Emerging evidence supports a developmental origin for the metabolic syndrome in the context of polycystic ovary syndrome (PCOS) in which the fetal environment programs both reproductive and metabolic abnormalities that will occur in adulthood. To explore the role of developmental androgen excess in programming metabolic dysfunction in adulthood, we reported a mouse model system in which neonates were androgenized with testosterone. We compared female mice with neonatal exposure to testosterone (NTF) with control females (CF), control males (CM), and male mice with neonatal testosterone exposure (NTM). NTF develop many of the features of metabolic syndrome observed in women with PCOS. These features include increased food intake and lean mass, visceral adiposity with enlarged adipocytes, hypoadiponectinemia, decreased osteocalcin activity, insulin resistance, pre-diabetes, and hypertension. NTF also develop a novel form of leptin resistance independent of STAT3.

In contrast, littermate NTM develop a phenotype of hypogonadotropic hypogonadism with decreased lean mass and food intake. These NTM mice exhibit subcutaneous adiposity without cardiometabolic alterations. We discuss the relevance of this mouse model of developmental androgenization to the metabolic syndrome and its clinical implications to human metabolic diseases.

The intrauterine environment in which the mammalian fetus develops plays an important role in programming the function of its physiological systems in adult life as well as in the development of adult diseases. There is emerging evidence for a developmental origin of obesity in which the fetal environment programs metabolic abnormalities that appear in adulthood. The polycystic ovary syndrome (PCOS), the most common endocrine disorder of premenopausal women, shares the same cluster of features that induce the metabolic syndrome (Mets). These include visceral obesity, insulin resistance, glucose intolerance, and hypertension. Thus, many consider PCOS as a subtype of Mets with added reproductive abnormalities in women. Similarly, PCOS and the MetS are believed to share a developmental origin in which maternal and fetal androgen excess during pregnancy programs both reproductive and metabolic abnormalities in the offspring.

To explore the role of androgen in programming metabolic dysfunction, we used mice neonatally androgenized with testosterone. We exposed littermate male and female C57BL/6 mice to testosterone (T) or vehicle at neonatal days 1 and 2 by subcutaneous injection (NT). When these mice matured, we compared female mice exposed to neonatal testosterone (NTF) with control females (CF), control males (CM), and male mice exposed to neonatal testosterone (NTM). Importantly, littermate males and females were androgenized at the same time, thereby facilitating comparison of their phenotypes in adulthood.

NTF displayed masculinization of lean tissues with increased cardiac, skeletal muscle, and kidney masses. NTF mice
showed increased and dysfunctional white adipose tissue with an increased number of enlarged and insulin resistant adipocytes as well as hypoadiponectinemia. They also displayed bone dysfunction with decreased undercarboxylated and active osteocalcin. In contrast, littermate NTM displayed an early reduction in lean mass and had a delayed increase in subcutaneous white adipose tissue without accompanying decreases in adiponectin or active osteocalcin. They also showed decreased locomotor activity and energy expenditure that could be related to decreased lean mass.

NTF displayed adipose tissue hyperplasia that demonstrated more cells in the adipose depots were committed to the adipocyte lineage. However, classical genetic markers of adipose differentiation (CEBPs and PPARy) were not increased. Like muscle and bone, adipose tissue is mesodermal in origin. In mice, adipose tissue development occurs during the first two weeks of neonatal life. The observation that developmental exposure to testosterone programed WAT, muscle, and bone suggested a possible effect of testosterone on mesenchymal stem cells. Indeed, we observed that in NTF, expression of developmental genes involved in depot-specific identity was altered in SC adipose tissue. Specifically, mRNA expression the homeobox gene HoxA5, the cell surface proteoglycan Gpc4, and the transcription factor Tbx15 were all increased. High levels of Tbx15 and Gpc4 expression in human SC adipose tissue are markers of visceral fat accumulation.

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.

NTF also exhibited increased sympathetic tone to SC and perigonadal fat. It is still unknown, however, whether the origin of this increased fat sympathetic tone is central or peripheral. Indeed, in mice, activation of central sympathetic efferent to adipose tissue is known to reduce cell numbers and decrease both lipogenesis and fat mass. Since we observed increased sympathetic tone in WAT that was accompanied by decreased lipogenesis and increased adipose tissue mass and cell number, the increased sympathetic tone may not be causative of the adiposity. Rather, it may reflect an adaptive mechanism that counters chronic fat accumulation.

NTF exhibited dysfunctional regulation of energy homeostasis. First, they exhibited decreased oxygen consumption and energy expenditure, which clearly favored adipose tissue accumulation. Second, NTF displayed reduced POMC expression and decreased intensity of neuronal projections from POMC neurons within the ARC that led to the increased food intake. The pro-opiomelanocortin (POMC) neurons of the hypothalamic arcuate nucleus (ARC) are critical to suppress energy intake and, to a large extent, POMC innervation of hypothalamic feeding circuits develops neonatally. NTF also exhibited hyperleptinemia and decreased ability of leptin to upregulate POMC, suppress food intake and prevent adipose tissue accumulation. These observations were independent of signal transducer and activator of transcription 3.

Unlike NTF, adult littermate NTM did not show alterations of the melanocortin system. Rather, they displayed hypogonadotropic hypogonadism with decreased serum testosterone and gonadotropins. Hypothalamic Kiss1 neurons are believed to be critical in the onset of puberty and are a target of leptin. Adult NTM showed lower hypothalamic Kiss1 expression and failure of leptin 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 in males.

Overall, NTF develop many of the features of metabolic syndrome observed in women with PCOS. These features include increased food intake and lean mass, visceral adiposity with enlarged adipocytes, hypoadiponectinemia, decreased osteocalcin activity, insulin resistance, pre-diabetes, and hypertension. In contrast, littermate male mice develop a mild metabolic phenotype with decreased lean mass and food intake and SC adiposity without cardiometabolic alterations.

This sex dimorphism underscores the potential for sex differences in metabolic diseases arising not from sex hormones but rather, from sex chromosomes, especially from the complements of sex-linked genes outside the testis-determining gene Sry.

A key question that arises from this work is whether the mouse is an appropriate model of developmental androgenization. We believe it is since this model of neonatal androgenization with testosterone is widely considered an established paradigm for centrally programmed masculinization of sexual behavior and reproductive physiology. Although testosterone is used at supraphysiological doses, it is an experimental feature that exaggerates the programming effect of the steroid. The NT mouse is also a reference model that facilitates study of energy homeostasis and adipose tissue programming. Human and non-human primates are both precocial species that give birth to mature young. In humans and primates, synaptogenesis of hypothalamic centers controlling body adiposity and peripheral adipose tissue development occur during the second trimester of pregnancy. Conversely, the mouse is an altricial species that gives birth to immature young. In mice, developmental plasticity of hypothalamic circuits controlling adiposity and peripheral adipose tissue development occur during the first two weeks of neonatal life. Thus, with regard to programming the hypothalamic and peripheral control adipose tissue, the mouse first week of neonatal life parallels human fetal development during the second trimester of pregnancy (Fig. 1). This additional window of neonatal developmental plasticity in the mouse provides an experimental advantage that allows manipulation of the neonatal sex steroid milieu in the presence or absence of androgen receptor (AR) or/and estrogen receptors (ER). This approach has a direct effect on the offspring while remaining comparable to human pregnancy. Furthermore, there are several other arguments supporting the relevance of the neonatal hyperandrogenism mouse model to human and non-human primates. First, women with prenatal androgen excess because of adrenal hyperplasia or virilizing tumors develop PCOS like reproductive symptoms as well as central obesity and insulin resistance in adults despite normalization of androgen excess with treatment. Similarly, female rhesus monkeys exposed to prenatal androgen excess manifest a predominant abdominal.
visceral fat accumulation during adulthood, independent of obesity, reflecting a masculinized pattern of fat accumulation.25 Importantly, NT exposure in female rats increases visceral adiposity distribution in adults24,25 and this is true in mice, as noted above.6 Second, in humans, females from opposite sex twin pairs exposed to prenatal testosterone from testes of a male co-twin develop masculinized eating behaviors as adults,26 a finding we observed in our mice.8 Thus, perinatal, transient, and moderate androgen excess in female humans, primates, and rodents reprograms their genetic predisposition to obesity and MetS in adulthood in a similar manner.

This study has clinical relevance on a number of levels. Over the last decade the prevalence of obesity has increased more in women than in men.27 Furthermore, the prevalence of visceral obesity among people with a MetS is two to ten times higher in women worldwide.27-32 This female predominance in visceral obesity is observed in all races, across all age groups and in both urban and rural areas. In particular, since a visceral fat distribution is considered a male pattern, the increased prevalence of visceral obesity in women suggests a masculinization of adipose tissue distribution.3 Thus, the issue of developmental androgenization is of critical importance and warrants greater attention because of increasing human exposure to environmental factors that interact with androgen and estrogen receptor systems.33,34 For example, a recent study found significant androgen activity in 35% of water sources from several states in 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.35 This relatively widespread androgen contamination from pharmaceutical and other sources is of increasing concern. There is a need for in vivo models to understand the pathological relevance of this contamination. The model explored here—neonatal exposure to androgen at supraphysiological doses in mice—represents a useful tool that may be of potential value in testing for clinically relevant water contamination in in vivo models.

Another unexplored aspect of this model is the potential effect of perinatal androgenization on the gut microbiome of the offspring. The gut microbiome has recently emerged as a critical factor in fat storage and the development of obesity.36,37 Estrogen and androgen affect the innate immune system that is expressed in the gut mucosa and that helps defend against infection.38 In addition, bacteria from human microbiota play an important role in the metabolism of sex hormones like estrogen and androgen.39 Therefore, perinatal nutritional exposure to substances interacting with androgen or estrogen receptors from food or environmental disruptors could also program malfunction of the innate immune system, change the microbiome and the metabolism of sex steroids with indirect effect on programming metabolism in adulthood. Further studies are needed to address this issue.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.
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