (interquartile range), and all other data are shown as means ± SE.

**RESULTS**

**Morphometrics**

**Dams.** While pregnancy induced the expected increase in weight gain, dams receiving TP injections exhibited accelerated body weight gain when compared with concurrent controls (Fig. 2). The body weights of TP-injected dams exceeded those of control dams by ∼25% at 80 days of gestation (P <0.029). TP-injected dams maintained this body weight until term delivery and did not differ from gestational controls in terms of body weight in the second half of gestation. This difference in the temporal progression of body weight represented an over fourfold weight gain in TP-injected dams vs. controls during TP treatment (P <0.001, Fig. 2), although similar rates of weight gain occurred in both groups before and after TP treatment, except for a transiently diminished (P <0.015) weight gain for 40 days immediately after TP injections (80–120 days of gestation; Fig. 2). During this latter time interval, TP-injected dams lost an average of 0.08 kg/40 days, whereas control dam body weight increased by an average of 0.60 kg/40 days.

**Fetuses.** Normal trends in fetal growth were observed across gestation in both groups. Ultrasound biometrics included biparietal diameter and femur length and were within the normative range at all time points assessed compared with historical controls (n ~ 150) (70), although PA females exhibited an
overall increase in fetal head size compared with concurrent control female fetuses ($P < 0.043$, Table 1).

**Infants.** As previously reported, PA female monkeys had birth weights within the normative range when compared with historical controls ($n = 130$) (70) at delivery by cesarean section (Fig. 3). PA female infants, however, demonstrated approximately a 10% increase in body weight compared with concurrent controls by 8 wk postnatal age, both by absolute weight and percent birth weight (type of female × age interactions: $P < 0.005$ and $P < 0.016$, respectively). Crown-rump lengths at delivery by cesarean section and at 9 wk of postnatal age were similar between control and PA infants (Fig. 3), as were femur lengths at 9 wk postnatal age (Fig. 3). The body mass index of infants, defined as the body weight relative to crown-rump length, did not differ significantly between female infant groups (cesarean section: control, $11.9 ± 0.4$ kg/m$^2$ and PA, $12.2 ± 0.3$ kg/m$^2$; $P = 0.55$; 8 wk postnatal age: control, $15.2 ± 0.4$ kg/m$^2$ and PA $15.8 ± 0.3$ kg/m$^2$; $P = 0.24$). Organ weights at tissue harvest, corrected for body weight, also were similar between female infant groups at 8 wk postnatal age (Table 2).

**Glucoregulation**

**Dams.** Basal (overnight-fasted) circulating glucose and insulin levels did not differ between oil- and TP-injected dams between 40 days gestation and term (Figs. 4 and 5). In both groups combined, basal glucose levels did not vary across this time period (time effect, $P < 0.70$), whereas between 60 and 120 days gestation ($P = 0.018–0.001$) basal insulin levels increased above the values at study initiation (40 days gestation; time effect, $P < 0.001$). Gestational peak values in maternal basal insulin levels occurred at a similar median of 80

### Table 1. Fetal biparietal diameter and femur length in five concurrent control and nine PA female rhesus monkeys determined from digitized caliper measurement during transabdominal ultrasonography of oil- or TP-injected gravid dams, respectively

<table>
<thead>
<tr>
<th>Gestational Age, days</th>
<th>Biparietal Diameter, mm</th>
<th>Femur Length, mm</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
<td>PA*</td>
</tr>
<tr>
<td>60</td>
<td>17.0 ± 0.4</td>
<td>17.9 ± 0.3</td>
</tr>
<tr>
<td>80</td>
<td>27.0 ± 0.5</td>
<td>27.9 ± 0.4</td>
</tr>
<tr>
<td>100</td>
<td>34.0 ± 0.6</td>
<td>35.9 ± 0.4</td>
</tr>
<tr>
<td>120</td>
<td>40.6 ± 0.6</td>
<td>41.6 ± 0.5</td>
</tr>
<tr>
<td>140</td>
<td>45.6 ± 0.7</td>
<td>46.0 ± 0.5</td>
</tr>
</tbody>
</table>

Data are means ± SE. PA, prenatally androgenized. *$P < 0.043$ vs. control females, overall type of female effect across all days of gestation.

Fig. 3. Mean ± SE body weight (A), %birth weight (B), crown-rump length (C), and femur length (D) in 5 concurrent control (○) and 9 prenatally androgenized (PA) (■) female infants. #*$P < 0.04$ and *$P < 0.05$ vs. control infants at 8 wk postnatal age.

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days of gestation in both groups of dams, with basal insulin levels in TP-injected dams tending \( (P < 0.067) \) to be \( \sim 130\% \) higher [379.3 \( \pm \) 190.2, 756.6 \( \mu \)U/ml; backtransformed mean (95% confidence limits)] than those in controls [164.8 \( \pm \) 108.8, 279.6 \( \mu \)U/ml]. Maternal basal insulin levels only returned to early gestation values at 140 days gestation (third trimester). During the ivGTT (80 days of gestation), glucose disappearance rate diminished by \( \sim 60\% \) \( (P \leq 0.03) \) and AUC\(_{\text{glucose}} \) tended \( (P \leq 0.057) \) to increase by \( \sim 20\% \) in TP-injected dams (Table 3), indicating impaired glucose tolerance in the latter dams despite elevated AUC\(_{\text{insulin}} \) values \( (P \leq 0.019) \). There were no significant differences between oil- and TP-injected dams with respect to SI, disposition index, and acute insulin response to glucose (Table 3). Glucose disappearance rate was negatively correlated \( (r^2 = 0.50, P < 0.033) \) with dam preconception body weight in TP- but not oil-injected dams (Fig. 6), whereas none of the other glucose or insulin parameters was correlated with glucose disappearance in either group of dams. There were no between-group differences in maternal FFA parameters during the ivGTT (Table 3) or basal FFA levels during gestation (data not shown).

During the intravenous glucagon-tolbutamide test at 140 days of gestation, there were no significant female-type differences for any glucose or insulin parameters (Table 3). In contrast, during ivGTT at 140 days gestation, both basal FFA and AUC\(_{\text{FFA}} \) values in PA fetuses were \( \sim 29\%–36\% \) (\( P \leq 0.03–0.036 \)), respectively, of controls (Table 3).

**Fetuses.** Overall, circulating glucose and insulin levels did not differ between control and PA female fetuses between 80 and 140 days gestation, nor were there gestational changes in circulating insulin (time effect, \( P > 0.17 \)) levels (Figs. 4 and 5). Circulating glucose levels, however, did change across gestation (time effect, \( P < 0.021 \), with values at 80 days gestation exceeding all later gestational values (Fig. 4). Unique to prenatal TP exposure, preconception dam body weight, and circulating glucose levels and glucose clearance rates in TP-injected dams at 80 days gestation, significantly influenced PA fetal glucose levels. PA fetal glucose levels at 80 days gestation showed a strong, positive correlation with the preconception body weight of their dams \( (r^2 = 0.72, P < 0.004) \), implicating dam adiposity before conception as a key contributor in the glucoregulatory associations found between PA fetuses and their TP-injected dams. PA fetal glucose levels showed a similarly strong negative correlation with glucose disappearance rates \( (r^2 = 0.64, P < 0.01) \) and a positive correlation with simultaneous basal glucose values \( (r^2 = 0.98, P < 0.001) \) in their TP-injected dams, with four of the nine (44%) PA fetuses exhibiting serum glucose levels (54.6–92.3 mg/dl) above concurrent control maximal values (52.4 mg/dl; Fig. 7). The positive correlation between fetal and maternal glucose levels persisted at 100 days gestation \( (r^2 = 0.62, P < 0.021) \) for PA fetuses and TP-injected dams only. There were no significant associations between fetal insulin levels and maternal glucose or insulin concentrations despite 44% of PA fetuses having serum insulin levels (11.7–31.3 and 22.5–32.5 \( \mu \)U/ml) above respective control maximal values (11.3 and 22.4 \( \mu \)U/ml) on 100 and 120 days gestation, respectively (Fig. 7).

**Table 2. Infant organ weights corrected for infant body weight in five concurrent control and nine PA female rhesus monkeys determined at tissue harvest at 9 wk postnatal age**

<table>
<thead>
<tr>
<th>Organ</th>
<th>Control (mg/kg) ( \pm ) SE</th>
<th>PA (mg/kg) ( \pm ) SE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Liver</td>
<td>( 21.8 \pm 1.3 )</td>
<td>( 24.2 \pm 1.0 )</td>
</tr>
<tr>
<td>Spleen</td>
<td>( 1.7 \pm 1.0 )</td>
<td>( 1.7 \pm 1.0 )</td>
</tr>
<tr>
<td>Thymus</td>
<td>( 4.5 \pm 0.5 )</td>
<td>( 4.7 \pm 0.3 )</td>
</tr>
<tr>
<td>Right adrenal</td>
<td>( 0.2 \pm 0.1 )</td>
<td>( 0.2 \pm 0.1 )</td>
</tr>
<tr>
<td>Left adrenal</td>
<td>( 0.3 \pm 0.1 )</td>
<td>( 0.2 \pm 0.1 )</td>
</tr>
<tr>
<td>Right kidney</td>
<td>( 2.9 \pm 0.1 )</td>
<td>( 2.7 \pm 0.1 )</td>
</tr>
<tr>
<td>Left kidney</td>
<td>( 2.9 \pm 0.1 )</td>
<td>( 2.6 \pm 0.1 )</td>
</tr>
<tr>
<td>Right ovary</td>
<td>( 0.6 \pm 0.1 )</td>
<td>( 0.5 \pm 0.1 )</td>
</tr>
<tr>
<td>Left ovary</td>
<td>( 0.6 \pm 0.1 )</td>
<td>( 0.6 \pm 0.1 )</td>
</tr>
</tbody>
</table>

Data are means \( \pm \) SE.

**Fig. 4.** Mean (95% confidence limits) of basal circulating glucose levels in 5 concurrent control (○) and 9 TP-exposed (●) dams between 40 and 160 days gestation (A), fetuses between 40 and 140 days gestation (B), and infants between postnatal days 1 and 45 (C). *\( P < 0.025 \) vs. 100, 120, and 140 days gestation, both female fetal groups combined. The duration of gestational treatment is indicated by the open (control) and filled (TP-treated) boxes designating the female groups. ***\( P < 0.001 \) vs. postnatal days 30 and 45, both female infant groups combined.
Infants. In both female groups combined and typical of newborns (Fig. 4), basal circulating glucose levels reached a nadir (time effect, $P < 0.001$) on the first postnatal day of life vs. values on both postnatal days 30 and 45. Basal glucose levels in five out of nine (55%) PA infants, however, ranged from 29 to 36 mg/dl, below the minimum level (38 mg/dl) found in control infants on the first postnatal day (Fig. 7). Consistent with this finding, PA but not control infants demonstrated a negative correlation ($r^2 = 0.45$, $P < 0.048$) between glucose levels on postnatal day 1 and their fetal glucose levels on 80 days of gestation, significantly associating elevated fetal glucose levels with initially low newborn infant glucose values in the androgen-exposed group. In contrast, basal circulating insulin levels did not vary between infant groups or across postnatal days (time effect, $P < 0.70$; Fig. 5). During the intravenous glucose-tolbutamide test at postnatal day 45, $S_I$ and the disposition index in PA infants, however, both increased to $\sim 230\%$ ($P < 0.02$) and $300\%$ ($P < 0.03$), respectively, above control values (Table 3). Increased disposition index values were consistent with $\sim 20\%$ lower ($P \leq 0.013$) AUC$_{glucose}$ values in PA than in control infants. The acute insulin responses to tolbutamide in PA infants were diminished ($P \leq 0.014$) compared with controls. There were no other significant differences between PA and control infants during the intravenous glucose-tolbutamide test, including FFA parameters (Table 3).

**DISCUSSION**

In women and ewes, preconception adiposity and enhanced maternal weight gain can impair glucose regulation during pregnancy, causing increased fetal growth and altered glycemic control in the offspring [humans (19, 37, 43) and sheep (31)]. In this study, despite similar preconception body weights of all dams, body weight before conception [as a measure of female rhesus monkey adiposity (30)] was negatively correlated with glucose clearance in TP-injected monkey dams following accelerated maternal weight gain during TP treatment, suggesting that preconception adiposity interacts with TP-induced maternal weight gain to disrupt maternal glucose regulation. Correspondingly, PA female monkey offspring exposed to fetal androgen excess demonstrated a subtle increase in fetal head growth compared with concurrent controls, along with indications of transient fetal hyperglycemia and relative hyperinsulinemia in neonates. PA female infants demonstrate increased $S_I$ and relative increases in insulin release following glucose challenge, unlike the diminished $S_I$ and reduced insulin release following glucose challenge found in adult PA female monkeys (1, 29) and diminished $S_I$ observed in young female PA sheep (51) as well as adult PA female rats (25). Consequently, PA fetal and neonatal monkeys may exhibit these metabolic abnormalities more from TP-induced maternal glucose intolerance than from direct actions of hyperandrogenism on fetal target tissues.

Such metabolic mechanisms underlying the ability of fetal androgen excess to induce PCOS-like metabolic phenotypes in female rhesus monkeys may explain differences in PCOS-like traits between various fetal androgen excess models (1, 33). In our study, early to midgestation treatment of rhesus monkey dams with TP [intentionally exceeding placental/hepatic steroid deactivation and aromatization to produce fetal male levels of testosterone in the female fetuses (2)] modestly accelerates maternal weight gain to elevate circulating glucose and insulin levels following glucose challenge and to diminish glucose clearance relative to preconception adiposity, likely adding a metabolic perturbation of postprandial hyperglycemia to in utero androgen exposure. Postprandial hyperglycemia is now recognized as an important pathophysiological component in cardiovascular disease (15, 20, 54), glucose intolerant and diabetic pregnancies (12, 48), and type 2 diabetes (8, 46, 59, 66). In addition, gestational maternal postprandial hyperglycemia has recently been shown to be a sensitive marker for maternal glucoregulatory impairment and postpartum development of metabolic syndrome (61). Such TP-induced maternal metabolic disruption may explain why both adult female (4, 29) and male (16) offspring of TP-injected monkey dams exhibit insulin resistance and pancreatic $\beta$-cell defects, as two sequelae of a transient hyperglycemic gestational environment (5, 35, 36), rather than
fetal androgen excess per se (2, 58). As in human pregnancies, relatively mild changes in gestational glucoregulation, as manifest in the present TP-injected dams and PA fetuses and infants, may have consequences for adult metabolic dysfunction (7, 18, 19, 62, 68) and total body fat (30) in female rhesus monkeys, the TP-accelerated weight gain in dams in the present study may well represent increased fat-free mass (44).

In addition, we observed impaired glucoregulation in TP-injected monkey dams, via diminished glucose clearance during midgestational ivGTT, equivalent to mild to moderate glucose intolerance found in obese pregnant rhesus monkeys (43). The degree of glucose intolerance shown by TP-injected dams resembles that of obese, untreated rhesus dams with preconception body weights ~2–5 kg (~30–70%) greater than those of the dams included in this study, implying that androgen excess may exaggerate the adverse metabolic consequence of adiposity accumulated before and during pregnancy (43), even though such adiposity may be within the range of normal body weight.

### Impaired Maternal Glucoregulation

As expected, testosterone treatment of rhesus monkey dams induced subtle hyperinsulinemia presumably from testosterone-induced insulin resistance (21, 27, 56, 74), with increased pancreatic insulin responsiveness to glucose (elevated AUC_{insulin}) and a tendency for increased basal insulin levels, but neither prevented glucose intolerance (diminished glucose clearance) in TP-injected

### Increased Maternal Weight Gain During Gestation

The modestly accelerated maternal weight gain found in this study emulates that found by Kemnitz and colleagues (44) when they administered TP at 2 mg·kg\(^{-1}\)·day\(^{-1}\) to gonadectomized adult FA and control female rhesus monkeys and induced increased fat-free mass. The TP dose in the latter study closely mimics the ~1.8–2.3 mg TP·kg\(^{-1}\)·day\(^{-1}\) range employed in the present study (15 mg·dam\(^{-1}\)·day\(^{-1}\); Fig. 1). Accelerated weight gain in rhesus monkey dams has potential adverse consequences on the fetus, as evident in human studies in which women in the highest quartile of weight gain during pregnancy are more likely to manifest maternal hyperglycemia and impaired glucose tolerance (37), and offspring born to overweight women are more likely to manifest metabolic syndrome in adulthood (19). Although body weight is positively correlated with body mass index (68) and total body fat (30) in female rhesus monkeys, the
dams at 80 days of gestation. Because no additional glucose challenges were performed on the dams, it is not known whether maternal glucoregulatory impairments persist beyond the period of TP treatment. In this regard, insignificantly diminished $S_i$ in TP- vs. oil-injected dams likely represents heterogeneity of $S_i$ in controls from variability of pregnancy-related insulin resistance in rhesus monkeys (43, 55). Because preconception maternal body weight negatively correlates with maternal glucose disappearance in TP-injected dams alone, prepregnancy adiposity may additionally impair glucoregulatory function (19, 31, 37, 43), rendering TP-injected dams particularly susceptible to testosterone-induced insulin resistance. As previously shown in female rats (25), monkeys (47), and sheep (51), hypercaloric, diet-induced obesity mimics the diminished glucoregulatory effects of testosterone treatment so that the combination of increased adiposity and testosterone treatment may exaggerate impaired glucoregulation induced by testosterone treatment alone. Consistent with this hypothesis, increased lipolysis from daily TP injections of adult female rhesus monkeys (44) may contribute to diminished maternal glucose clearance (6, 38, 73).

Fetal Glucose, Insulin, and Growth

In hyperglycemia, excess glucose crosses the placenta by facilitated diffusion and induces compensatory hyperinsulinemia in the fetus (31, 34, 52), whereas circulating maternal insulin does not cross the placenta in any significant amount (41). Transient gestational maternal postprandial hyperglycemia, as found in TP-injected dams in this study, likely has an analogous effect on exposed female fetuses. Such a transient increase in transplacental glucose transport in TP-injected dams can explain how (1) circulating glucose levels in 44% of PA female fetuses exceed those of all fetal controls at 80 days gestation (first fetal sample day, last day of dam treatment) and (2) PA female fetal glucose levels positively correlate with those in their respective dams (at both 80 and 100 days gestation), while negatively correlating with dam glucose clearance (at 80 days gestation). As a consequence of such fetal hyperglycemia, providing principal energy substrate, and likely subtle increases in fetal insulin, as a growth promoter (31, 34, 52), PA monkey fetuses exhibited modestly enhanced biparietal diameter compared with concurrent controls during and after androgen excess treatment of their dams.

In such a fetal environment, circulating FFA levels in PA female fetuses are only ~30% of those found in controls by late gestation. Their respective dams, however, exhibit circulating FFA levels similar to those found in control dams. Although increasing glucose load on human placental tissue in vitro does not alter placental transport of fatty acids (50), gestational diabetes alters placental processing of fatty acids and lipids that may diminish (11) or enhance (57) their release to the fetus. It is thus unclear whether diminished circulating FFA levels in PA female fetuses reflect reduced placental delivery or enhanced fetal extraction. Either alteration in FFA availability to PA female fetuses may perturb early acquisition of fat stores without fetal growth restriction, as observed in this study. In humans, early acquisition of body fat in fetuses and infants leads to increased prevalence of adult adiposity and metabolic syndrome (17, 19).

**Fig. 6.** Negative relationship between ivGTT-determined glucose clearance ($K_G$) in dams at 80 days of gestation and dam preconception body weight ($\bullet$, oil-injected control dams; $\circ$, TP-injected dams).

**Fig. 7.** Individual values for fetal circulating levels of glucose (mg/dl) ($A$) and fetal circulating levels of insulin ($\mu$U/ml) ($B$) between 80 and 140 days of gestation and infant circulating levels of glucose (mg/dl) between postnatal days 1 and 45 ($C$) in 5 concurrent control (open symbols) and 9 PA (filled symbols) infants showing the PA values that exceed control ranges. The same individuals designated by the same symbol appear in all three panels, as either fetus or infant.
Neonatal Glucose, Insulin, and Growth

Increases in PA fetal dimensions, combined with increased dam weight gain during pregnancy, would be expected to increase the postnatal risk of metabolic derangements in mammalian offspring (7, 17, 19, 31, 35). A degree of neonatal hypoglycemia was found in PA neonates on postnatal day 1 when 55% exhibited serum glucose levels of 29–36 mg/dl, all of which were lower than the minimum level of 38 mg/dl found in concurrent controls. With this modest glucose difference, PA neonates also demonstrated a negative correlation between their first postnatal day circulating glucose levels and those obtained from fetal blood samples at 80 days gestation, significantly associating low postnatal glucose levels with high fetal glucose levels in PA females, alone. Analogous associations between newborn hypoglycemia and maternal hyperglycemia have also been found in human pregnancies, including modest maternal hyperglycemia (17, 35).

Insulin hypersecretion following glucose challenge in PA 45-day-old infant monkeys, as shown by excessive acute insulin responses to glucose relative to Si (i.e., increased disposition index), are biologically relevant, as confirmed by increased glucose clearance (diminished AUCglucose), and may represent persistent pancreatic insulin hypersecretion entrained by transient maternal postprandial hyperglycemia (36, 53). The mechanism of exaggerated Si in PA compared with control infant monkeys is unknown but may reflect increased skeletal muscle insulin metabolic signaling, as manifest in neonatal lambs with fetal growth restriction (49), or increased adipose St, as observed in children with increased fetal growth and early postnatal weight gain (14). Relatively excessive insulin release (increased disposition index) in an insulin-sensitive environment (elevated Si) likely explains why a modest increase in weight gain occurs in PA infants at ~8 wk of age, particularly because insulin’s anabolic action (7, 10) may synergize with PA infant hyperandrogenism (2) to increase lipogenesis and muscle protein synthesis (7, 22, 75), thereby enhancing insulin-sensitive tissue mass in PA monkey neonates.

Clinical Relevance

The present study shows that fetal pancreatic β-cells entrained by transient maternal postprandial hyperglycemia cause relative insulin hypersecretion in neonatal life, linking pregnancy-related metabolic antecedents with metabolic dysfunction in PA female rhesus monkeys (4) and possibly in PCOS women. Cresswell and colleagues (23) have associated the development of polycystic ovaries with increased birth weight and mother adiposity during pregnancy, indicating an increased likelihood for postnatal hyperinsulinemia from insulin resistance. Studies of two PCOS populations of Spanish descent (26, 65) have proposed that hyperinsulinemia accompanying postnatal catch-up growth amplifies the development of PCOS traits. In support of this, preadolescent and adolescent daughters of PCOS women have subtle hyperinsulinemia from insulin resistance without apparent changes in glucose parameters preceding an obvious PCOS phenotype (45, 64), whereas preadolescent daughters of glucose-intolerant PCOS women have greater insulin resistance and inferior insulin secretory compensation (D. H. Geller, personal communication). Postnatal insulin defects may thus provide an important developmental component in the expression of PCOS phenotype.

That such developmental origins for PCOS may be strongly influenced by clinically unrecognizable events in the postnatal environment, yet go virtually unrecognized until adulthood, raises profound health care concerns regarding the effect of maternal obesity and/or increased pregnancy weight gain on the transgenerational development of obesity as a susceptibility factor to later adult chronic diseases. With a progressive epidemic of obesity likely to induce metabolic abnormalities amplifying fetal androgen excess programming, the need for primate models to understand the developmental origins of PCOS-like reproductive and metabolic dysfunction is crucial to develop new clinical strategies that treat abnormalities during maternal-fetal development and postnatal life development to reduce the risk of adult disease.

ACKNOWLEDGMENTS

We thank J. M. Turk, S. J. Muller, J. R. Lange, and Assay Services of the Wisconsin National Primate Research Center (WNPRC) for assistance with endocrine determinations; members of the animal care staff at the California National Primate Research Center (CNPRC) for expert technical assistance; Dr. R. N. Bergmann for advice on Minimal Model derivations; and R. A. Becker and J. L. Peterson at WNPRC for assistance with preparation of the figures and manuscript, respectively.

GRANTS

This work was supported by National Institutes of Health Grants P50 HD-044405, P51 RR-00167 (WNPRC base operating grant), and RR-00169 (CNPRC base operating grant) and was partly conducted at a facility (WNPRC) constructed with support from Research Facilities Improvement Program Grant nos. RR-15459-01 and RR-020141-01.

DISCLOSURES

No conflicts of interest are declared by the authors.

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