Hypertension caused by prenatal testosterone excess in female sheep

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King AJ, Olivier NB, Mohankumar PS, Lee JS, Padmanabhan V, Fink GD. Hypertension caused by prenatal testosterone excess in female sheep. Am J Physiol Endocrinol Metab 292: E1837–E1841, 2007. First published February 27, 2007; doi:10.1152/ajpendo.00668.2006.—Polycystic ovary syndrome (PCOS), a leading cause of infertility, affects ~10% of women of reproductive age. The etiology and pathophysiology of PCOS are poorly understood. PCOS is multifaceted and includes reproductive abnormalities and components of the metabolic syndrome such as insulin resistance, obesity, dyslipidemia, and hypertension. Exposure to excess testosterone (T) during the prenatal period may predispose individuals to PCOS phenotype. The goal of this study was to determine whether hypertension and dyslipidemia occur in a well-characterized model of PCOS produced by prenatal treatment of sheep with T. Radiotelemetry was used to measure blood pressure over a 24-h period in conscious, undisturbed animals. To normalize circulating estradiol levels across treatment, control (n = 4) and prenatal T-treated (100 mg T propionate im twice weekly from days 30 to 90 of fetal life, n = 4) 2-yr-old females were ovariectomized, instrumented with a radiotelemetry transmitter, and clamped with early follicular phase levels of estrogen using an implant. Six days later, a 24-h recording period commenced. Prenatal T-treated sheep were hypertensive compared with control sheep, and heart rate tended to be higher. T-treated sheep had hyperglycemia, insulin resistance, hypernatremia, and hyperchloremia, and both total and LDL cholesterol tended to be higher. Plasma aldosterone and epinephrine were significantly lower in T-treated sheep, whereas norepinephrine was unchanged. This first-ever use of radiotelemetry blood pressure recordings in sheep demonstrates that mild hypertension, a risk factor reported in some women with PCOS, is also a feature of the sheep model of PCOS produced by prenatal T treatment.

intrauterine programming; polycystic ovary syndrome; dyslipidemia; hyperglycemia; hypernatremia

A suboptimal intrauterine environment, resulting in impaired fetal growth, is associated with a higher incidence of cardiovascular, metabolic, and reproductive disorders in adult life (26, 34). An abnormal intrauterine hormonal milieu can clearly influence fetal growth and development and has been identified as one factor known to cause intrauterine programming (25, 26). For instance, excessive prenatal exposure to glucocorticoids causes hypertension, insulin resistance, and other metabolic abnormalities in numerous animal models, including sheep (10, 11, 27, 46). The sex steroids are well-established intrauterine programming agents in many species, including sheep (36). Exposure to excess testosterone (T) during the prenatal period causes phenotypic masculinization, intrauterine growth retardation (7, 32, 33, 42), reproductive disruptions, and insulin resistance (39) and recreates the polycystic ovary syndrome (PCOS) phenotype in female sheep (36).

PCOS, which affects ~10% of women of reproductive age (8), is a multifaceted condition comprised of infertility, dysfunctional uterine bleeding, and components of the metabolic syndrome, including obesity. These women are at risk for type II diabetes mellitus, dyslipidemia, hypertension, and other cardiovascular diseases (3). Although T treatment during fetal life recapitulates many of these features in female sheep, the cardiovascular effects have not been studied. The goal of this study was to determine whether hypertension and dyslipidemia occur in this well-characterized model of PCOS.

METHODS

Animal preparation. All protocols were approved by the Animal Use Committee at the University of Michigan. In total, eight age-matched 2-yr-old female Suffolk sheep were studied. Control (n = 4) and prenatal T-treated (100 mg T propionate im twice weekly from days 30 to 90 of fetal life, n = 4) sheep were ovariectomized and replaced with early follicular phase levels of estradiol. Estradiol replacement was by subcutaneous implantation in the axillary region of a 10-mm Silastic implant (0.33 cm id, 0.46 cm OD; Dow Corning, Midland, MI) containing a packed column of crystalline 17β-estradiol (Sigma, St. Louis, MO) and sealed with Silastic adhesive Type A (Dow Corning). The purpose of ovariectomy and hormone replacement was to ensure a consistent estradiol environment in all animals. Earlier studies (14) have implicated potential blood pressure regulating effects of sex hormones. Ovary-intact prenatal T-treated females have higher circulating levels of estradiol than controls (2) because of their multifollicular ovarian morphology (45). This implant is expected to produce chronic serum levels of estradiol of ~1 pg/ml (23, 24). Anesthesia was induced by administering 20–30 ml of pentobarbital sodium intravenously (50 mg/ml Nembutal Na solution; Abbott Laboratories, Chicago, IL) and the animals were intubated to maintain a plane of anesthesia with 1–2% halothane (Halocarbon Laboratories, River Edge, NJ).

Arterial pressure measurement. During the same surgery, the catheter of a radiotelemetry-based pressure transmitter [TA11PA-D70; Data Sciences International (DSI)] was implanted into the femoral artery and the body of the transmitter placed subcutaneously at the inner thigh. Six days later, to allow a sufficient postoperative recovery period, sheep were housed in individual small rectangular pens (3 × 4 ft) with free access to food and water. A radiotelemetry receiver (RPC-1; DSI) was mounted on the pen and connected to a data exchange matrix and computerized data acquisition program (Dataquest ART 3.0; DSI) to monitor arterial pressure remotely. Starting at noon, a 24-h continuous blood pressure recording period commenced. Mean arterial pressure (MAP), systolic blood pressure...
(SBP), diastolic blood pressure (DBP), heart rate (HR) and body temperature were sampled for 10 s every minute for the 24-h period. Relative physical activity level was also measured utilizing the radiotelemetry system. An activity count is generated by the data exchange matrix in response to a change in the strength of the telemetry signal, as the animal moves relative to the receiver. Activity is quantified as counts/min.

Blood sampling and analysis. After completion of the study, unfasted blood samples were collected for lipid profile, aldosterone, catecholamine, glucose, and electrolyte determination. A commercial clinical pathology laboratory (Charles River Laboratories, Wilmington, MA) measured total cholesterol, triglycerides, low-density lipoproteins (LDL), and high-density lipoproteins in serum samples. Aldosterone was measured in plasma by radioimmunoassay (Diagnostic Center for Population and Animal Health, Michigan State University, East Lansing, MI). Plasma norepinephrine and epinephrine were measured using HPLC and electrochemical detection. Plasma electrolytes and glucose were measured with specific ion-selective electrodes (NOVA 16; Nova Biomedical, Waltham, MA).

Statistical Analysis. Statistical analysis was performed by comparing control and T-treated sheep by Student’s t-test. A P value of <0.05 was considered statistically significant. All results are presented as means ± SE.

RESULTS

Hemodynamics. The 24-h average MAP, SBP, DBP, pulse pressure (PP), HR, body temperature, and activity level measured by radiotelemetry in control and prenatally T-treated female sheep is shown in Table 1. Arterial pressure was ~10 mmHg higher in sheep treated with T during the prenatal period. This difference was statistically significant (P < 0.05). HR also tended to be higher in prenatal T-treated sheep by ~10 beats/min; however, this difference was not statistically significant (P = 0.1) in this small group of animals. Body temperature was similar in both groups. Activity level tended to be higher in the prenatal T-treated group, but this was not statistically significant, and the counts of activity measured in this study were relatively low in both groups. The 1-h MAP and HR averages for the 24-h recording period in control and prenatally T-treated female sheep are shown in Fig. 1 and demonstrate that differences in MAP and HR between groups were evident throughout the recording period.

Blood sample analysis. Lipid profile analysis in control and prenatal T-treated female sheep is shown in Table 2. Total (P = 0.17) and LDL (P = 0.08) cholesterol tended to be higher in the prenatal T-treated group. Plasma aldosterone, norepinephrine, and epinephrine are shown in Fig. 2. Sheep treated with T during the prenatal period had significantly lower plasma aldosterone and epinephrine levels compared with control sheep (P < 0.05); however, plasma norepinephrine was not different between the groups.

Plasma electrolyte and glucose concentrations are shown in table 3. Sheep treated with T during the prenatal period had statistically significant elevations in plasma Na⁺, Cl⁻, and glucose compared with the control group (P < 0.05). There were no statistically significant differences in body weight (control 54 ± 3 kg, T treated 56 ± 5 kg), heart, kidney, adrenal, or liver weight between the two groups.

DISCUSSION

The major new finding of this study is that prenatal exposure to excess T causes mild hypertension in adult female sheep. Exposure to excess T during the prenatal period in sheep recapitulates many of the components of PCOS (36), a disease also characterized by a clustering of cardiovascular risk factors (8). Although the model has been exploited extensively to understand the developmental origin of neuroendocrine and ovarian dysfunction, as well as insulin resistance, the cardiovascular consequences have not been explored.

Interestingly, the magnitude of the hypertension resulting from prenatal T excess was similar to what has been previously

Table 1. Twenty-four-hour averages of physiological parameters measured by radiotelemetry in prenatal T-treated and control sheep

<table>
<thead>
<tr>
<th>Group</th>
<th>MAP, mmHg</th>
<th>SBP, mmHg</th>
<th>DBP, mmHg</th>
<th>PP, mmHg</th>
<th>HR, beats/min</th>
<th>Activity, counts/min</th>
<th>Temp, °C</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>90.9 ± 2.8</td>
<td>115.1 ± 4.5</td>
<td>79.2 ± 2.3</td>
<td>35.9 ± 3</td>
<td>73.5 ± 5.9</td>
<td>1.7 ± 0.4</td>
<td>38.7 ± 0.2</td>
</tr>
<tr>
<td>T treated</td>
<td>100.5 ± 2.7*</td>
<td>125.0 ± 2.8*</td>
<td>88.9 ± 2.8*</td>
<td>35.9 ± 0.3</td>
<td>82.5 ± 7.4</td>
<td>2.8 ± 0.5</td>
<td>38.9 ± 0.1</td>
</tr>
</tbody>
</table>

Values are means ± SE. T treated, prenatal testosterone treated; MAP, mean arterial pressure; SBP, systolic blood pressure; DBP, diastolic blood pressure; PP, pulse pressure; HR, heart rate; Temp, body temperature. *P < 0.05, prenatal T-treated group compared with control group.
reported for fetal programming with dexamethasone in sheep (10, 11). Phenotypically, intrauterine programming by T shares many features with prenatal glucocorticoid excess, including intrauterine growth retardation, insulin resistance, and hypertension (26). The intrauterine programming effects of glucocorticoids on adult cardiovascular function have been well documented in both rodent and ovine models (10–12). Overactivity of the renin-angiotensin-aldosterone system (RAAS) plays a key role in the pathogenesis of hypertension programmed by prenatal glucocorticoid exposure (10, 11, 13, 21, 22, 29, 35, 48). In particular, it appears that activation of the intrinsic brain RAAS and not the peripheral RAAS is critical (13, 37, 41). It has also been suggested (41, 43) that sympathetic nervous system overactivity may be involved. Whether the hypertension seen in response to prenatal T excess involves similar mechanisms remains to be elucidated.

The elevated HR seen in prenatal T-treated sheep does support the possibility that sympathetic nervous system activation may be involved (30). A recent study using analysis of HR variability to assess cardiac autonomic function (47) showed increased sympathetic and decreased parasympathetic frequency components in young women with PCOS. However, the finding of normal plasma norepinephrine levels in these sheep indicates that global sympathetic activity is unlikely to be increased. Plasma cathecolamines are only a rough index of global sympathetic activity, and more accurate assessments of sympathetic transmitter release require utilization of radio-isotope dilution measurements of total body or regional norepinephrine spillovers (15) or direct sympathetic nerve recordings (17). This is particularly important given that regionalized sympathetic activation has been convincingly demonstrated by direct nerve recordings in rabbits (38), and several authors (1, 16, 18–20) have elucidated the importance of regionalized sympathetic activation in human cardiovascular disease. Reduced plasma norepinephrine levels have been reported in women with PCOS (28).

Surprisingly, plasma aldosterone levels were significantly decreased in sheep treated with T during the prenatal period. The findings of our study are consistent with a recent report (5) documenting normal plasma aldosterone levels in sheep to be 300–500 pmol/l. Therefore, it is unlikely that peripherally circulating aldosterone is involved in the pathogenesis of hypertension seen in this model, although the role of tissue specific RAAS remains to be investigated. Women with PCOS demonstrate an insulin resistance-related, but very modest, increase in serum aldosterone (4). Prenatal T treatment in sheep does cause insulin resistance (39). Therefore, the reason

### Table 2. Lipid profiles in control and prenatal T-treated sheep

<table>
<thead>
<tr>
<th>Group</th>
<th>Cholesterol, mg/dl</th>
<th>Triglycerides, mg/dl</th>
<th>HDL, mg/dl</th>
<th>LDL, mg/dl</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>54.6±3.5</td>
<td>14.1±1.4</td>
<td>39.8±1.6</td>
<td>12.0±1.2</td>
</tr>
<tr>
<td>T treated</td>
<td>63.2±4.3</td>
<td>14.6±2.2</td>
<td>42.0±2.4</td>
<td>16.3±1.7</td>
</tr>
<tr>
<td></td>
<td>Values are means ± SE.</td>
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</table>

### Table 3. Plasma electrolytes and glucose in control and prenatal T-treated sheep

<table>
<thead>
<tr>
<th>Group</th>
<th>Na⁺, mmol/l</th>
<th>K⁺, mmol/l</th>
<th>Cl⁻, mmol/l</th>
<th>Glucose, mg/dl</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>138.8±3.3</td>
<td>4.4±0.1</td>
<td>108.3±2.2</td>
<td>59.5±1.8</td>
</tr>
<tr>
<td>T treated</td>
<td>149.5±2.1*</td>
<td>4.4±0.3</td>
<td>114.5±1.3*</td>
<td>66.8±1.8*</td>
</tr>
<tr>
<td></td>
<td>Values are means ± SE. *P &lt; 0.05 prenatal T-treated group compared with control group.</td>
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Fig. 2. Plasma aldosterone (A), norepinephrine (B), and epinephrine (C) in control and prenatal T-treated sheep. *P < 0.05 compared with control sheep.
for the discrepancy in plasma aldosterone levels between the experimental model and human clinical condition is unclear.

Prenatal T-treated sheep were hypernatremic and hyperchloremic compared with controls. Although the physiological mechanism is unclear in this study, prenatal programming of hypernatremia and systemic arterial hypertension has been documented in sheep and appears to be due to alterations in the plasma osmolality threshold for arginine vasopressin release (9, 40). Abnormalities in arginine vasopressin secretion have also been reported in women with PCOS (6). Sheep treated with T in the prenatal period were mildly hyperglycemic compared with control animals. Insulin resistance has been well described in this model, although baseline fasting plasma glucose levels were previously reported (39) to be unaffected by prenatal T treatment during the prepubertal period.

Prenatal exposure to excess T tended to increase total and LDL cholesterol, although the increases observed in this small group of animals are not statistically significant. Dyslipidemia is the most common metabolic abnormality in PCOS (8). Approximately 70% of PCOS patients have dyslipidemia characterized by increased total and LDL cholesterol and triglycerides (8, 31, 44). Although the lipid trends seen in these sheep are consistent with the changes expected in lean PCOS women, they differ from those of the metabolic syndrome found in obese PCOS women (31). This suggests that prenatal exposure to excess T may alter serum LDL levels in a manner similar to that of lean PCOS women independently of obesity.

Another important finding of this study is that radiotelemetry technology can be readily applied to sheep for dynamic blood pressure recordings in conscious, undisturbed animals with no complications. To our knowledge this is the first report of the use of radiotelemetry to measure blood pressure in this species. In this study, blood pressure differences were greater during the lights-on period (~11 mmHg) than the lights-off period (~7 mmHg). Dynamic blood pressure recordings in unstressed animals by radiotelemetry can allow for detection of these subtle differences. Radiotelemetry is probably the most versatile method for chronic direct arterial pressure measurements.

PCOS is associated with a clustering of cardiovascular risk factors, including insulin resistance, dyslipidemia, hypertension, and obesity in reproductive age women (8). It has been hypothesized (8) that these factors interact to produce inflammation, oxidative stress, and endothelial dysfunction, leading to clinical cardiovascular disease. It is now well documented that prenatal exposure to excess T recapitulates many of the reproductive and metabolic abnormalities of PCOS, including insulin resistance, in adult female sheep. We have now shown that prenatal exposure to excess T causes mild hypertension and lipid abnormalities in this ovine model of PCOS. Therefore, this animal model provides the opportunity to test the above hypothesis and investigate the relationship between PCOS and cardiovascular disease.

GRANTS

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REFERENCES


