Developmental androgen excess disrupts reproduction and energy homeostasis in adult male mice

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Abstract

Polycystic ovary syndrome is a common endocrine disorder in females of reproductive age and is believed to have a developmental origin in which gestational androgenization programs reproductive and metabolic abnormalities in offspring. During gestation, both male and female fetuses are exposed to potential androgen excess. In this study, we determined the consequences of developmental androgenization in male mice exposed to neonatal testosterone (NTM). Adult NTM displayed hypogonadotropic hypogonadism with decreased serum testosterone and gonadotropin concentrations. Hypothalamic Kiss1 neurons are believed to be critical to the onset of puberty and are the target of leptin. Adult NTM exhibited lower hypothalamic Kiss1 expression and a failure of leptin to upregulate Kiss1 expression. NTM displayed an early reduction in lean mass, decreased locomotor activity, and decreased energy expenditure. They displayed a delayed increase in subcutaneous white adipose tissue amounts. Thus, excessive neonatal androgenization disrupts reproduction and energy homeostasis and predisposes to hypogonadism and obesity in adult male mice.

Key Words

- androgens
- reproduction
- energy homeostasis

Introduction

Polycystic ovary syndrome (PCOS) is the most common endocrine disorder in females of reproductive age and is believed to have a developmental origin in which maternal and fetal androgen excess during pregnancy programs both the reproductive and metabolic abnormalities of the offspring (Abbott et al. 2005, Xita & Tsatsoulis 2006, 2010). Female primates and rodents exposed to androgen excess during critical periods of perinatal development go on to exhibit a predominantly visceral fat accumulation with accompanying insulin resistance during adulthood (Nilsson et al. 1998, Eisner et al. 2003, Alexanderson et al. 2007). During fetal life, both male and female fetuses are exposed to a potential source of androgen excess. In fact, brothers of women with PCOS develop insulin resistance compared
with control men (Yildiz et al. 2003, Sam et al. 2008), an observation that is compatible with the hypothesis involving a common developmental origin characterized by excess androgen exposure. In addition, male primates exposed to prenatal androgen excess exhibit insulin resistance as adults, emulating prenatally exposed females (Bruns et al. 2004). Furthermore, male sheep and rats exposed to prenatal androgen excess display signs of hypogonadism and feminization (Wolf et al. 2002, Recabarren et al. 2008). The issue of developmental androgenization is of critical importance and should be paid more attention because of increasing human exposure to environmental factors that interact with androgen and estrogen receptor systems (Hotchkiss et al. 2007, Chen et al. 2008). An obvious question arises as to whether developmental exposure to androgen from endogenous or exogenous sources could program both reproductive and metabolic abnormalities in male offspring as it does in females. There is extensive evidence supporting brain programming of behavior and physiology by perinatal testosterone (MacLusky & Naftolin 1981, Arnold & Gorski 1984, Simerly 2002, Morris et al. 2004, Negri-Cesi et al. 2008, Wu et al. 2009). For example, the Kiss1 gene encodes for kisspeptins that are instrumental in triggering puberty (Seminara et al. 2003, d’Anglemont de Tassigny et al. 2007). Male rodents express lower levels of Kiss1 in the hypothalamus, and in females, perinatal testosterone exposure suppresses Kiss1 expression, thereby preventing the preovulatory surge of gonadotropins (Kauffman et al. 2007). Human and nonhuman primates are precocial species that give birth to mature young. In both groups, synaptogenesis of hypothalamic centers that control energy homeostasis and adipose tissue development occurs during the second trimester of pregnancy (Ailhaud et al. 1992, Koutcherov et al. 2002, Gesta et al. 2007). By contrast, the mouse is an altricial species that gives birth to immature young. In mice, the development of hypothalamic circuits that control adiposity and adipose tissue development occurs during the first 2 weeks of neonatal life (Ailhaud et al. 1992, Bouret et al. 2004, Gesta et al. 2007). In male rodents, testosterone mediates many aspects of sexual differentiation of the brain during a restricted developmental neonatal period ending on day 10 (MacLusky & Naftolin 1981, Arnold & Gorski 1984). Thus, during a critical period corresponding to late pregnancy in humans, androgen excess could program reproductive and metabolic abnormalities that would later appear in adult male rodents. In this study, we used the male mouse model neonatally androgenized with testosterone as a means to understand the role of developmental androgen excess-induced reproductive and metabolic abnormalities in males.

Materials and methods

Animals

Mice neonatally injected with testosterone (NT) were obtained by injecting C57BL/6 pups with 100 μg testosterone enanthate (Steraloids, Inc., Newport, RI, USA) subcutaneously in the neck in sesame oil (volume 20 μl) on neonatal days 1 and 2 (birth date = day 0). Control pups of the same age were injected with a vehicle in sesame oil. The mice were fed a standard rodent chow (Harlan Teklad, code 7912). 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 Animals.

Metabolic studies

Serum leptin and adiponectin concentrations were measured using ELISA (Linco Research, Inc., St Louis, MO, USA). Serum testosterone (Siemens Medical Solutions Diagnostics, Los Angeles, CA, USA), 17β-estradiol (E2; Beckman Coulter, Inc., Fullerton, CA, USA), and follicle-stimulating hormone (FSH) concentrations were measured using RIA (Gay et al. 1970). Serum gonadotropin luteinizing hormone (LH) concentrations were measured using sandwich ELISA (Haavisto et al. 1993).

Gene expression analysis by real-time quantitative PCR

Gene expression was quantified in tissues by real-time quantitative PCR and normalized to β-actin expression. Briefly, total RNA was extracted in TRIzol Reagent (Invitrogen). One microgram of RNA was reverse-transcribed using the iScript cDNA synthesis kit (Bio-Rad Laboratories) with random hexamers. Primer sequences are available upon request.

In vivo leptin stimulation

The mice were housed individually in cages for 1 week for the purpose of acclimatization. Food intake was measured daily for 1 week to obtain basal values. Leptin (25 μg/20 g i.p.; National Hormone and Peptide Program (NHPP)) was injected daily for 4 days. During this period, food intake and body weight were measured daily. For hypothalamic Kiss1 expression studies, PBS or leptin (3 μg/g) was injected intraperitoneally after a 24-h fast. After 6 h, the mice were killed and their hypothalami were isolated. Hypothalami were then frozen in liquid N2 and stored at −80°C until assayed.
Euglycemic–hyperinsulinemic clamp

The rate of whole-body glucose utilization (mg/kg×min) was determined under hyperinsulinemic-euglycemic conditions (5.5 mM) as described herein. Insulin was infused at a rate of 18 mU/kg×min for 3 h and HPLC-purified D-(3H)3-glucose (NEN LifeScience, Boston, MA, USA) was simultaneously infused at a rate of 30 Ci/kg×min to ensure detectable plasma D-(3H)3-glucose enrichment. Throughout the infusion period, blood glucose concentrations were assessed using blood samples (1.5 μl) collected from the tip of the tail vein when needed using a blood glucose meter. Euglycemia was maintained by periodically adjusting a variable infusion of 16.5% glucose. Plasma glucose concentrations and D-(3H) 3-glucose-specific activity were determined using 5 μl of blood sampled from the tip of the tail vein using heparinized microcapillaries every 10 min during the final hour of the infusion period.

Indirect calorimetry

Respiratory metabolism was measured by indirect calorimetry using the Oxymax Comprehensive Laboratory Animal Monitoring System (CLAMS; Columbus Instruments, Columbus, OH, USA). The mice were evaluated for oxygen consumption (VO2) and carbon dioxide production (VCO2) over a 24-h period. Heat production (kcal/h) was calculated according to the following formula: (VO2−(3.815+1.232×RQ)).

Locomotor activity

Locomotor activity was assessed by the infrared beam break method using an Opto-Varimetrix-3 sensor system, and water intake was measured using the Feed-Scale System (Columbus Instruments).

Table 1  Serum reproductive hormone concentrations. Serum testosterone (n=16–25), LH (n=13), FSH (n=5–8), and estradiol (n=19–23) concentrations. Values represent means±S.E.M. NTF and CM vs CF. n=3–11 (12 weeks old)

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<th>CM</th>
<th>NTF</th>
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<tr>
<td>Testosterone (ng/dl)</td>
<td>34.9±4.3</td>
<td>23.6±2.7*</td>
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<tr>
<td>LH (ng/ml)</td>
<td>0.15±0.03</td>
<td>0.09±0.01*</td>
</tr>
<tr>
<td>FSH (ng/ml)</td>
<td>33.3±1.4</td>
<td>19.0±1.6*</td>
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<tr>
<td>E2 (pg/ml)</td>
<td>24.7±2.5</td>
<td>26.7±2.7</td>
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*P<0.05 and †P<0.001.

Measurement of adipocyte size

Perigonadal adipose tissue was fixed in 10% formalin (v/v; Sigma–Aldrich) embedded in paraffin, sectioned, and stained with hematoxylin and eosin (H&E). Adipocyte surface area was quantified from H&E-stained adipose tissue sections using the Image J software (National Institute of Health, Bethesda, MD, USA). The mean adipocyte surface area (size) was calculated from 600 cells per mouse. We used an average of four mice per group.

Fertility test

Fertility was assessed by mating experimental males with C57BL/6 females purchased from Jackson Laboratory (Bar Harbor, ME, USA). The mice were allowed to mate for a 30-day period, and pairs were monitored regularly for signs of pregnancy. The pregnancy ratio was calculated by the number of pregnant female mice undergoing parturition over the total female mice in each group.
Androgens disrupt reproduction and energy balance

**Table**: Muscular and Skeletal Characteristics

<table>
<thead>
<tr>
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<th>CM</th>
<th>NTM</th>
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<tbody>
<tr>
<td>Skeletal (mg)</td>
<td>174.6 ± 3.0</td>
<td>165.1 ± 3.1*</td>
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<tr>
<td>Heart (mg)</td>
<td>128.0 ± 1.9</td>
<td>114.1 ± 2.8***</td>
</tr>
<tr>
<td>Kidney (mg)</td>
<td>375.7 ± 8.8</td>
<td>307.8 ± 7.8***</td>
</tr>
<tr>
<td>Lean mass (g)</td>
<td>20.2 ± 0.2</td>
<td>18.7 ± 0.4**</td>
</tr>
<tr>
<td>Fat mass (g)</td>
<td>3.6 ± 0.1</td>
<td>5.1 ± 0.2**</td>
</tr>
<tr>
<td>Bone mass (BV/TV)</td>
<td>13.6 ± 0.4</td>
<td>10.2 ± 0.6***</td>
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</tbody>
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**Figure**: Subcutaneous fat weight (mg) and Visceral:subcutaneous fat ratio

**Figure**: Body weight (g) and Age (weeks)

**Figure**: Food intake (g) and Food intake (cumulative) (g)

**Figure**: Cumulative activity (beam breaks × 104)

**Figure**: Heat (kcal/h) and Heat (kcal/kg BW per h)

**Figure**: Relative gene expression (Npy, Agrp, Orexin)

**Figure**: Serum adiponectin (µg/ml)

**Figure**: Total fat pad weight (mg)

**Figure**: Muscle, Skeletal (mg), Heart (mg), Kidney (mg), Lean mass (g), Fat mass (g), Bone mass (BV/TV)

**Figure**: Adipocyte size (µm²)

**Figure**: Journal of Endocrinology

**DOI**: 10.1530/JOE-13-0230

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Food intake measurement

The mice were housed individually for 1 week to get acclimatized to the new environment. Food intake was measured daily for 1 week following acclimatization. For the measurement of food intake following prolonged fasting, the mice were fasted for 24 h, and food intake was measured at the indicated time points.

Osteocalcin concentration measurement

Serum osteocalcin concentrations were measured using ELISA as described previously (Ferron et al. 2010). GLU13 OCN (active form) concentrations were calculated by subtraction of GLA13 OCN (inactive form) concentrations from total OCN concentrations.

Statistical analysis

Results are presented as means ± S.E.M., unless otherwise stated. Data were analyzed using unpaired Student’s t-test. A value of \( P < 0.05 \) was considered to be statistically significant.

Results

Hypogonadotropic hypogonadism and altered hypothalamic KISS1 regulation in NTM

To determine the pathophysiological consequences of neonatal androgenization on reproduction and metabolism in males, we compared littermate control male mice (CM) with male mice exposed to neonatal testosterone (NTM). We used testosterone enanthate to induce prolonged testosterone exposure for 2 weeks. Adult NTM displayed hypogonadotropic hypogonadism compared with CM, with small testes and prostate (Fig. 1A) and decreased serum testosterone, LH, and FSH concentrations. No change was observed in E2 concentrations (Table 1). As expected, NTM were less fertile than CM (Fig. 1B). We had previously shown that NT exposure in female mice suppresses hypothalamic Kiss1 expression to levels observed in CM (Nohara et al. 2011). KISS1 neurons of the hypothalamus are believed to be a major trigger for the onset of puberty (Navarro & Tena-Sempere 2012), and in mice lacking a functional Kiss1 gene hypogonadotropic hypogonadism develops (d’Anglemont de Tassigny et al. 2007) as in NTM. Thus, to explain the central hypogonadism, we quantified hypothalamic Kiss1 expression. As expected, NTM exhibited lower hypothalamic Kiss1 mRNA expression than CM (Fig. 1C). KISS1 neurons of the hypothalamus are also the target of leptin (Tena-Sempere 2006). Ob/Ob mice are leptin deficient and exhibit lower hypothalamic Kiss1 expression, a phenotype that can be rescued by leptin injection (Smith et al. 2006). Therefore, we tested the ability of leptin to upregulate hypothalamic Kiss1 expression in NTM. Under fasting conditions, NTM displayed lower hypothalamic Kiss1 expression than CM (Fig. 1D). Leptin successfully increased hypothalamic Kiss1 expression in CM. However, it failed to upregulate Kiss1 expression in NTM (Fig. 1D).

Altered energy homeostasis and body composition in NTM

NTM displayed an alteration in body composition characterized by lower body weight in early life, followed by accelerated growth leading to normal body weight in adulthood (Fig. 2A). At 44 weeks of age, body weight did not differ between the groups (CM: 32.9 ± 1.1; NTM: 33.5 ± 0.9; mean ± S.E.M.). However, adult NTM had reduced lean mass, including skeletal muscle, heart, kidney, and bone weight, accompanied by increased fat mass (Fig. 2B). Increased fat pad weight was mostly observed in subcutaneous fat depots without parallel increases in visceral fat depots (Fig. 2C). This resulted in...
a relatively decreased index of visceral fat consistent with a feminized fat distribution (Fig. 2D). Although overall adiposity increased in NTM, adipocyte size did not change significantly in NTM compared with that in their CM counterparts (Fig. 2E). Adiponectin concentrations decrease in obesity (Weyer et al. 2001) and in littermate NT female mice (Nohara et al. 2013). Despite higher adiposity, serum adiponectin concentrations were not significantly increased in NTM, consistent with the absence of visceral adiposity and large adipocytes (Fig. 2F).

NTM displayed hypophagia at 5 weeks and a trend toward decreased food intake in adulthood (Fig. 2G and H). Decreased food intake was more apparent in adult NTM following a 24-h fast (Fig. 2I). Consistent with the early decrease in food intake, at 5 weeks, NTM exhibited decreased body weight with reduced lean mass, but no alteration in fat mass (Fig. 2J, K and L). Also consistent with a primary decrease in food intake, the expression of hypothalamic orexigenic neuropeptides Npy, Agrp, and orexin (Fig. 2M) was upregulated, probably as a compensatory mechanism. Conversely, the expression of anorexigenic factors Pomp/Cartpt, Mc4r, and Mch was unaltered (Supplementary Figure 2a, see section on supplementary data given at the end of this article). NTM displayed decreased locomotor activity (Fig. 2N) and lower energy expenditure (Fig. 2O) even when these measures were normalized for total body mass (Fig. 2P). Since the expression of the uncoupling protein 1 (Ucp1) in BAT was normal (Fig. 2Q), the decreased energy expenditure appeared to be a consequence of reduced lean mass and locomotor activity.

Consistent with increased adiposity, in adults, serum leptin concentrations were increased in NTM than in CM (Fig. 3A), suggesting a state of leptin resistance. To evaluate leptin sensitivity in these mice, we carried out an i.p. leptin tolerance test. Consistent with the hyperleptinemia, 1 day after leptin injection, in NTM, leptin failed to decrease food intake and suppress body weight (Fig. 3B and C). The effect of leptin on the level of expression of first-order neuropeptide mRNAs Pomp, Cartpt, Npy, and Agrp was not contributive (Supplementary Figure 2a, see section on supplementary data given at the end of this article).

Normal glucose homeostasis and insulin sensitivity in NTM

Adult NTM exhibited no significant alteration in blood glucose or insulin concentrations in either the fasting or ad libidum conditions (Table 2). They exhibited insulin sensitivity under euglycemic–hyperinsulinemic clamp conditions similar to CM (Table 2). Thus, NTM exhibited
Table 2 Metabolic parameters of glucose metabolism. Values represent means±S.E.M. CM vs NTM. n = 3–27

<table>
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<tr>
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<th>CM</th>
<th>NTM</th>
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<tr>
<td>Fasting glucose (mg/dl)</td>
<td>112.7±5.78</td>
<td>117.8±7.10NS</td>
</tr>
<tr>
<td>Fed glucose (mg/dl)</td>
<td>231.4±8.49</td>
<td>240.0±12.86NS</td>
</tr>
<tr>
<td>Fasting insulin (ng/ml)</td>
<td>0.34±0.02</td>
<td>0.42±0.04NS</td>
</tr>
<tr>
<td>Fed insulin (ng/ml)</td>
<td>2.78±0.30</td>
<td>2.80±0.36NS</td>
</tr>
<tr>
<td>Clamp GiR (mg/kg×min)</td>
<td>86.22±7.05</td>
<td>87.83±7.86NS</td>
</tr>
<tr>
<td>4-month GTT AUC (mg/dl per min×1000)</td>
<td>16.91±1.02</td>
<td>16.45±0.88NS</td>
</tr>
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GTT, glucose tolerance test.

Discussion

In male mammals including humans, the testes produce two perinatal testosterone surges that are critical to masculinize the organism (Corbiet et al. 1992). In this study, we used testosterone enanthate to induce 2 weeks of prolonged nonphysiological testosterone exposure in neonatal male mice. In males, developmental exposure to androgens programs KiSS1 neuron dysfunction that is characterized by lower hypothalamic Kiss1 expression and failure of leptin to stimulate hypothalamic Kiss1 expression. This is associated with hypothalamic hypogonadism with testosterone deficiency and late-onset subcutaneous adiposity without features of insulin resistance.

The importance of kisspeptins in the control of the onset of puberty was initially suggested by the observation that inactivating mutations of their G-protein-coupled receptor GPR54 prevented the onset of puberty in humans and mice (Seminara et al. 2003). In addition, mice without functional Kiss1 develop hypothalamic hypogonadism (d’Anglemont de Tassigny et al. 2007). Furthermore, administration of an antagonist to kisspeptin actions delays puberty in female mice (Pineda et al. 2010). Thus, although we did not assess the timing of puberty in neonatally androgenized male mice, decreased hypothalamic Kiss1 expression is likely to be instrumental in the development of hypothalamic hypogonadism with decreased gonadotropin concentrations, androgen deficiency, and decreased fertility One limitation of our approach is the quantification of Kiss1 mRNA in the whole hypothalamus. In rodents, Kiss1 expression takes place in two populations of hypothalamic neurons in the anteroventral periventricular nucleus (AVPV) and arcuate nucleus (ARC). However, male rodents exhibit dramatically lower levels of Kiss1 expression in the AVPV compared with the females (Kauffman et al. 2007).
Leptin has been considered to be a candidate for the metabolic regulation of Kiss1 neurons (Tena-Sempere et al. 2006). Obese leptin-deficient ob/ob mice are infertile (Chehab et al. 1996). In these mice, leptin replacement increases Kiss1 expression in the ARC (Smith et al. 2006) and restores fertility (Chehab et al. 1996). Neonatally androgenized male mice exhibit decreased hypothalamic Kiss1 expression. In these mice, leptin fails to upregulate Kiss1 expression. This supports the hypothesis that neonatal exposure to excess testosterone has either decreased Kiss1 neuronal cell number or programmed an acquired leptin resistance to stimulate Kiss1 expression, thereby leading to central hypogonadism. The current experiments were not designed to determine whether alteration in Kiss1 expression occurred in Kiss1 or afferent neurons. Indeed, previous evidence suggests that leptin acts directly on Kiss1 neurons – which express leptin receptor (LepR) – to control Kiss1 function (Smith et al. 2006). More recent evidence, however, suggests that the mode of action of leptin on Kiss1 neurons is indirect, supported by the observation that genetic deletion of LepR selectively from hypothalamic Kiss1 neurons in mice has no effect on puberty and fertility (Donato et al. 2011). However, re-expression of LepR in premammillary nucleus neurons in the LepR null mice has been observed to be sufficient to induce puberty. In addition, an unidentified population of LepRb neurons has been identified in close contact with, but afferent to, ARC and anterovertricular Kiss1 neurons (Louis et al. 2011). Thus, neonatal androgenization may also have programmed leptin resistance in Kiss1 afferent neurons.

We had previously observed that NTM exposure in female mice programs alteration of Kiss1 neuronal function, leptin resistance, increased energy intake, and increased visceral adiposity in adults (Nohara et al. 2011, 2013). By contrast, neonatally androgenized adult male mice exhibit decreased energy intake, decreased locomotor activity, and predominant subcutaneous fat deposition. Decreased food intake is probably a primary event, since it is observed early in life, and is associated with a compensatory but inefficient increase in the concentrations of hypothalamic orexigenic neuropeptides without alteration in those of anorexigenic neuropeptides. Decreased lean mass is also present early – at 4 weeks – a period when no fat accumulation is observed. This finding supports the hypothesis that decreases in food intake, lean mass, and locomotor activity are collectively linked to a demasculinization of behavior and appear first. These characteristics then lead to a secondary decrease in energy expenditure that favors the accumulation of subcutaneous fat. It is likely that this phenotype of demasculinization of behavior and body composition is secondary to androgen deficiency. Indeed, a phenotype of disrupted energy homeostasis and fat distribution similar to that of neonatally androgenized male mice is observed in androgen receptor (AR) knockout mice. These mice exhibit reduced spontaneous activity, decreased energy expenditure, and predominant subcutaneous fat accumulation (Sato et al. 2003, Fan et al. 2005). Moreover, male mice lacking DNA-binding-dependent AR signaling exhibit decreased lean mass and food intake with reduced voluntary activity, and they exhibit a predominant increase in subcutaneous fat without accompanying changes in insulin sensitivity. Furthermore, in both cases, adiponectin concentrations are increased in a manner similar to that observed in neonatally androgenized male mice (Fan et al. 2005, Rana et al. 2011). The absence of decreased or increased adiponectin concentrations might help retain insulin sensitivity and glucose homeostasis in NTM as in AR knockout mice.

Neonatally androgenized female mice develop many of the features of the metabolic syndrome observed in women with PCOS (Nohara et al. 2011, 2013). These features include increased food intake and lean mass, visceral adiposity with enlarged adipocytes, hypoadiponectinemia, decreased osteocalcin activity, insulin resistance, pre-diabetes, and hypertension. By contrast, littermate male mice develop a mild metabolic phenotype with decreased lean mass and food intake and subcutaneous adiposity without cardiometabolic alterations. This observation underscores the potential for sex differences in metabolic diseases arising from the complement of sex-linked genes outside the testis-determining gene Sry.

This study has clinical relevance on a number of levels. A recent study has found significant androgen activity in 35% of water sources in several states of the US, suggesting that, at least in certain areas, there is a high risk for human exposure to androgens that may result in alterations of the fetal or neonatal environment (Stavreva et al. 2012). This relatively widespread androgen contamination from pharmaceutical and other sources is of increasing concern. There is a need for in vivo models to test the pathophysiological relevance of such contamination. The model explored here – neonatal exposure to androgens at supraphysiological doses in male mice – represents a novel tool that may be of potential value in testing for clinically relevant water contamination in in vivo models.


Supplementary data
This is linked to the online version of the paper at http://dx.doi.org/10.1530/JOE-13-0230.

Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

Funding
F M-J received research support from Pfizer, Inc. All other authors declare no competing financial interests. This work was supported by grants from the National Institutes of Health (PS0 HD044405, RO1 DK074970-01), the American Heart Association (11IRG5570010), and the March of Dimes (6-FY07-678).

Author contribution statement
K N and F M-J designed the experiments; K N, S L, M S M, A W, and M F carried out the experiments; K N, G K, R B, and F M-J analyzed the data; K N and F M-J wrote the article.

Acknowledgements
The authors thank the cores of the University of Virginia Center for Research in Reproduction (NICHD grant U54-HD288934) for measurements of gonadal hormones, the Rodent Metabolic Phenotyping Core of Northwestern Comprehensive Center on Obesity for measurements of body composition, and the Seattle Mouse Metabolic Phenotype Core (MMPC grant U24-DK076126) for measurements of energy expenditure.

References


Chehab FF, Lim ME & Lu R 1996 Correlation of the sterility defect in homozygous obese female mice with treatment by the human recombinant leptin. Nature Genetics12 318–320. (doi:10.1038/ng0396-318)


Research

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219:3 268

Received in final form 27 August 2013
Accepted 1 October 2013
Accepted Preprint published online 1 October 2013


