Developmental androgen excess programs sympathetic tone and adipose tissue dysfunction and predisposes to a cardiometabolic syndrome in female mice

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Nohara K, Waraich RS, Liu S, Ferron M, Waget A, Meyers MS, Karsenty G, Burcelin R, Mauvais-Jarvis F. Developmental androgen excess programs sympathetic tone and adipose tissue dysfunction and predisposes to a cardiometabolic syndrome in female mice. Am J Physiol Endocrinol Metab 304: E1321–E1330, 2013. First published April 23, 2013; doi:10.1152/ajpendo.00620.2012.—Among women, the polycystic ovarian syndrome (PCOS) is the most common endocrine disorder in women of reproductive age, is considered to be a form of metabolic syndrome (MetS), and is characterized by reproductive abnormalities (51). Like women with MetS, women with PCOS have visceral adiposity, enlarged adipocytes, hypoadiponectinemia, insulin resistance, glucose intolerance, increased inactivating osteocalcin, and hypertension. Excess fetal exposure to androgens has been hypothesized to play a role in the pathogenesis of PCOS. Previously, we showed that neonatal exposure to the androgen testosterone (NT) programs leptin resistance in adult female mice. Here, we studied the impact of NT on lean and adipose tissues, sympathetic tone in cardiometabolic tissues, and the development of metabolic dysfunction in mice. Neonatally androgenized adult female mice (NTF) displayed masculinization of lean tissues with increased cardiac and skeletal muscle as well as kidney masses. NTF mice showed increased and dysfunctional white adipose tissue with increased sympathetic tone in both visceral and subcutaneous fat as well as increased number of enlarged and insulin-resistant adipocytes that displayed altered expression of developmental genes and hypoadiponectinemia. NTF exhibited dysfunctional brown adipose tissue with increased mass and decreased energy expenditure. They also displayed decreased undercarboxylated and active osteocalcin and were predisposed to obesity during chronic androgen excess. NTF showed increased renal sympathetic tone associated with increased blood pressure, and they developed glucose intolerance and insulin resistance. Thus, developmental exposure to testosterone in female mice programs features of cardiometabolic dysfunction, as can be observed in women with PCOS, including increased sympathetic tone, visceral adiposity, insulin resistance, prediabetes, and hypertension. 

androgen; obesity; adiponectin; insulin resistance; osteocalcin

THE POLYCYSTIC OVARIAN SYNDROME (PCOS) is the most common endocrine disorder in women of reproductive age, is considered to be a form of metabolic syndrome (MetS), and is characterized by reproductive abnormalities (51). Like women with MetS, women with PCOS have visceral adiposity, enlarged adipocytes, hypoadiponectinemia, insulin resistance, and hypertension (17, 32). The sympathetic nervous system is believed to play a role in the pathogenesis of both PCOS and MetS (22). Women with PCOS and MetS display increased sympathetic tone (48). Furthermore, PCOS and MetS are believed to have a common developmental origin in which maternal androgen excess during pregnancy programs both the reproductive and metabolic abnormalities of the offspring (1, 51, 52). First, adult women with prenatatal androgen excess due to adrenal hyperplasia or virilizing tumors develop PCOS-like reproductive symptoms, central obesity, and insulin resistance despite normalizing androgen excess with treatment after birth (5, 23). In addition, female rhesus monkeys exposed to prenatal androgen excess manifest a predominant abdominal visceral fat accumulation during adulthood that reflects a masculinized pattern of fat accumulation (15). Similarly, perinatal testosterone exposure increases adiposity in adult female rats (3, 38). Given the emergence of environmental substances with androgenic actions in fetal androgenization (10, 25), the question arises as to whether developmental androgen excess can program the cardiometabolic features of PCOS. Since developmental testosterone exposure masculinizes the structure and function of the hypothalamus in females (4, 33, 36, 47, 49), the question also arises as to whether developmental androgen excess programs masculinization of metabolism. Using a mouse model, we reported previously that neonatal androgenization of female mice masculinized the organization of proopiocortin, thereby resulting in failure of leptin to suppress food intake (41). In this report, we further explored the phenotype of mice neonatally androgenized with testosterone to understand the role of developmental androgen excess in the metabolic abnormalities of women with PCOS. We addressed two questions. 1) In females, does developmental androgen excess program a masculinization of metabolism that is focused on lean and adipose tissues? 2) Does developmental androgen excess program increased sympathetic tone?

MATERIALS AND METHODS

Experimental animals. Female mice exposed neonatally to testosterone and control mice were produced by injecting C57BL/6 pups with testosterone (100 μg/pup; Steraloids, Newport, RI) or vehicle subcutaneously in sesame oil (Sigma-Aldrich, St. Louis, MO) at neonatal days 1 and 2, as described previously (41). Control pups of the same age were injected with vehicle in sesame oil. Mice were studied on a standard rodent chow. For steroid hormone treatment studies, testosterone (12.5 mg/60 days) or estradiol pellets (0.48 mg/60 days) (Innovative Research of America, Sarasota, FL) were inserted under the skin in the sham-
operated or ovariectomized mice. All animal experiments were approved by the Northwestern University Animal Care and Use Committee in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals.

Metabolic studies. Serum insulin and adiponectin levels were measured by ELISA (Linco Research, St. Charles, MO). Serum triglyceride (Sigma-Aldrich, St. Louis, MO) and serum-free fatty acid (Wako Chemicals, Nissanstr, Neuss, Germany) levels were measured by an enzymatic colorimetric assay. For glucose tolerance tests (2 g/kg) and blood glucose determination, blood samples were obtained from mouse tails, and venous blood glucose levels were determined using an automatic glucose monitor (30).

**Fig. 1.** Neonatal exposure to the androgen testosterone (NT) masculinizes lean tissues and adipose tissues. A: body weight from CF, NTF, and CM mice was determined at the indicated time points (n = 14–28). B: skeletal muscle, heart, and kidney weights were measured at 32 wk (10–23). Body composition was measured by NMR at 20 wk (n = 8). C: 4 fat pad weights [subcutaneous (SC), mesenteric (Mes), perigonadal (PG), and perirenal (PR)] were measured at 8 wk, and visceral fat index was calculated by dividing the total visceral fat pad weight by subcutaneous fat pad weight (n = 6–21). D: 4 fat pad weights were measured at 32 wk; visceral fat index was calculated as described in C (n = 8–23). E and F: lean (E) and fat mass (F) from young control female mice (CF) and mice exposed neonatally to testosterone (NTF) were determined at the indicated time points by quantitative NMR (n = 7–10). Results represent means ± SE. *P < 0.05, **P < 0.01; ***P < 0.001. NTF, CM vs. CF.
Measurement of adipocyte size and number. Perigonadal (PG) adipose tissue was fixed in 10% formalin (Sigma-Aldrich), embedded in paraffin, sectioned, and stained with hematoxylin and eosin. Adipocyte surface area was quantified from hematoxylin and eosin-stained adipose tissue sections using Image J software (National Institutes of Health, Bethesda, MD). The mean adipocyte surface area (size) was calculated from 600 cells/mouse. The relative adipocyte number was then calculated by dividing perigonadal fat pad weight by the mean adipocyte size in each mouse. We used an average of four mice per group.

Euglycemic hyperinsulinemic clamp. The rate of whole body glucose utilization (mg kg⁻¹ min⁻¹) was determined in hyperinsulinemic euglycemic conditions (5.5 mM) as described. Insulin was infused at a rate of 18 mg kg⁻¹ min⁻¹ for 3 h, and HPLC-purified d-[3H]3-glucose (NEN LifeScience, Boston, MA) was infused simultaneously into triglycerides. The labeled triglycerides are extracted using a tail vein every 10 min during the last hour of the infusion.

### Table 1. NE turnover in WAT

<table>
<thead>
<tr>
<th></th>
<th>CF</th>
<th>NTF</th>
<th>CM</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>SC</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tissue weight, mg</td>
<td>143 ± 13.8</td>
<td>165.4 ± 10.4</td>
<td>174.3 ± 9.4</td>
<td>NS</td>
</tr>
<tr>
<td>Tissue NE, ng</td>
<td>153.3 ± 15.9</td>
<td>200.2 ± 8.6</td>
<td>189.5 ± 20.4</td>
<td>NS</td>
</tr>
<tr>
<td>Fraction NE turnover, %/h</td>
<td>13.0 ± 2.5</td>
<td>17.5 ± 2.7</td>
<td>16.2 ± 3.3</td>
<td>NS</td>
</tr>
<tr>
<td>Total NE turnover, ng/h</td>
<td>19.9 ± 5.9</td>
<td>35.1 ± 6.8</td>
<td>30.8 ± 9.6</td>
<td>&lt;0.05 (CF vs. NTF)</td>
</tr>
<tr>
<td>PR</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tissue weight, mg</td>
<td>47.5 ± 4.4</td>
<td>55.4 ± 4.4</td>
<td>92.4 ± 6.2</td>
<td>&lt;0.0001 (CM vs. CF, NTF)</td>
</tr>
<tr>
<td>Tissue NE, ng</td>
<td>21.1 ± 1.8</td>
<td>19.3 ± 4.1</td>
<td>36.4 ± 6.5</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Fraction NE turnover, %/h</td>
<td>9.9 ± 5.3</td>
<td>17.3 ± 3.4</td>
<td>NS</td>
<td></td>
</tr>
<tr>
<td>Total NE turnover, ng/h</td>
<td>3.2 ± 0.7</td>
<td>1.9 ± 1.4</td>
<td>6.3 ± 2.4</td>
<td>&lt;0.05 (CM vs. CF, NTF)</td>
</tr>
<tr>
<td>PG</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tissue weight, mg</td>
<td>305.7 ± 27.1</td>
<td>391.5 ± 31.6</td>
<td>296.4 ± 16.4</td>
<td>&lt;0.025 (CM vs. NTF)</td>
</tr>
<tr>
<td>Tissue NE, ng</td>
<td>98.4 ± 14.5</td>
<td>126.2 ± 11.2</td>
<td>84.5 ± 7.9</td>
<td>&lt;0.1</td>
</tr>
<tr>
<td>Fraction NE turnover, %/h</td>
<td>11.5 ± 2.9</td>
<td>17.7 ± 3.4</td>
<td>10.6 ± 2.7</td>
<td>NS</td>
</tr>
<tr>
<td>Total NE turnover, ng/h</td>
<td>11.4 ± 4.5</td>
<td>22.3 ± 6.2</td>
<td>8.9 ± 3.1</td>
<td>&lt;0.05 (NTF vs. CF)</td>
</tr>
<tr>
<td>Mes</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tissue weight, mg</td>
<td>93.6 ± 6.8</td>
<td>115.6 ± 8.5</td>
<td>122.4 ± 4.7</td>
<td>&lt;0.025 (CF vs. CM)</td>
</tr>
<tr>
<td>Tissue NE, ng</td>
<td>319.5 ± 36.9</td>
<td>311.3 ± 80.9</td>
<td>273.4 ± 57.8</td>
<td>NS</td>
</tr>
<tr>
<td>Fraction NE turnover, %/h</td>
<td>11.8 ± 2.9</td>
<td>10.9 ± 4.1</td>
<td>9.9 ± 2.9</td>
<td>NS</td>
</tr>
<tr>
<td>Total NE turnover, ng/h</td>
<td>37.7 ± 3.6</td>
<td>34.0 ± 21.7</td>
<td>27.1 ± 13.7</td>
<td>NS</td>
</tr>
</tbody>
</table>

Values represent means ± SE; n = 7–8. NE, norepinephrine; WAT, white adipose tissue; CF, control female mice; NTF, mice exposed neonatally to testosterone; CM, control male mice; SC, subcutaneous; PR, perirenal; PG, perigonadal; Mes, mesenteric; NS, nonsignificant. Visceral fat index = (PR + PG + Mes)/SC.
Statistical analysis. Results are presented as means ± SE unless stated otherwise. Data were analyzed using Student’s t-test, one-way ANOVA followed by post hoc analysis using Dunnett’s multiple comparison tests, or two-way ANOVA followed by post hoc analysis using Bonferroni test as appropriate. A value of $P < 0.05$ was considered statistically significant.

RESULTS

To determine the pathophysiological consequences of neonatal androgenization, we compared littermate control female mice (CF) with female mice exposed neonatally to testosterone (NTF). We also studied age-matched littermate control male mice (CM) to control for the effect of masculinization. We used testosterone enanthate to induce prolonged testosterone exposure for 2 wk [testosterone level at postnatal day 10 (ng/dl); CF: 11.5 ± 2.2; NTF: 107.6 ± 29.1; CM: 34.1 ± 15.3]. However, in adults, NTF and CF showed similar serum testosterone and $17\beta$-estradiol (E$_2$) concentrations (41). Consistent with the increased food intake observed in our previous study (41), NTF, like CM, exhibited an increase in body weight in the prepubertal period, followed by a stable increase after 7 wk (Fig. 1A). NTF mice displayed a masculinization of lean androgen-sensitive tissues, with elevation in skeletal muscle, heart, and kidney weights (Fig. 1B). Masculinization of body weight and lean tissue mass were characterized by linear trends such that CF showed the lowest values, followed by NTF and CM, which exhibited the highest values (Fig. 1, A and B).

Altered white adipose tissue sympathetic tone and adipose dysfunction in NTF mice. CM accumulated proportionally more visceral fat in mesenteric, perigonadal, and retroperitoneal areas than CF mice. NTF mice showed an early masculinization of white adipose tissue (WAT), with a predominantly visceral distribution that exacerbated with age (Fig. 1, C and D). Note that in NTF the increase in lean mass compared with CF was observed as early as 4 wk of age (Fig. 1E). In contrast, the increase in fat mass was observed 2 wk later at 6 wk of age (Fig. 1F). The sympathetic nervous system is involved in adipose distribution and function (8, 40) and can be programmed in early life (53). We used NE turnover as a measurement of sympathetic outflow to WAT. CM showed higher NE turnover than both CF and NTF in perirenal depots but no significant sex dimorphism in other depots (Table 1). Increased NE turnover was associated with an age-dependent increase in perirenal fat in CM compared with both CF and NTF (compare Fig. 1, C and D, with Table 1). Conversely, NTF developed a preferential increase in NE turnover in subcutaneous (SC) and PG depots compared with CF that was indicative of increased sympathetic nervous system outflow in these depots (Table 1). Notably, in NTF, the increased NE turnover in PG depots was higher than in CM and was associated with an age-dependent increase in PG fat in NTF compared with CM (compare Fig. 1, C and D, with Table 1). Thus, in CM and NTF, the increased sympathetic tone in WAT was associated with increased size of the depot. Note that the group of mice used for the NE turnover

![Figure 2. Adipocyte morphology and function in NTF.](https://example.com/figure2.png)

**Fig. 2.** Adipocyte morphology and function in NTF. *A:* adipocyte area distribution and average in 32-wk-old PG fat ($n = 4$). *B:* lipogenic gene expression was measured in PG fat of 8-wk-old fed mice by quantitative PCR ($n = 5–12$). *C:* in vivo lipogenesis was measured by $^3$H-labeled glucose incorporation into triglyceride in PG fat during euglycemic hyperinsulinemic clamp in 24-wk-old mice ($n = 7$). *D:* glucose infusion rate during euglycemic hyperinsulinemic clamp in 24-wk-old mice ($n = 6–11$). *E:* serum adiponectin concentrations in 12- to 24-wk-old mice ($n = 21–27$). Results represent means ± SE. *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$. NTF, CM vs. CF. Srebpf, gene encoding the sterol regulatory element-binding protein-1c; Fasn, gene encoding fatty acid synthase; Acaca, gene encoding acetyl-CoA carboxylase.
Table 2. **Metabolic parameters**

<table>
<thead>
<tr>
<th></th>
<th>CF</th>
<th>NTF</th>
<th>CM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fasting glucose, mg/dl</td>
<td>113.00 ± 3.41</td>
<td>128.33 ± 8.61</td>
<td>119.00 ± 1.73</td>
</tr>
<tr>
<td>Fed glucose, mg/dl</td>
<td>156.20 ± 8.67</td>
<td>161.67 ± 4.13</td>
<td>185.00 ± 8.50</td>
</tr>
<tr>
<td>Fasting insulin, ng/ml</td>
<td>0.21 ± 0.03</td>
<td>0.34 ± 0.02**</td>
<td>0.34 ± 0.02*</td>
</tr>
<tr>
<td>Fed insulin, ng/ml</td>
<td>0.63 ± 0.01</td>
<td>1.60 ± 0.31*</td>
<td>2.78 ± 0.30***</td>
</tr>
<tr>
<td>GTT 2-h glucose, mg/dl</td>
<td>107.80 ± 5.60</td>
<td>136.33 ± 6.76*</td>
<td>127.33 ± 13.87</td>
</tr>
<tr>
<td>GTT AUC (×1000)</td>
<td>20.70 ± 0.86</td>
<td>24.03 ± 0.52*</td>
<td>24.41 ± 0.86*</td>
</tr>
</tbody>
</table>

Values represent means ± SE; n = 3–8. GTT, glucose tolerance test; AUC, area under the curve. *P < 0.05; **P < 0.01; ***P < 0.001. CF vs. NTF or CM.

The study in Table 1 exhibited a milder phenotype than the groups of mice used in the rest of the study and shown in Fig. 1. As a result, the fat pads used for the NE turnover study did not show weight differences across groups when adjusted for body weight.

With a focus on PG depots, we observed that adipocytes from CM were larger (Fig. 2A), and they showed decreased expression of genes involved in de novo lipogenesis, such as *Srebp1* (encoding the sterol regulatory element-binding protein-1c) and its target genes *Fasn* (encoding fatty acid synthase) and *Acaca* (encoding acetyl-CoA carboxylase) (Fig. 2B). We also observed reduced in vivo glucose incorporation into triglycerides (Fig. 2C). The decrease in insulin-dependent lipogenesis in CM was associated with decreased insulin sensitivity in euglycemic hyperinsulinemic clamp conditions compared with CF (Fig. 2D). We observed that adipocytes from NTF, like those of CM mice, were enlarged (Fig. 2A) and had lower expression of genes involved in de novo lipogenesis. They also had lower lipogenic capacity (Fig. 2C), which was associated with trend toward reduced insulin sensitivity (Fig. 2D). Since glucose incorporation into triglyceride was measured during hyperinsulinemic clamp conditions, these data demonstrate that compared with WAT of CF, WAT of CM and NTF is insulin resistant. The serum concentration of adiponectin was sexually dimorphic and lower in CM than in CF mice (Fig. 2E). Importantly, serum adiponectin concentrations were also lower in NTF, consistent with the observed “masculinization” of adipose tissue (Fig. 2E).

NTF showed no significant alteration in fasting or random fed blood glucose levels (Table 2). However, consistent with insulin resistance observed in clamp conditions, NTF and CM exhibited higher serum insulin levels under both fasting and fed conditions (Table 2). Insulin resistance during clamp and hyperinsulinemia were characterized by linear trends such that CF showed the lowest values, followed by NTF and CM, which exhibited the highest values (Fig. 2D and Table 2). In addition, NTF and CM exhibited glucose intolerance compared with CF (Table 2). Consistent with these data, postchallenge glucose was significantly higher in NTF and CM compared with CF (Table 2).

**Decreased osteocalcin activity in NTF mice.** Bone-derived osteocalcin has been identified as an endocrine regulator of adiponectin production (31). Osteocalcin is present in the serum in carboxylated or undercarboxylated form, yet only the undercarboxylated form of osteocalcin acts as a hormone (18, 31). There were no significant differences among groups in total osteocalcin or the carboxylated inactive form of osteocalcin, GLA13 (Fig. 3, A and B). However, compared with CF, in CM we observed a decrease in the undercarboxylated active form of osteocalcin, GLA13 (Fig. 3C), as well as the ratio of active to inactive osteocalcin (Fig. 3D). We also observed a decrease in the active form of osteocalcin, GLU13, and the ratio of active to inactive osteocalcin in NTF (Fig. 3, C and D). Thus, males have decreased undercarboxylated active osteocalcin, and this pattern can be programmed in females by developmental androgen exposure.

**Altered adipose phenotypical identity in NTF mice.** We found that NTF (but not CM) showed adipose hyperplasia, suggesting that in NTF adipose depots, more cells were committed to adipocyte lineage (Fig. 4A). It has been suggested recently that programmed developmental differences in patterning genes play a role in the differential development of various adipose tissue depots during obesity (20). We focused on expression of three genes, *HoxA5*, *glypican 4* (*Gpc4*), and *T-box 15* (*Tbx15*), that have been correlated with fat distribution and obesity (20). In CF, PG depots expressed higher levels of *Gpc4* (Fig. 4C) and *HoxA5* (Fig. 4D), whereas SC depots have higher levels of *Tbx15* (Fig. 4B), as described previously (20). Since no sexual dimorphism has been described for this gene (20), we did not study males. NTF had a dramatic increase in *Tbx15*, *Gpc4*, and *HoxA5* expression in SC depots, whereas *HoxA5* expression was decreased in PG depots (Fig. 4, B–D). Together, these findings suggest that NTF have an acquired loss of adipose cell phenotypic identity in these depots.

**Brown adipose tissue dysfunction in NTF mice.** Leptin’s role in controlling fat mass includes activation of sympathetic outflow to brown adipocytes, leading to the induction of the uncoupling protein-1 (UCP1) with stimulation of thermogenesis and energy expenditure as fat mass increases (11). In NTF, BAT mass increased similarly to CM mice (Fig. 5A). To explore sympathetic outflow to brown adipose tissue (BAT) under basal conditions, we quantified NE turnover and expres-
We observed reduced energy expenditure relative to lean resistance that suppresses food intake and reduces body weight. In addition to reduced energy expenditure, we observed reduced energy expenditure relative to lean mass and reduced energy expenditure relative to whole body weight in NTF (Fig. 5, E and F). Thus, since NTF were hyperleptinemic (41), there was a relative reduction in leptin’s ability to activate sympathetic tone in BAT.

Androgen excess predisposes to adiposity in NTF mice. Because NTF develop adiposity despite normal testosterone and E2 serum concentrations (41), we wondered whether NT programs the sensitivity to androgen or estrogen with regard to control of fat mass. We explored whether ovariectomy (OVX) followed by testosterone and E2 replacement altered the obesity phenotype. In CF, OVX induced a dramatic increase in visceral and SC adiposity (Fig. 6, A–C) as well as whole body fat mass (Fig. 6D). However, in NTF, OVX had no effect on additional fat accumulation (Fig. 6, A–D). We then explored the effect of E2 supplementation. In both OVX CF and NTF, E2 treatment reduced adiposity to levels similar to sham-operated CF mice, thereby demonstrating that CF and NTF had the same sensitivity to E2 with regard to suppressing adiposity (Fig. 6, A–D).

We next looked at the effect of testosterone exposure in OVX mice. Testosterone suppressed adiposity in OVX CF (Fig. 6, A–D). However, testosterone did not suppress adiposity in OVX NTF (Fig. 6, A–D). Since NTF do not exhibit increased serum testosterone levels (41), we further explored whether the raising of testosterone levels in NTF, as was observed in women with PCOS, would further predispose to adiposity. Three weeks of exposure to testosterone dramatically increased (+57%) both abdominal and SC fat pads in NTF (Fig. 6, E–I).
against the backdrop of a minor increase in overall body weight (10%) (Fig. 6J). These findings suggested that NTF are predisposed to obesity during chronic androgen excess.

Increases in renal sympathetic tone and hypertension in NTF mice. Since NTF showed alteration in sympathetic outflow in WAT, we also analyzed NE turnover in heart and kidney. Heart NE turnover was similar in all three groups of mice. However, NE turnover was increased in kidney of NTF compared with both CF and CM (Table 3). Consistent with this phenotype, NTF showed increased diastolic and mean blood pressure without an increase in heart rate (Table 3).

DISCUSSION

Neonatally androgenized female mice develop a masculinized body composition with increased lean and fat mass, as
Developmental androgen excess programs deregulation of sympathetic tone to SC and perigonadal fat in females that is not a mere masculinization. Indeed, males have increased sympathetic tone in retroperitoneal fat, whereas androgenized females have increased sympathetic tone in SC and perigonadal fat. Activation of central sympathetic efferent to adipose tissue is known to decrease lipogenesis and fat mass (8, 40) as well as reduce cell numbers in mouse adipose tissue (7). Since we observed increased sympathetic tone in WAT accompanied by decreased lipogenesis and increased adipose tissue mass and cell number, the increased sympathetic tone may reflect an adaptive mechanism to counter chronic fat accumulation. How can androgenized female mice retain adiposity despite suppressed lipogenesis? Several mechanisms could be in play. First, the combination of increased energy intake (41) and decreased energy expenditure favors adiposity. Androgenized female mice show reduced energy expenditure in the presence of increased BAT mass but in the absence of increased BAT sympathetic activity. Since the androgenized female mice also exhibit hyperleptinemia, which is not observed in males and is inconsistent with masculinization (41), this suggests that androgenized female mice have acquired a state of BAT hypofunction with failure of leptin to upregulate the expression of the thermogenic protein UCP1. Second, there is programming of adipocyte number and identity that is not masculinization. We observed that expression of developmental genes involved in depot-specific identity is altered in SC adipose tissue. Specifically, HoxA5, Gpc4, and Tbx15 expression are increased. High levels of Tbx15 and Gpc4 expression in SC adipose tissue are markers of visceral fat accumulation in humans (20). Thus, neonatal androgenization may have altered a developmental program of gene expression that leads to loss of adipose cell phenotypic identity in females’ SC and visceral depots. Interestingly, testosterone suppresses preadipocyte growth in ovariectomized female rodents (26), and we have observed that testosterone suppresses adipose tissue mass in ovariectomized control females. However, testosterone fails to suppress adiposity in ovariectomized neonatally androgenized females. Furthermore, neonatally androgenized females develop obesity when chronically treated with testosterone. Thus, neonatal androgenization may have programmed increased androgen sensitivity of adipose tissue in a manner that favors adiposity during hyperandrogenemia. In such a two-hit hypothesis, developmental androgen excess increases adipose mass and also favors androgen-induced adiposity, thereby setting the stage for the metabolic syndrome that characterizes women with PCOS.

Table 3. Cardiovacular parameters

<table>
<thead>
<tr>
<th>Parameter</th>
<th>CF</th>
<th>NTF</th>
<th>CM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blood pressure (diastolic)</td>
<td>106.42 ± 4.05</td>
<td>123.38 ± 4.76*</td>
<td>111.67 ± 3.81</td>
</tr>
<tr>
<td>Blood pressure (systolic)</td>
<td>140.18 ± 3.92</td>
<td>154.48 ± 5.02</td>
<td>144.74 ± 3.89</td>
</tr>
<tr>
<td>Blood pressure (mean)</td>
<td>117.32 ± 3.99</td>
<td>133.42 ± 4.829</td>
<td>122.33 ± 3.82</td>
</tr>
<tr>
<td>Heart rate/min</td>
<td>83.60 ± 25.05</td>
<td>803.23 ± 28.76</td>
<td>793.96 ± 18.67</td>
</tr>
<tr>
<td>NE turnover</td>
<td>47.6 ± 8.8</td>
<td>47.8 ± 10.9</td>
<td>62.6 ± 12.9</td>
</tr>
<tr>
<td>Tissue NE, ng</td>
<td>471.6 ± 23.7</td>
<td>483.3 ± 12.4</td>
<td>530.7 ± 21.3</td>
</tr>
<tr>
<td>Fraction NE turnover, %/h</td>
<td>10.1 ± 1.4</td>
<td>9.9 ± 2.0</td>
<td>11.8 ± 2.0</td>
</tr>
<tr>
<td>Total NE turnover, ng/h</td>
<td>47.6 ± 8.8</td>
<td>47.8 ± 10.9</td>
<td>62.6 ± 12.9</td>
</tr>
<tr>
<td>Kidney</td>
<td>870.9 ± 36.2</td>
<td>897.8 ± 37.2</td>
<td>824.4 ± 46.3</td>
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<tr>
<td>Tissue NE, ng</td>
<td>14.7 ± 1.3</td>
<td>21.0 ± 3.7</td>
<td>13.7 ± 1.7</td>
</tr>
<tr>
<td>Fraction NE turnover, %/h</td>
<td>127.9 ± 17.0</td>
<td>188.6 ± 40.6*</td>
<td>112.9 ± 20.0</td>
</tr>
</tbody>
</table>

Values represent means ± SE; n = 7–8. *P < 0.05. CF vs. NTF or CM.

observed in males despite normal testosterone levels (41). One could argue that this phenotype results from an overall increase in growth. We believe that this is not the case since neonatally androgenized female mice have different timings of lean and fat mass growth. More importantly, they develop classical features of cardiometabolic dysfunction that lead to a metabolic syndrome in humans and are not mere masculinization, as we will discuss below. Female mice that are neonatally androgenized, a period corresponding to the second trimester of pregnancy in humans regarding the synaptogenesis of hypothalamic centers controlling adiposity and adipose tissue development (2, 6, 21, 29), develop many of the metabolic features observed in women with PCOS. These features include increased sympathetic tone, decreased energy expenditure, visceral adiposity with enlarged adipocytes and hypoadiponectinemia, decreased osteocalcin activity, insulin resistance, and hypertension.

Male and neonatally androgenized female mice display adipocyte hypertrophy and decreased insulin-dependent de novo lipogenesis compared with female control mice. De novo lipogenesis correlates with smaller adipose cell size in humans and promotes insulin sensitivity (45). Thus, the decreased de novo lipogenesis in fat of males and neonatally androgenized female mice may explain their increased adipose cell size and insulin resistance (34). Similarly, males have reduced concentrations of serum adiponectin that are dependent on serum testosterone concentrations (39). Neonatally androgenized female mice have acquired a decreased or “masculinized” serum adiponectin concentration. The mechanism of this decreased adiponectin set point is unknown, although it could involve dysfunction of larger adipocytes in males and neonatally androgenized female mice. Indeed, larger adipocytes and lower adiponectin are observed in women with PCOS (35). The skeleton has been identified as an endocrine regulator of both insulin sensitivity and adiponectin via osteocalcin secretion (31). It is the undercarboxylated form of osteocalcin that acts as a hormone (18, 31). We observed a previously unknown sex dimorphism in which males have decreased undercarboxylated active osteocalcin. Neonatally androgenized female mice develop a “masculinized,” low-undercarboxylated active osteocalcin. In humans, low-carboxylated osteocalcin is associated with insulin resistance (46). Thus, low-carboxylated osteocalcin could play a role in insulin resistance in male and neonatally androgenized female mice. Osteocalcin activity also correlates with adiponectin levels in mice, with reduced serum osteocalcin levels being associated with decreased adiponectin levels (18). Thus, in neonatally androgenized female mice, the reduced undercarboxylated active osteocalcin could explain the decrease in serum adiponectin levels. Interestingly, women with PCOS exhibit higher levels of carboxylated inactive osteocalcin (14). Thus, the neonatally androgenized female mice have yet another similarity with PCOS women, thereby suggesting a role for developmental androgen programming in osteocalcin carboxylation.
Neonatally androgenized female mice have developed an increased renal sympathetic tone associated with increased blood pressure that is not masculinization. The role of renal sympathetic tone in the pathophysiology of salt-sensitive hypertension has been known for some time (13, 27, 49). In obesity, there is evidence for selective leptin resistance. Indeed, during high-fat feeding (44), in the agouti obese mouse (12), and in obese mice with Bardet-Biedl syndrome (42), there is selective impairment of leptin suppression of food intake and regulation of body weight with maintenance of leptin’s ability to stimulate the sympathetic nervous system. Thus, hyperleptinemia increases renal sympathetic tone, thereby leading to hypertension (24, 43). In neonatally androgenized mice, hyperleptinemia (41) may stimulate renal sympathetic outflow. Prenatal testosterone excess also causes hypertension in female sheep, but the mechanism is unknown (28). Since neonatally androgenized female mice have elevated sympathetic outflow to kidneys but not to the heart, as well as no increased heart rate, it is likely that neonatally androgenized females have developed hypertension via hyperactivation of renal sympathetic outflow.

In summary, neonatally androgenized female mice develop many features of cardiometabolic dysfunction observed in women with PCOS and that are inconsistent with masculinization. This study reinforces the hypothesis that PCOS originates from developmental androgen excess and shows that the mouse is an excellent model to dissect the role of developmental androgens in the pathogenesis of the cardiovascular and metabolic abnormalities of PCOS.

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DISCLOSURES

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AUTHOR CONTRIBUTIONS

K.N., R.S.W., R.B., and F.M.-J. contributed to the conception and design of the research; K.N., R.S.W., S.L., M.F., A.W., and M.S.M. performed the experiments; K.N., R.S.W., S.L., M.F., G.K., R.B., and F.M.-J. analyzed the data; K.N., R.B., and F.M.-J. interpreted the results of the experiments; K.N., R.S.W., and M.S.M. prepared the figures; K.N. and F.M.-J. drafted the manuscript; K.N., R.S.W., S.L., M.F., G.K., and F.M.-J. edited and revised the manuscript; K.N., R.S.W., S.L., M.F., A.W., M.S.M., G.K., R.B., and F.M.-J. approved the final version of the manuscript.

REFERENCES

23. Hague WM, Adams J, Rodda C, Brook CG, de Bruyn R, Grant DB, Jacobs HS. The prevalence of polycystic ovaries in patients with congen-