HHS Public Access

Author manuscript

Mol Cell Endocrinol. Author manuscript; available in PMC 2019 April 15.

Published in final edited form as:

Mol Cell Endocrinol. 2018 April 15; 465: 92–102. doi:10.1016/j.mce.2017.09.001.

The impact of androgen actions in neurons on metabolic health and disease

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Abstract

The male hormone testosterone exerts different effects on glucose and energy homeostasis in males and females. Testosterone deficiency predisposes males to visceral obesity, insulin resistance and type 2 diabetes. However, testosterone excess predisposes females to similar metabolic dysfunction. Here, we review the effects of testosterone actions in the central nervous system on metabolic function in males and females. In particular, we highlight changes within the hypothalamus that control glucose and energy homeostasis. We distinguish the organizational effects of testosterone in the programming of neural circuitry during development from the activational effects of testosterone during adulthood. Finally, we explore potential sites where androgen might be acting to impact metabolism within the central nervous system.

Keywords

Androgen Receptor (AR); Sex Differences; Testosterone; Hypothalamus; Central Nervous System (CNS); Metabolism; Obesity; Insulin Resistance; Polycystic Ovarian Syndrome (PCOS); Arcuate Nucleus (ARC)

1. Introduction

Understanding sex differences in glucose and energy homeostasis is fundamental for the development of precision medicine for the treatment of metabolic disorders. One of the most striking sexually dimorphic aspects of metabolic homeostasis concerns the differential effects of testosterone on glucose and energy homeostasis in males and females (Escobar-Morreale et al., 2014; Navarro et al., 2015). In men, testosterone deficiency produces visceral obesity and insulin resistance and increases the risk for type 2 diabetes (T2D) (Ding et al., 2006; Escobar-Morreale et al., 2014; Navarro et al., 2015; Zitzmann et al., 2006,

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Zitzmann, 2009). In women with polycystic ovary syndrome (PCOS), testosterone excess impairs insulin action, favors adiposity, and increases risk for T2D (Ding et al., 2006; Goodman et al., 2015; Legro et al., 1999; Mater et al., 2015; Phillips et al., 2000). Considering that a significant number of men experience testosterone deficiency and up to 10% of reproductive-aged women exhibit testosterone excess, it is important to understand the mechanisms through which testosterone differentially impacts metabolism in men and women (Azziz et al., 2004; Legro et al., 1999; Mulligan et al., 2006; Sam, 2007).

Sex differences in physiology result from genetic differences in sex chromosomes which produces differences in concentrations of circulating sex hormones that act during development and adulthood (Wallen, 2009). During development, the testis-determining *Sry* gene on the Y chromosome causes the development of testes in males. This sets up a lifelong difference in the blood concentrations of sex steroids between males and females, which are critical factors that contribute to the development of sex differences in tissues (Arnold, 2017). Although sex steroids are critical in determining sex differences in many tissues including the brain, the sex chromosomes themselves also play a role (Arnold, 2017). For example, the *Sry* gene itself affects sexual differentiation in the brain (Arnold, 2017). However, this review will focus on the hormone testosterone. During the development of male mammals, the testes produce a testosterone surge (prenatal in humans and primates and neonatal in rodents) that masculinizes the reproductive tract and the organization of neural circuits that will activate male behavior at puberty. There are critical windows during which hormones such as testosterone program the organization of the brain, the results of which persist even in the absence of hormone (Arnold, 2017; Morris et al, 2004; Simerly, 2002; Wallen, 2009).

The critical periods for the development of the hypothalamus, the area of the brain most directly involved in the regulation of metabolic homeostasis, differ between rodents and primates (Abramovich, 1974; Bouret et al., 2004; Bouret et al., 2012; Corbier et al., 1992; Koutcherov et al., 2002; Morris et al., 2004; Simerly, 2002; Wallen, 2009). Although hypothalamic neurogenesis occurs during prenatal life in rodents, synaptogenesis within the hypothalamus and development of peripheral adipose tissue occur during neonatal days 1–14 (Bouret et al., 2004; Bouret et al., 2012; Corbier et al., 1992). Therefore prenatal androgenization of rodents could potentially alter the number of a given type of hypothalamic neuron, but would not affect hypothalamic circuit formation. In primates and other precocial species such as sheep, neurogenesis occurs during the first trimester of pregnancy, while synaptogenesis and development of adipose tissue occur during the second trimester. (Abramovich, 1974; Bouret et al., 2012; Koutcherov et al., 2002). Therefore, androgens would need to be administered prenatally in these precocial species to exert effects on the organization of the hypothalamus. Despite these timing differences, there are many similarities in the development and organization of human and rodent hypothalami, making the rodent a reliable model for the exploration of metabolic regulation by testosterone (Altman and Bayer, 1986; Enderlin et al., 1987). During adulthood, hormones act transiently on circuitry that was formed during development to elicit behavioral and/or physiological responses (Arnold, 2017; Morris et al, 2004; Simerly, 2002; Wallen, 2009). This review will focus on how testosterone action in the central nervous system (CNS)

differentially affects metabolic homeostasis in men and women during development and adulthood.

2. Androgen receptor expression in the brain

The enzyme 5-α-reductase converts testosterone to its more active metabolite dihydrotestosterone (DHT) which exerts its actions via the androgen receptor (AR) (Celotti et al., 1992; Colciago et al., 2009; Thigpen et al., 1993; Torres and Ortega, 2003). Testosterone itself can also act at the AR, but it is a weaker agonist than DHT (Bhasin et al., 2006). Testosterone also acts as a reservoir for estrogens as the enzyme aromatase can convert testosterone to the estrogen estradiol (E2) which exerts its actions via estrogen receptors (ERs) (Celotti et al., 1992; Colciago et al., 2009; Garcia-Segura, 2008). Both enzymes are expressed in the brain and therefore the effect of testosterone in the brain can result from actions at either AR or ERs (Celotti et al., 1992; Colciago et al., 2009; Garcia-Segura, 2008; Thigpen et al., 1993; Torres and Ortega, 2003). The AR is present in several nuclei of the hypothalamus, a region of the brain known to regulate glucose and energy homeostasis. During development (up to 15 days post-birth), female and male mice show similar levels of AR expression in the arcuate nucleus (ARC), ventromedial hypothalamus (VMH), anteroventral periventricular nucleus (AVPV), and medial preoptic nucleus (MPN) (Brock et al., 2015). In adults, males have higher levels of AR expression in all (reported) regions of the brain (Brock et al., 2015; Lu et al., 1999; Shah et al., 2004; Simerly et al., 1990). Outside of the hypothalamus, males exhibit robust expression of the AR in the medial amygdala (MeA), lateral septum (LS) and bed nucleus of the stria terminalis (BNST) (Brock et al., 2015; Lu et al., 1999; Shah et al., 2004; Simerly, 1990). While AR expression is higher in males, AR expression in the female brain is still fairly strong in both the hypothalamic and extra-hypothalamic nuclei noted above (Lu et al., 1999; Shah et al., 2004; Simerly, 1990). Although the extra-hypothalamic sites mentioned here are traditionally known to be involved in reproduction, recent findings indicate they may also be involved in some aspects of metabolic regulation (Hu et al., 2016; Liu et al., 2013; Meek et al., 2016; Terrill et al., 2016). Within the hypothalamus, the AR is also expressed in the dorsomedial hypothalamus (DMH), paraventricular nucleus (PVN), suprachiasmatic nucleus (SCN), and lateral hypothalamus (LH) of males, with very little expression in females (Simerly et al., 1990; Fan et al., 2008). The sites discussed here, some of which will be explored in further detail later in the review, are all potential areas where androgens could be acting to impact metabolic homeostasis in males and females.

3. The organizational effects of androgens during development predispose females to obesity and type 2 diabetes

Androgen excess and anovulation characterize PCOS, a common endocrine disorder that affects reproductive-aged females (Ehrmann, 2005). Prenatal androgen excess in monkeys and sheep programs the development of a PCOS-like phenotype in female offspring, supporting the hypothesis that PCOS results at least partially from the programming effect of prenatal androgen excess (Abbot et al., 2002; Cardoso et al., 2015; Dumesic et al., 2007). In the U.S., 80% of women with PCOS are obese (Sam, 2007). Moreover, many women with

PCOS display insulin resistance and glucose intolerance (Dunaif et al., 1989; Ehrmann et al., 1999; Sahin et al., 2014). Women who have been exposed to androgen excess prenatally due to virilizing tumors or adrenal hyperplasia develop obesity and insulin resistance during adulthood, despite the normalization of androgen levels by treatment with anti-androgens (Barnes et al., 1994; Hague et al., 1990). This supports the idea that prenatal androgen excess in female fetuses programs metabolic dysfunction that will later develop in adulthood. In fact, models of prenatal androgen excess in monkeys and sheep have been used to validate the paradigm that prenatal testosterone excess predisposes adult female offspring to obesity, insulin resistance, and glucose intolerance (Abbot et al., 1998; De Haan et al., 1990; Hansen et al., 1995; Eisner et al., 2000; Padmanabhan et al., 2010; Recabarren et al., 2005;). Models of neonatal androgen excess in rodents also support the hypothesis that testosterone excess during development predisposes females to obesity, insulin resistance, and glucose intolerance during adulthood (Alexanderson et al., 2007; Nilsson et al., 1998; Nohara et al., 2011, 2013a). The difference in the timing of androgen exposure between monkeys and sheep versus rodents corresponds to the difference in the timing of hypothalamic circuitry formation in these species (Bouret et al., 2004; Bouret et al., 2012; Corbier et al., 1992).

Developmental androgen excess clearly impacts energy balance. Moreover, evidence suggests that the effects of testosterone excess during development are due at least in part to androgen acting in the CNS. For example, women with PCOS display sympathetic hyperactivity, which could contribute to the development of obesity (Lansdown and Rees, 2012). Interestingly, neonatal testosterone excess in female mice also programs sympathetic hyperactivity and obesity during adulthood (Nohara et al., 2013a). These mice exhibit reduced energy expenditure, increased fat mass and increased norepinephrine turnover in white adipose tissue, a marker of sympathetic activity (Nohara et al., 2013a). These results suggest that neonatal testosterone exposure programs the enhanced sympathetic activity that predisposes female mice to the development of obesity (Nohara et al., 2013a). From these results alone, it is unclear if testosterone programs sympathetic activity via action at ARs after conversion to DHT, or via action at ERs after conversion to E2. However, neonatal exposure to E2 increases fat mass in adult female mice, while neonatal exposure to DHT does not (Nohara et al., 2011). This suggests that neonatal testosterone excess predisposes female mice to obesity via aromatization into E2. This conclusion is supported by a study in a rat model of PCOS in which females are exposed neonatally to a long-acting estrogen, producing enhanced sympathetic output that precedes the development of other PCOS characteristics (Lara et al., 1993). Nevertheless, female neuronal ARKO mice exposed to DHT prepubertally do not develop obesity and dyslipidemia while littermate controls exposed to DHT during the same period do, suggesting a role for the neuronal AR in the development of obesity and leptin resistance in females (Caldwell et al., 2017). In these mice, exon 3, which contains the DNA binding domain of the AR, is knocked out, suggesting that nuclear actions of the AR mediate these effects (Caldwell et al., 2017). Perhaps activation of ERs in the neonatal female mouse brain is responsible for the programming of sympathetic hyperactivity that may predispose females to obesity, while activation of ARs in the prepubertal female mouse brain is responsible for the programming of the hypothalamus that predisposes them to obesity. Indeed, these mice were administered

DHT at 3 weeks of age, just as puberty is starting to commence (Caldwell et al., 2017). Puberty is beginning to be recognized as an additional critical period where hormones exert organizational effects on the brain, so E2 could be acting during the neonatal critical period, while DHT could be acting during the pubertal critical period (Romeo, 2003). Still neonatal DHT does exert lasting effects in the CNS. Indeed, neonatal exposure to DHT increases food intake in adult female mice (Nohara et al., 2011). This increased food intake is accompanied by decreased expression of the anorexigenic peptide pro-opiomelanocortin (POMC) in the ARC and the fibers projecting from the ARC (Nohara et al., 2011). This appears to be a masculinization of the melanocortin system that regulates feeding behavior, as food intake and POMC expression in females exposed neonatally to DHT were similar to that of littermate males (Nohara et al., 2011). These results suggest that testosterone is converted to DHT in the brain, which acts at the AR to program a male pattern of feeding behavior by reducing POMC expression. Similarly, human females from opposite-sex twin pairs, who are exposed to prenatal testosterone from the testes of the male twin, exhibit male-like eating behavior as adults (Culbert et al., 2008). However, we do not know if this is the result of action at ARs or ERs in humans. Together, these results suggest that testosterone acts in the brain to program male-like feeding behavior during female development in both rodents and humans.

A number of studies in various species demonstrate that developmental testosterone excess causes insulin resistance in female mammals (Abbot et al., 1998; Alexanderson et al., 2007; Eisner et al., 2000; Nilsson et al., 1998; Padmanabhan et al., 2010; Recabarren et al., 2005). In female rats, neonatal exposure to either testosterone or DHT leads to insulin resistance, suggesting that the AR is involved (Alexanderson et al., 2007; Nilsson et al., 1998). Whether testosterone action at central AR is involved in programming insulin resistance in rodents is unknown. Interestingly, prenatal androgen excess predisposes female mice to impaired glucose tolerance and reduced glucose-stimulated insulin secretion (GSIS), but not to insulin resistance (Roland et al., 2010). These results highlight the importance of the timing of the DHT administration during development. The authors show that DHT acts directly on primary islets of female mice to reduce GSIS, thereby suggesting that the effects of prenatal androgenization in mice are due to action in the pancreas (Roland et al., 2010). Although hypothalamic neurocircuitry in mice is thought to start developing after birth, a recent publication challenges this concept. (Bouret et al., 2004; Corbier et al., 1992;). Indeed, female mice treated on prenatal days 16–18 with DHT have enhanced GABAergic synaptic input onto GnRH neurons of the medial septum, rostral preoptic area, and anterior hypothalamus (Moore et al., 2015). The authors show that this GABAergic input originates from the ARC, suggesting that prenatal androgen does in fact program hypothalamic neurocircuitry in mice. Moreover, this programming occurs in a nucleus known to modulate metabolic homeostasis. Thus, future studies are needed to explore the extent to which these DHT-induced changes in ARC neurocircuitry impact metabolic homeostasis. Nevertheless, the results of this study suggest that the critical period for hypothalamic circuitry development begins earlier in development than is traditionally thought (Bouret et al., 2004; Campbell and Heberison, 2014; Moore et al., 2015). Alternatively, the prenatal DHT treatment could have resulted in elevated DHT levels during the postnatal period, which could be responsible for the changes in neurocircuitry. In ewes, prenatal androgen excess

also impacts the pancreas. Unlike in mice, prenatal testosterone excess leads to increased GSIS, increased insulin secretion during a glucose tolerance test, and an increased number of β-cells in adult ewes (Rae et al., 2013; Ramaswamy et al., 2016). Moreover, the number of β-cells was already increased at day 90 of gestation, suggesting that testosterone programmed an increase in β-cell number during gestation that led to increased insulin secretion later in life (Ramaswamy et al., 2016). It is unclear if this difference between sheep and mice reflects a true species difference or if it is due to the fact that the mice were exposed to prenatal DHT while the sheep were exposed to prenatal testosterone since both fetal levels of testosterone and estradiol were elevated in the sheep. However, prenatal treatment with the non-steroidal synthetic ER agonist, diethylstilbestrol, did not alter β-cell number or insulin secretion, suggesting that these effects are due to action at the AR (Ramaswamy et al., 2016). This suggests that the differences between the effects of prenatal androgen exposure in sheep and mice are indeed due to species differences in the effects of prenatal AR activation.

Although it is clear that prenatal androgen excess impacts the pancreas, we cannot rule out the likelihood that prenatal androgen excess also programs the hypothalamus to alter insulin sensitivity. In ewes and rhesus monkeys, the hypothalamus develops during the prenatal period, so prenatal androgen could potentially influence the development of hypothalamic circuitry to alter control of peripheral insulin sensitivity (Bouret et al., 2012). Prenatal exposure to testosterone leads to insulin resistance in rhesus monkeys (Abbot et al., 1998; Eisner et al., 2000). However, it is unclear if this effect is due to conversion of testosterone to DHT which acts at ARs, or to E2 which acts at ERs. In ewes, prenatal DHT does cause insulin resistance, suggesting that the AR is involved (Padmanabhan et al., 2010; Recabarren et al., 2005). Despite a number of studies showing that prenatal androgenization causes insulin resistance, the extent to which AR in CNS neurons is involved in this effect is unknown. Nevertheless, it is clear that prenatal androgen excess alters gene expression and connectivity in hypothalamic neurons known to be involved in the regulation of glucose and energy homeostasis. Female ewes exposed prenatally to DHT exhibit an increased number of neurons expressing the orexigenic peptide agouti-related peptide (AgRP), with no change in the number of neurons expressing POMC (Sheppard et al., 2011). This is in contrast to DHT-treated female mice, who show reduced POMC expression (Nohara et al., 2011). This demonstrates another species difference in the way developmental testosterone programs hypothalamic dysfunction. In the ewe, prenatal testosterone also increased AgRP projections to the DMH, PVN, LH, and MPN. This effect was blocked by the AR antagonist flutamide, suggesting that the increase in AgRP projections was due to action at the AR (Sheppard et al., 2011). Interestingly, prenatal testosterone treatment in ewes reduced colocalization between the insulin receptor and AgRP in an AR-dependent manner (Cernea et al., 2016). Since mice lacking the insulin receptor from AgRP neurons have impaired suppression of hepatic glucose production (Könner et al., 2007), one can speculate that reduced insulin receptor expression in AgRP neurons would produce central insulin resistance that would impair suppression of hepatic glucose production. Perhaps the increase in AgRP neuron number and projections to hypothalamic nuclei involved in glucose homeostasis is an attempt to compensate for the reduced expression of the insulin receptor in AgRP neurons that results from DHT exposure. Therefore, developmental testosterone excess could be

causing hepatic insulin resistance in the adult ewe by programming AgRP neurons. However, this hypothesis has yet to be tested. The metabolic phenotypes associated with developmental androgen excess in the different species discussed in this section are summarized in Table 1. The metabolic phenotypes associated with androgen programming the female hypothalamus are highlighted in Figure 1.

4. Androgen actions during development program male metabolism

Somewhat surprisingly, neonatal DHT exposure leads to decreased food intake and decreased locomotor activity in adult male mice (Nohara et al., 2013b). This is accompanied by a compensatory increase in hypothalamic expression of the orexigenic peptides AgRP, NPY, and orexin that is nevertheless insufficient to normalize food intake. This then leads to a secondary reduction in energy expenditure and an accumulation of subcutaneous fat (Nohara et al., 2013b). These mice also display leptin resistance. Androgen is likely acting centrally to induce leptin resistance, because leptin fails to upregulate hypothalamic Kiss1 expression in these mice (Nohara et al., 2013b). These results demonstrate that excess DHT acts in the developing male brain to alter hypothalamic circuitry involved in energy homeostasis, resulting in decreased food intake, decreased energy expenditure, and increased subcutaneous fat during adulthood. These results demonstrate that too much neonatal androgen in the CNS can have a detrimental effect in males. Additionally, treatment of neonatal male rats with DHT causes mild hyperglycemia, but it is not known if DHT acts centrally or peripherally to elicit this effect (Lazic et al., 2011). Nevertheless, physiological levels of androgen during development are needed to properly program the hypothalamic circuitry that controls energy homeostasis.

Physiological levels of neonatal androgens in male mice program the activity of Kiss1 neurons that regulate POMC and AgRP neuronal activity (Nestor et al., 2016). To demonstrate this, the authors used castrated or intact males expressing Channel rhodopsin in YFP-labelled Kiss1 neurons. The authors used light to activate these Kiss1 neurons and record the activity of downstream neurons, which could later be characterized as AgRP or POMC neurons. Using this technique, they demonstrated that Kiss1 neurons form monosynaptic glutamatergic connections with both AgRP and POMC neurons. Through action at different metabotropic glutamate receptors, glutamate released from Kiss1 neurons excites POMC neurons and inhibits AgRP neurons (Nestor et al., 2016). Kiss1 neurons of castrated males showed increased expression of the vesicular glutamate transporter, suggesting increased glutamate release (Nestor et al., 2016). This was confirmed by a decrease in the paired pulse ratio in castrated males. Therefore, castrated males lacking testosterone have increased excitation of POMC neurons and decreased excitation of AgRP neurons compared to intact males. These results suggest that testosterone programs Kiss1 neurons of the ARC to release less glutamate. This is in agreement with the idea that testosterone inhibits ARC Kiss1 neurons (Smith et al., 2005; Navarro et al., 2011). Because Kiss1 neurons express both ARs and ERs, it is unclear whether Kiss1 neurons are programmed by testosterone converted to E2 acting at ERs, or to DHT acting at ARs (Smith et al., 2005). Nevertheless, these results give direct evidence that testosterone programs the neural circuitry controlling glucose and energy homeostasis in males.

5. The effects of androgens in the CNS during adulthood on obesity and type 2 diabetes

Androgen deficiency predisposes men to obesity and T2D (Ding et al., 2006; Escobar-Morreale et al., 2014; Navarro et al., 2015; Oh et al., 2012; Zitzmann et al., 2009; Zitzmann et al., 2006). Indeed, men with prostate cancer who undergo androgen deprivation therapy have an increased risk for the development of T2D and obesity (Keating et al., 2006; Navarro et al., 2015; Zitzmann et al., 2009). Animal models demonstrate that a lack of androgen signaling in males causes obesity, insulin resistance, and glucose intolerance (Fan et al., 2005, 2008; Fernando et al., 2010; Holmang and Bjorntorp, 1992; Lin et al., 2008, 2005; Navarro et al., 2016; Yu et al., 2013). In particular, testosterone action in liver, muscle, and insulin producing beta-cells is essential for glucose and energy homeostasis in males (Fernando et al., 2010; Lin et al., 2008; Navarro et al., 2016). There is also evidence that testosterone action in the brain contributes to metabolic homeostasis at least in part via AR (Fan et al., 2008; Yu et al., 2013). In humans, males with a transcriptional variant of the AR that has low activity display obesity and hyperinsulinemia (Zitzmann et al., 2003). However, in men undergoing testosterone replacement therapy, inhibition of aromatase, the enzyme that converts testosterone to E2, prevented testosterone-induced fat loss, suggesting that the ER is also involved (Finkelstein et al., 2013). To better understand the role of central AR in the control of glucose and energy homeostasis, we must turn to knockout models. Unfortunately, many of these models involve a knockout in which the AR gene is eliminated during development, with the absence of the gene persisting throughout life. This prevents one from distinguishing the organizational and activational effects of androgens. Therefore, one cannot determine if a given phenotype is due to the absence of AR signaling during development or during adulthood.

The absence of AR in male mice disrupts energy homeostasis, leading to late-onset obesity (Fan et al., 2005; Yu et al., 2013). Global AR knockout (ARKO) male mice display late-onset obesity that results from decreased energy expenditure earlier in life (Fan et al., 2005). Neuronal AR knockout (NARKO) male mice also display late-onset obesity (Yu et al., 2013). Although energy expenditure was not assessed in NARKO mice, data from global ARKO mice suggest that obesity results from the combination of reduced metabolic rate, reduced locomotor activity, and reduced thermogenesis, leading to reduced overall energy expenditure (Fan et al., 2005). Male NARKO mice also display ectopic fat accumulation in liver, which could be secondary to the reduced energy expenditure (Yu et al., 2013).

The absence of AR signaling in males also produces central leptin resistance (Fan et al., 2008). Global ARKO male mice exhibit a failure of centrally administered leptin to reduce food intake and body weight (Fan et al., 2008). This is accompanied by a failure of leptin to properly activate signal transducer and activator of transcription 3 (STAT3), a signaling molecule downstream of the leptin receptor that is critical for leptin's metabolic actions, in the ARC and VMH. In an in vitro model, the authors show that AR activation enhances leptin-induced STAT3 activation. The AR and the leptin receptor are co-expressed in several hypothalamic nuclei involved in metabolic homeostasis, including the SCN, ARC, DMH, VMH, PVN, LH, and PMN (Fan et al., 2008). However, it is most likely that androgen is

failing to signal in leptin receptor-expressing neurons of the ARC or VMH in the global ARKO male mice because leptin fails to cause STAT3 activation in these nuclei (Fan et al., 2008). Nevertheless, the possibility that androgen is acting in neurons upstream of the leptin receptor-expressing neurons cannot be ruled out.

The absence of central AR signaling in males disrupts glucose homeostasis, leading to late-onset insulin resistance and glucose intolerance (Yu et al., 2013). As mentioned above, the absence of AR signaling in liver, muscle, and β-cells produces insulin resistance and glucose intolerance in early adulthood (Fernando et al., 2010; Lin et al., 2008; Navarro et al., 2016). NARKO male mice do not show insulin resistance and glucose intolerance until 36 weeks of age (Yu et al., 2013). These mice display elevated liver phosphoenolpyruvate carboxykinase 1 (PCK1) mRNA, suggestive of enhanced gluconeogenesis and hepatic insulin resistance (Yu et al., 2013). This apparent liver insulin resistance could result from hypothalamic insulin resistance, as intravenous insulin failed to activate AKT in NARKO male hypothalami (Yu et al., 2013). In addition, DHT inhibits nuclear factor-κB (NFκB) and protein-tyrosine phosphatase 1B (PTP1B), inhibitors of insulin signaling in cultured neurons. Consistent with hypothalamic insulin resistance, NARKO male mice exhibit elevated levels of NFκB and PTP1B (Yu et al., 2013). Therefore, the absence of androgenic signaling leads to high levels of these inhibitors of insulin signaling, causing hypothalamic insulin resistance. This hypothalamic insulin resistance results in increased levels of AgRP, which likely contributes at least in part to the phenotypes of obesity, peripheral insulin resistance, and glucose intolerance (Yu et al., 2013). It is unclear if these effects are due to the absence of androgenic signaling during development which programs metabolic dysfunction in later life, or if they are due to the absence of androgenic signaling in later life. Nevertheless, it is clear that the neuronal AR is involved in the maintenance of metabolic homeostasis in males. This makes it a promising target for the treatment of obesity and T2D in older men with androgen deficiency (Figure 2).

Clearly, central androgen signaling is beneficial to male metabolism in mice. However, supraphysiological doses of testosterone can have negative effects on male metabolism. In rodents, castrated rats given physiological doses of testosterone had improved insulin sensitivity while castrated rats given high doses of testosterone did not (Holmang and Bjorntorp, 1992). In humans, athletes taking high doses of anabolic steroids exhibit insulin resistance and glucose intolerance compared to athletes who do not and sedentary normal weight men (Cohen et al., 1987). Thus, there appears to be a parabolic relationship between androgen signaling and metabolic function, with both low and high levels of signaling being detrimental to metabolism. This curve is shifted far to the right for males, indicating that males require higher androgen levels than females for optimal metabolic function

Androgen excess predisposes females to metabolic dysfunction and T2D (Ding et al., 2006; Escobar-Morreale et al., 2014; Legro et al., 1999 Navarro et al., 2015; Page-Wilson et al., 2009). As discussed in the previous section, women with PCOS exhibit androgen excess throughout life (Abbot et al., 2002; Dunaif et al., 1989; Sahin et al., 2014). Therefore, in addition to the programming effects of developmental androgen excess on female metabolism, androgen excess during adulthood can contribute to metabolic dysfunction. Moreover, 50–90% of women with PCOS display insulin resistance and glucose intolerance

(Barber et al., 2016; Dunaif et al., 1989: Ehrmann et al., 1999; Sahin et al., 2014; Sam, 2007; Venkatesan et al., 2001). In these women, the degree of insulin resistance is correlated with testosterone levels, suggesting that testosterone is instrumental in inducing insulin resistance (Sahin et al., 2014). Female to male transsexuals who use testosterone supplements develop insulin resistance, demonstrating that androgen excess during adulthood causes metabolic dysfunction in females (Polderman et al., 1994). This conclusion is further supported by the finding that anti-androgen treatment during adulthood partially reverses insulin resistance in women with functional hyperandrogenemia (Moghetti et al., 1996). Evidence from rodent models also supports the conclusion that androgen excess during adulthood impairs metabolic homeostasis in females (Andrisse et al., 2017; Nohara et al., 2014).

Androgen excess in adult female mice causes resistance to leptin's ability to reduce body weight, leading to obesity (Nohara et al., 2014). These effects are paralleled by a failure of leptin to induce uncoupling protein 1 (UCP1) expression in brown adipose tissue (BAT), which is associated with decreased thermogenesis (Nohara et al., 2014). This suggests an impairment between the central leptin signal and BAT. Additionally, similar to leptin, the melanocortin agonist melanotan II (MTII) fails to reduce body weight in DHT-treated females, suggesting androgen-induced impairment of the melanocortin system (Nohara et al., 2014). These DHT-treated females also have reduced expression of POMC in nerve fibers projecting to the DMH (Nohara et al., 2014). Leptin is known to act in the DMH to increase sympathetic output to BAT, leading to increased thermogenesis (Enriori et al., 2011). Therefore, in female mice, androgens appear to be impairing melanocortin signaling in the DMH, leading to impaired communication between the DMH and BAT and thus reducing energy expenditure. In addition, in female rats, androgen excess reduces locomotor activity, which could also contribute to androgen excess-induced obesity (Feng et al, 2011). Unlike neonatal androgen excess, adult androgen excess in rodents has no apparent effect on food intake or the ability of leptin and MTII to reduce food intake (Nohara et al., 2014). The site of androgen action in the brain that causes these defects is unclear. Although POMC expression is altered in the ARC, only 3% of POMC neurons express AR, so these neurons are likely not the site of androgen action (Fodor and Delemarre-van de Waal, 2001). However, this study was conducted in male rats, so expression patterns could differ in female mice.

Androgen excess in adult female mice also causes hepatic insulin resistance (Andrisse et al., 2017). A pathophysiological dose of DHT in adult female mice elevated serum DHT levels twofold higher than those of control female mice. These DHT-treated mice showed metabolic and reproductive dysfunction with normal body weight/composition, thus confirming that the metabolic and reproductive phenotypes were induced from the androgen actions themselves, rather than as an indirect consequence of obesity (Andrisse et al, 2017). Livers of DHT-treated mice showed 1) increased gluconeogenic enzyme mRNA levels, 2) reduced insulin-stimulated FOXO1 (forkhead box protein O1) degradation, and 3) increased PCK and G6Pase (glucose-6-phosphatase) protein levels. The AR, with elevated DHT, interacts with PI3K (phosphatidylinositol 3-kinase) by binding to P^{85}-PI3K, dissociating the heterodimeric PI3K component P^{85} from P^{110}, thus decreasing P^{85}- P^{110} docking to insulin receptor substrate 1/2. This mechanism may account for the observation of lower insulin-

stimulated PI3K activity and reduced pAKT levels. The effects are hepatic AR-specific because inhibition of AR by flutamide in the presence of elevated androgen prevents the pathology in the hepatic cell line H2.35. Although this mechanism involves only the hepatocytes, one cannot eliminate the possibility that central androgen could be altering parasympathetic output to the liver to potentiate hepatic insulin resistance. The impact of androgen excess on female metabolism during adulthood is summarized in Figure 3.

Although too much androgen has detrimental effects in females, the absence of androgen signaling could have a negative impact on female metabolism. When fed a high-fat diet (HFD), global ARKO female mice develop more severe insulin resistance, glucose intolerance, and obesity compared to control mice fed HFD on an atherosclerosis-prone apolipoprotein E deficient background (Fagman et al., 2015). Surprisingly, the authors did not assess food intake or energy expenditure. Moreover, it is unclear if these phenotypes result from organizational or activational effects of androgens. Additionally, the extent to which central AR contributes to these phenotypes is unclear. Nevertheless, these results demonstrate that some level of androgen signaling is necessary for proper metabolic function in females.

6. Potential sites of androgen action in the CNS that contribute to metabolic homeostasis

Many questions remain unanswered as to where and how androgens act in the CNS to exert their multi-faceted effects on metabolism. One of the most pressing is to what extent androgens are acting at the same sites in males and females to affect metabolism. Are androgens impairing female metabolism through action in the same nuclei where they are benefiting male metabolism? Secondly, are the detrimental effects of excess androgens due to action at the same nucleus where physiological levels of androgens are required for proper metabolic function? The third important question is whether androgens during adulthood act upon pathways that were organized by androgens during development to affect metabolism. Do androgens during adulthood act in the same areas as androgens during development to alter metabolism? Fourthly, are androgens acting in the same nuclei in different species to impact metabolism? Unfortunately, we have no conclusive answers to these questions.

It is clear that androgens, during both development and adulthood, affect the melanocortin system of males and females. The most parsimonious explanation would be that androgens are acting within the ARC, where the components of the melanocortin system reside. However, it is very possible that androgens could be acting upstream or downstream of these neurons. In males, testosterone regulates the pathways between Kiss1 neurons and both POMC and AgRP neurons (Nestor et al., 2016). This is likely via action at the Kiss1 neurons themselves, or upstream neurons, as glutamate release from Kiss1 neurons is altered by castration (Nestor et al., 2016). POMC neurons are unlikely to be the site of action of androgens in males, as only 3% of these neurons express the AR in male rats (Fodor and Delemarre-van de Waal, 2001). Androgen excess in female mice decreases POMC expression in the hypothalamus, but there is little evidence to suggest colocalization between POMC and AR in mice (Nohara et al., 2014; Nohara et al., 2011). In contrast, there is

substantial colocalization between POMC and AR in the female ewe (Sheppard et al., 2011). Additionally, AgRP and AR are colocalized in the female ewe (Sheppard et al., 2011). In light of this evidence, it is possible that androgens act on AR in POMC and/or AgRP neurons to affect female metabolism. In female ewes, prenatal androgen excess increases the number of AgRP neurons while reducing colocalization between AgRP and the insulin receptor (Sheppard et al., 2011; Cernea et al., 2016). As discussed above, AgRP neurons regulate hepatic insulin sensitivity (Konner et al., 2007). POMC neurons expressing insulin and leptin receptors are also involved in hepatic insulin sensitivity (Berglund et al., 2012; Hill et al., 2010; Huo et al., 2009). In addition, both POMC and AgRP neurons are also involved in energy expenditure and food intake (Burke et al., 2016; Small et al., 2003). Together, these results suggest that androgen action at AR in POMC or AgRP neurons could contribute to alterations in food intake, energy expenditure, and hepatic insulin resistance in females. Although hepatic AR is sufficient to induce insulin resistance in female mice with androgen excess, androgen action at AgRP neurons of the ARC could potentiate this hepatic insulin resistance (Konner et al., 2007, Andrisse et al., 2017). Additionally, male mice lacking the neuronal AR who display late onset obesity and hepatic insulin resistance also show increased hypothalamic AgRP expression, further arguing for a relationship between hypothalamic AR action, AgRP neurons, hepatic insulin sensitivity, and obesity (Yu et al., 2013).

Adult female mice exposed to androgen excess exhibit reduced thermogenesis that results in obesity without changes in food intake (Nohara et al., 2014). This phenotype parallels that seen in female mice with impairment of insulin-expressing GABAergic ARC neurons (Kong et al., 2012). In PCOS models, it is believed that AR activation in ARC GABAergic neurons, upstream of GnRH neurons, is instrumental in enhancing GnRH pulsatility (Moore and Campbell, 2015). Therefore, these GABAergic neurons of the ARC could be the site at which androgens act in females to reduce thermogenesis and promote obesity. Notably, these insulin-expressing GABAergic neurons do not express POMC, so androgen action at these neurons would be distinct from their action at POMC neurons (Choudhury et al., 2005).

Stimulation of the VMH also enhances BAT thermogenesis (Perkins et al., 1981; Ramadori et al., 2011). Adult female mice exposed to DHT show obesity and impaired thermogenesis (Nohara et al, 2014). Therefore, one could hypothesize that the VMH is the site where excess androgen is acting during adulthood to impair female energy homeostasis. However, in adult female mice with androgen excess, POMC expression is reduced in fibers of the DMH (Nohara et al., 2014). As discussed above, leptin acts in the DMH to alter sympathetic output to BAT (Enriori et al., 2011) and in adult female mice exposed to androgen excess, leptin fails to upregulate BAT thermogenesis (Nohara et al., 2014). Thus, the DMH is another potential site where excess androgen could be acting in females to alter energy expenditure.

Conclusion

The studies summarized in this review demonstrate that androgen action in the hypothalamus programs and activates metabolism during development and adulthood, respectively. We highlight evidence that testosterone differentially affects male and female

energy and glucose homeostasis. Many questions remain regarding the exact neurons targeted by androgens in the hypothalamus, including the sex-specific mechanisms of action of androgens that affect metabolism. Gaining insight into these questions will help us further decipher how sex differences in metabolism develop and persist, and may allow us to develop precision medical care for better treatment of metabolic disorders.

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Highlights

1. The androgen receptor is expressed in regions of the hypothalamus that control metabolic homeostasis in males and females.
2. Androgen deficiency predisposes male rodents to type 2 diabetes and visceral obesity via loss of peripheral and hypothalamic AR actions.
3. Developmental androgen excess predisposes female animal models to metabolic dysfunction in adulthood via action at both hypothalamic neurons and peripheral tissues.
4. Adult androgen excess predisposes in female mice to obesity via impairment of central leptin signaling and BAT thermogenesis.

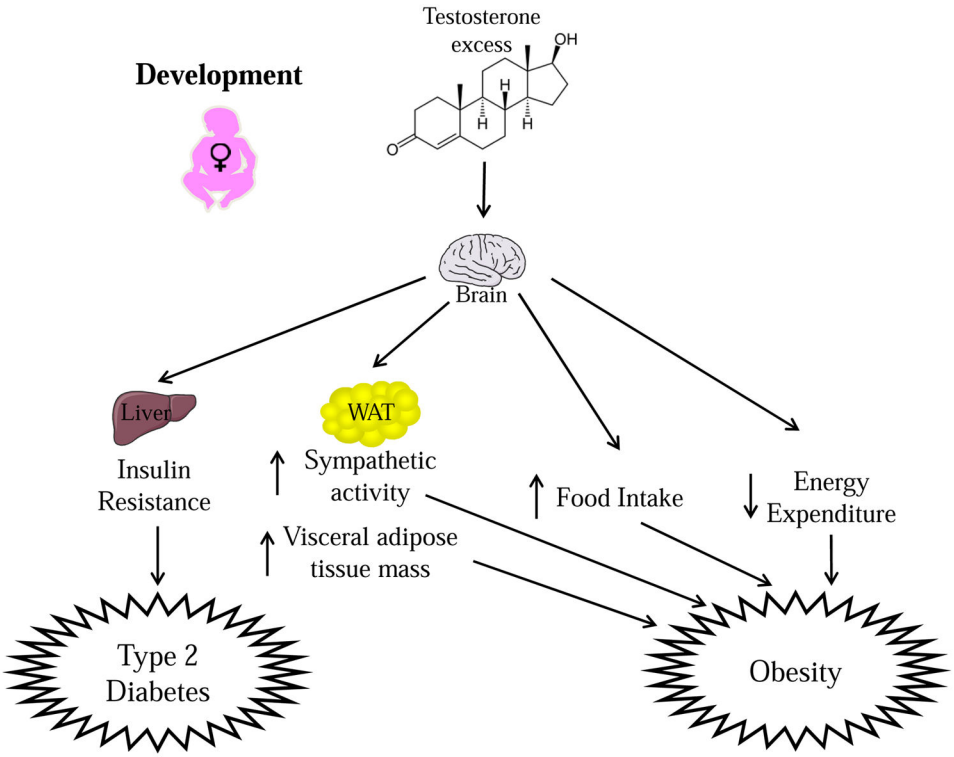

Figure 1. Testosterone excess during development predisposes females to Type 2 Diabetes (T2D) and Obesity

During development, testosterone acts in the brain to predispose females to T2D by causing insulin resistance. Testosterone excess during development also predisposes females to obesity by enhancing sympathetic output to white adipose tissue (WAT), increasing WAT mass and increasing food intake.

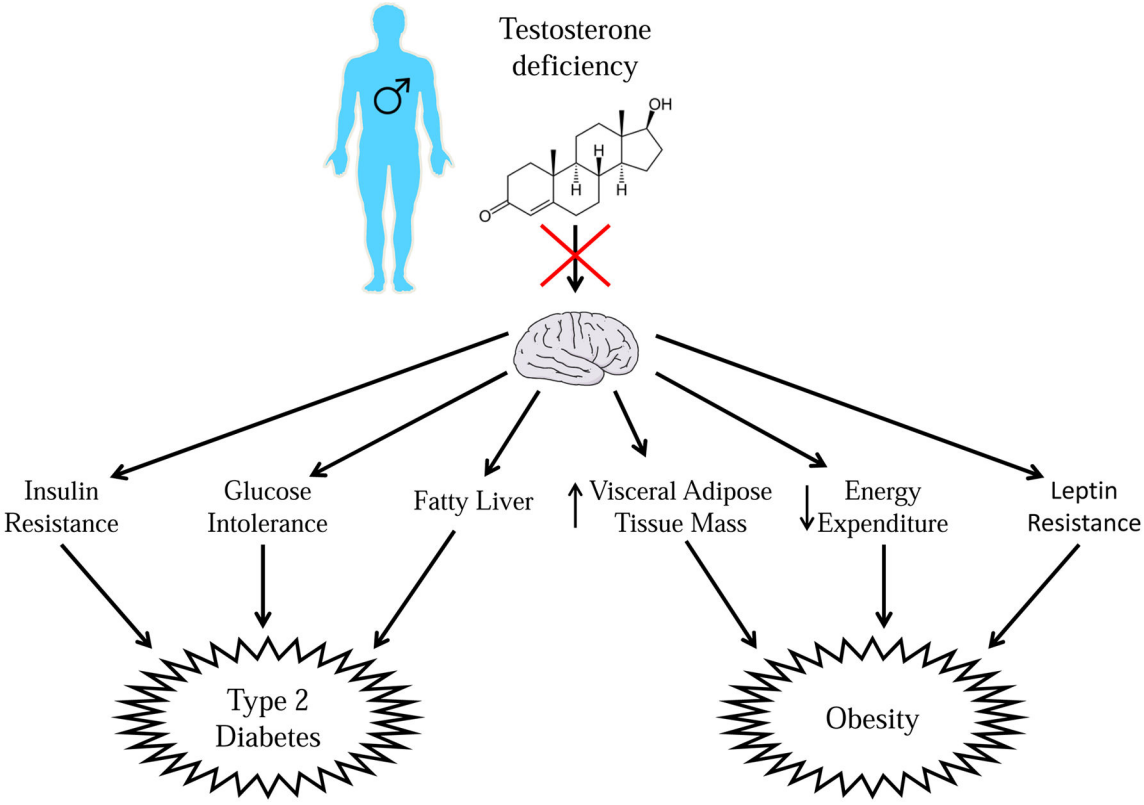

Figure 2. Testosterone deficiency predisposes males to type 2 diabetes and obesity

Testosterone deficiency during adulthood predisposes males to T2D by acting in the brain to contribute to insulin resistance, decreased glucose tolerance, and predisposing to fatty liver. Testosterone deficiency in adult males predisposes to obesity by acting in the brain to increase white adipose tissue mass, decrease energy expenditure, and cause leptin resistance

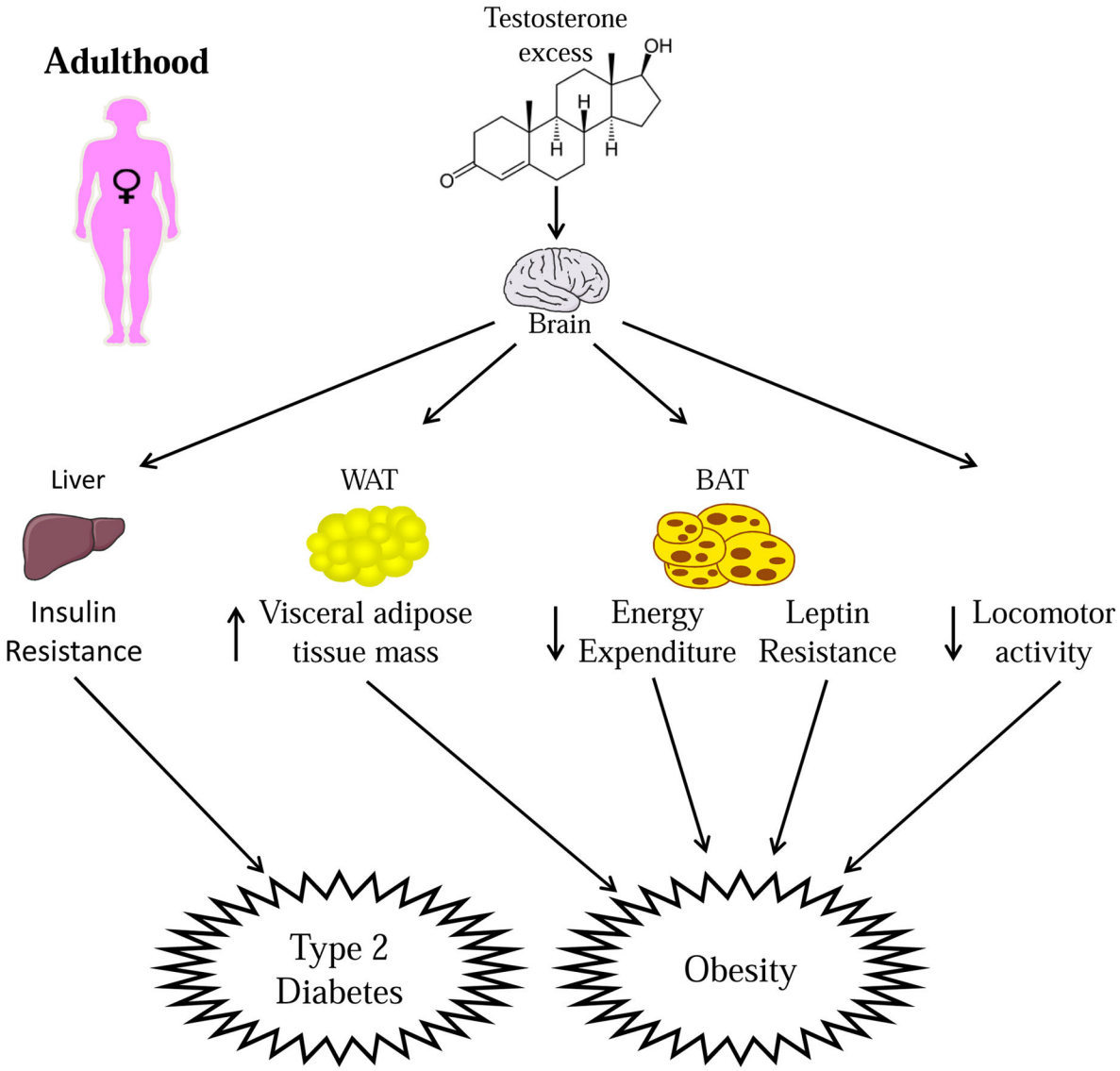

Figure 3. During adulthood, testosterone excess predisposes females to T2D by causing insulin resistance

Testosterone excess in adult females predisposes them to increased visceral WAT mass, decreased energy expenditure, leptin resistance and decreased locomotor activity.

Table 1

Animal models of developmental androgen excess and the resulting metabolic phenotypes. DHT=dihydrotestosterone, AR=Androgen Receptor, ER=Estrogen Receptor, ARC=Arcuate nucleus, AgRP=Agouti-Related Peptide, POMC=Proopiomelanocortin.

Androgen	Dose	Days of Exposure	Species	Metabolic Phenotype	Reference
Testosterone propionate	10mg daily for 15–35 days	Injections start at gestational day 40	Rhesus Macaque	Impaired β-cell function (decreased insulin secretion)	Eisner et al., 2000
Testosterone propionate	10mg daily for 15–35 days	Injections start at gestational day 100–115	Rhesus Macaque	Insulin Resistance	Eisner et al., 2000
Testosterone propionate	10mg for 15– 80 days	Injections start at gestational day 40–44	Rhesus Macaque	Increased visceral fat mass	Eisner et al., 2003
Testosterone propionate and DHT propionate	100mg	Gestational days 30–90 or 60–90	Sheep	Hyperinsulinemia during GTT, Insulin Resistance	Padmanabhan et al., 2010
Testosterone propionate	2g in silastic tubing	Tubing implanted at gestational days 40–60, remained until day 131	Sheep	Hyperinsulinemia, increased non- esterfied fatty acid in plasma	De Haan et al., 1990
Testosterone propionate	2g in silastic tubing	Tubing implanted between gestational days 40–70, remained until days 98–128	Sheep	Hyperinsulinemia, increased body weight	Hansen et al., 1995
Testosterone propionate	20mg each day	Injection into fetal flank on gestational days 62 and 82	Sheep	Hyperinsulinemia, increased β-cell number	Ramaswamy et al., 2016
Testosterone propionate	100mg	Injections twice weekly from gestational day 30 or 62 until day 102	Sheep	Enhanced glucose- stimulated insulin secretion, increased β-cell number (in both fetus and adolescent)	Rae et al., 2013
Testosterone propionate	60mg each day	Injections twice weekly from gestational days 30–90	Sheep	Insulin resistance in early life (5 weeks of age)	Recabarren et al., 2005
Testosterone propionate, Testosterone propionate + Flutamide, or DHT propionate	100mg testosterone or DHT, 15mg/kg flutamide	During gestational days 30–90 injections twice weekly for testosterone and DHT, and daily for flutamide	Sheep	Increased AgRP neuron number in ARC with testosterone and DHT, blocked by flutamide	Sheppard et al., 2011
Testosterone propionate or Testosterone propionate + Flutamide	100mg testosterone or DHT, 15mg/kg flutamide	During gestational days 30–90 injections twice weekly for testosterone and DHT, and daily for flutamide	Sheep	Reduced colocalization between insulin receptor and AgRP in ARC, blocked by flutamide	Cernea et al., 2016
Testosterone propionate	1mg	Injection 3 hours after birth	Rats	Insulin resistance, increased body weight	Nilsson et al., 1998
Testosterone propionate, DHT propionate, or estradiol benzoate	1mg testosterone or DHT, 0.5 mg estradiol	Injection 3 hours after birth	Rats	Insulin resistance (AR and ER), enlarged mesenteric adipocytes (ER)	Alexanderson et al., 2007
Testosterone, DHT, or estradiol	100ug testosterone or DHT, 50ug estradiol	Injections neonatal days 1 and 2	Mice	Increased food intake and decreased hypothalmic POMC (AR), leptin resistance and obesity (ER)	Nohara et al., 2011
Testosterone	100ug	Injections neonatal days 1 and 2	Mice	Obesity, sympathetic hyperactivity in white adipose tissue, reduced energy expenditure	Nohara et al., 2013a
DHT	250ug	Injections gestational days 16–18	Mice	Impaired glucose tolerance, reduced glucose-stimulated insulin secretion	Roland et al., 2010