The role of sex steroids in white adipose tissue adipocyte function

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Abstract

With the increasing knowledge that gender influences normal physiology, much biomedical research has begun to focus on the differential effects of sex on tissue function. Sexual dimorphism in mammals is due to the combined effects of both genetic and hormonal factors. Hormonal factors are mutable particularly in females in whom the estrous cycle dominates the hormonal milieu. Given the severity of the obesity epidemic and the fact that there are differences in the obesity rates in men and women, the role of sex in white adipose tissue function is being recognized as increasingly important. Although sex differences in white adipose tissue distribution are well established, the mechanisms affecting differential function of adipocytes within white adipose tissue in males and females remain largely understudied and poorly understood. One of the largest differences in the endocrine environment in males and females is the concentration of circulating androgens and estrogens. This review examines the effects of androgens and estrogens on lipolysis/lipogenesis, adipocyte differentiation, insulin sensitivity and adipokine production in adipocytes from white adipose tissue with a specific emphasis on the sexual dimorphism of adipocyte function in white adipose tissue during both health and disease.

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Introduction

Historically, the effects of gender have been largely ignored across biomedical research. Subsequent to this practice, studies utilizing immortalized cell lines, single-sex animal models or human patients representing only one sex have resulted in the inability to determine whether research findings are applicable to both sexes (Johnson et al. 2014). In recent years, concerns initially raised about the lack of women in clinical studies (Kim et al. 2010) have resulted in the National Institutes of Health (NIH) requiring sex balance in research involving cells and animal models (Clayton & Collins 2014). Furthermore, sex differences in the relative risk for diseases such as cancer, obesity, coronary heart disease and autoimmune diseases are well established (Becker et al. 2005).

Sexual dimorphism in mammals is the result of both genetic and hormonal factors (Money & Ehrhardt 1972). Genetic factors derived from chromosomes are relatively permanent, whereas hormonal factors are mutable, particularly in females in whom the hypothalamic–pituitary–gonadal axis and the menstrual cycle have predominantly controlled the hormonal milieu (Christensen et al. 2012). Women have a higher percentage body fat than men and tend to distribute their adipose tissue in the hips and thighs (‘gynecoid’), whereas men tend to distribute their adipose tissue abdominally (‘android’) (Karastergiou et al. 2012). Gynecoid adipose tissue distribution is associated with lower risks for type II diabetes and coronary artery disease (Manolopoulos et al. 2010). Recent evidence suggests that sex-specific single nucleotide polymorphisms (SNPs) help direct fat distribution in males and females and epigenetically regulate lipid homeostasis (Sung et al. 2016). Visceral adipocytes exhibit similar basal lipolysis but higher catecholamine-stimulated lipolysis compared to subcutaneous adipocytes in both sexes (Tchernof et al. 2006, Boivin et al. 2007). Lipoprotein lipase (LPL) activity and glucose uptake are lower in visceral adipocytes than those in subcutaneous adipocytes in women who also have smaller adipocytes, but is similar among adipocytes from these two white adipose tissue (WAT) depots in men (Edens et al. 1993, Boivin et al. 2007). Differences in adipocyte function within different WAT depots may be related to the differences in WAT distribution in women and men.

Disease states in women associated with elevated circulating androgen concentrations such as polycystic ovary syndrome (PCOS) or congenital adrenal hyperplasia may result in android adipose tissue accumulation and, subsequently, an increased propensity for insulin resistance and atherosclerosis (Sirmans & Pate 2014). By contrast, overweight men with visceral adipose tissue accumulation are at risk for hypogonadism, low androgen levels and type II diabetes (Dandona & Dhindsa 2011). Furthermore, menopause,
which is due to the onset of ovarian inactivity and results in decreased estrogen and progesterone levels, is accompanied by increases in overall adiposity, particularly due to visceral adipose tissue accumulation (Tchernof & Poehlman 1998). The aforementioned body transitions and disease states underscore the importance of sex steroid hormones to WAT adipocyte function.

Although sex differences in WAT distribution are well established, the mechanisms affecting differential WAT adipocyte function in males and females are poorly understood. Given the severity of the obesity epidemic and the fact that there are differences in the rate at which obesity affects men and women (Flegal et al. 2016), the role of sex in WAT adipocyte function is recognized as increasingly important. Although other factors besides sex steroid levels vary and account for differences between WAT adipocyte function in males and females, sex steroids play a central role in the regulation of many tissues in the body (Wierman 2007). Additionally, it is likely that sex differences in cell types other than adipocytes within WAT also account for differences in metabolism between males and females. However, this review focuses on the effect of sex steroids (androgens and estrogens) on WAT adipocyte function including lipolysis/lipogenesis, adipocyte differentiation, insulin sensitivity and adipokine production/secretion. Particular emphasis is paid in this review to the sexual dimorphism of WAT adipocyte function in the context of steroid hormones in both health (i.e. menopause) and disease (i.e. polycystic ovary syndrome in women, hypogonadism in men).

Estrogens

**Lipolysis and lipogenesis**

Lipolysis and lipogenesis of adipose tissue assists with energy release and energy storage in the body (Thomas et al. 1979) (Fig. 1). Estradiol (E2) renders its effects through binding to either classical estrogen receptors (ER), which are nuclear transcription factors expressed widely throughout the adipose tissue compartment (Mizutani et al. 1994, Dieudonné et al. 1998), or to non-classical ER like G-protein-coupled estrogen receptor (GPER), which are membrane-bound receptors (Prossnitz & Barton 2011). There are two classes of classical ERs, ER alpha (ERα) and ER beta (ERβ), each of which has a distinct tissue distribution (Koehler et al. 2005). In addition to differential tissue expression, the ligand binding domains of ERα and ERβ are unique, which allows selective ligands to target one or the other pathway (Koehler et al. 2005). With respect to reproductive tissues, ERα is uterotropic and found in the ovarian stroma and breast, whereas ERβ is in the ovarian granulosa cells (Korach et al. 2003). Current literature suggests that ERα modulates anti-lipogenesis, insulin sensitivity and glucose tolerance, and reduction of body weight and WAT mass (Blüher 2013). In contrast, ERβ appears to be detrimental for the maintenance of glucose and lipid homeostasis (Foryst-Ludwig & Kintscher 2010). Recent evidence also indicates a role for the non-genomic regulation of WAT lipogenesis by GPER, but the findings between publications differ (Haas et al. 2009, Isensee et al. 2009, Mårtensson et al. 2009, Sharma et al. 2013). Several studies have demonstrated that male GPER-deficient (GPERKO) mice suffer from increased WAT mass and decreased insulin sensitivity, whereas female GPERKO mice have decreased weights (Mårtensson et al. 2009, Sharma et al. 2013). Other studies have shown that both male and female GPERKO mice have increased WAT, particularly in the visceral compartment (Haas et al. 2009). Finally, other studies have found no differences in body weight and adiposity between GPERKO and wild-type male or female mice fed either a control or high-fat diet (Isensee et al. 2009). Research using knock-out rodent models has demonstrated that both

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**Figure 1** Overview of adipocyte metabolism. After a meal, energy enters the adipocyte through several mechanisms: 1) direct absorption of fatty acids (FA) from the bloodstream, 2) uptake of glucose through GLUT4 receptors on the adipocyte cell membrane and 3) cleavage of triglycerides (TG) into fatty acids via lipoprotein lipase (LPL) made by the adipocyte. Catecholamines stimulate release of fatty acids from triglycerides in the triglyceride pool by stimulating hormone-sensitive lipase (HSL; 4). Insulin stimulates uptake of glucose via GLUT4 (2) but inhibits lipolysis via HSL (5). Fatty acids can be released from the adipocyte after lipolysis (6).
cytosolic and membrane-bound sex steroid receptors in WAT are actively involved in the modulation of lipolysis and lipogenesis in adipocytes (Table 1). Estrogens play an active role in the lipogenic control of adipocytes in both sexes across the life span of individuals.

It is well established that estrogen regulates the metabolic status of WAT in females, but the mechanisms behind this axiom are not completely understood. In ad libitum fed mice, ovariectomy results in the accumulation of WAT, whereas estrogen replacement of ovariectomized (OVX) mice decreases WAT mass.

Table 1 Effects of estradiol on white adipose tissue adipocyte function of males and females.

<table>
<thead>
<tr>
<th>Steroid</th>
<th>Category of effect</th>
<th>Type of response</th>
<th>Males</th>
<th>Females</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Estradiol (E2)</td>
<td>Lipolysis/lipogenesis</td>
<td>Inhibition of lipogenesis (mouse)</td>
<td>+</td>
<td>+</td>
<td>D'Eon et al. 2005</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Increased lipolysis (mouse)</td>
<td>-</td>
<td>+</td>
<td>D'Eon et al. 2005</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Decreased adipocyte size (mouse)</td>
<td>?</td>
<td>+</td>
<td>D'Eon et al. 2005</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Suppresses LPL (human; mouse; pre-menopause)</td>
<td>?</td>
<td>+</td>
<td>Homma et al. 2000, Tchernol et al. 2004</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Increases LPL activity (human; post-menopause)</td>
<td>-</td>
<td>+</td>
<td>Tchernol et al. 2004</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Increases HSL activity (human)</td>
<td>?</td>
<td>+</td>
<td>Palin et al. 2003</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Increases catecholamine-induced lipolysis (human; mouse)</td>
<td>-</td>
<td>+</td>
<td>D'Eon et al. 2005, Homma et al. 2000</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Increases PPARγ function with ERβ stimulation (mouse)</td>
<td>?</td>
<td>+</td>
<td>Foryst-Ludwig et al. 2008</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Downregulation of lipogenic genes (human; post-menopause)</td>
<td>-</td>
<td>+</td>
<td>Lundholm et al. 2008, Santos et al. 2013</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Increased WAT mass with ERα stimulation (mouse)</td>
<td>+</td>
<td>+</td>
<td>Heine et al. 2000</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Decreased WAT mass with GPER stimulation (mouse)</td>
<td>-</td>
<td>+</td>
<td>Martensson et al. 2009, Sharma et al. 2013</td>
</tr>
<tr>
<td>Adipocyte differentiation</td>
<td>Adipogenesis (humans; women&gt;men; visceral&gt;SQ)</td>
<td>+</td>
<td>+</td>
<td>Anderson et al. 2001</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Adipogenesis (rat; SQ only)</td>
<td>-</td>
<td>+</td>
<td>Dieudonne et al. 2000</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Inhibit adipogenesis via GPER (3T3-L1 cells)</td>
<td>-</td>
<td>-</td>
<td>Zhu et al. 2013</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Inhibition of miR-125a, which inhibits ERα, promotes adipogenesis (pig)</td>
<td>?</td>
<td>?</td>
<td>Ji et al. 2014</td>
<td></td>
</tr>
<tr>
<td>Insulin sensitivity</td>
<td>High fat feeding decreases GLUT4 and ERα in visceral WAT (mouse-females; rat-ERα only, males)</td>
<td>+</td>
<td>+</td>
<td>Gorres et al. 2011, Metz et al. 2016</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Increased uptake of glucose via ERα stimulation and tyrosine phosphorylation of IRS1 (3T3-L1 cells, concentration dependent)</td>
<td>-</td>
<td>-</td>
<td>Muraki et al. 2006, Nagira et al. 2006</td>
<td></td>
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<tr>
<td></td>
<td>Activation of AMPK and Akt via estrogen receptor (3T3-L1 cells)</td>
<td>-</td>
<td>-</td>
<td>Kim et al. 2012</td>
<td></td>
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<tr>
<td></td>
<td>Upregulates miR-222 transcript levels with subsequent downregulation of GLUT4 transcript levels (human)</td>
<td>-</td>
<td>+</td>
<td>Shi et al. 2014</td>
<td></td>
</tr>
<tr>
<td>Leptin</td>
<td>Positively correlated with serum E2 levels (human; young women&gt;post-menopausal women&gt;men)</td>
<td>+</td>
<td>+</td>
<td>Hickey et al. 1996, Hong et al. 2007</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Increased secretion and transcript levels (rat; mouse)</td>
<td>+</td>
<td>+</td>
<td>Machinal et al. 1999, Van Sinderen et al. 2015</td>
<td></td>
</tr>
<tr>
<td></td>
<td>No effect on secretion (3T3-L1 cells)</td>
<td>-</td>
<td>-</td>
<td>Pektas et al. 2015</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Leptin SNP G-2548A (rs7799039) related to serum leptin concentrations and obesity in young women only (human)</td>
<td>-</td>
<td>-</td>
<td>Shahid et al. 2015</td>
<td></td>
</tr>
<tr>
<td>Adiponectin</td>
<td>Decreased secretion (3T3-L1 cells)</td>
<td>-</td>
<td>-</td>
<td>Pektas et al. 2015</td>
<td></td>
</tr>
<tr>
<td></td>
<td>No effect on secretion (SBGS cells)</td>
<td>-</td>
<td>-</td>
<td>Horenburg et al. 2008</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Adiponectin SNPs only associated with serum adiponectin concentrations and obesity in women (human)</td>
<td>-</td>
<td>+</td>
<td>Riestra et al. 2015</td>
<td></td>
</tr>
<tr>
<td>Resistin</td>
<td>Positively correlated with serum E2 levels (human; young women&gt;post-menopausal women)</td>
<td>-</td>
<td>+</td>
<td>Martos-Moreno et al. 2006</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Increased secretion (3T3-L1 cells)</td>
<td>-</td>
<td>-</td>
<td>Pektas et al. 2015</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Decreased transcript levels (rat)</td>
<td>-</td>
<td>-</td>
<td>Huang et al. 2005, Caja &amp; Puerta 2007</td>
<td></td>
</tr>
</tbody>
</table>

LPL, lipoprotein lipase; HSL, hormone-sensitive lipase; PPARγ, peroxisome proliferator-activated receptor gamma; ERβ, estrogen receptor β; WAT, white adipose tissue; ERα, estrogen receptor α; GPER, G-protein-coupled estrogen receptor; SQ, subcutaneous; ERα, estrogen-related receptor α; GLUT4, glucose transporter type 4; IRS1, insulin receptor substrate 1; AMPK, 5’AMP-activated protein kinase; Akt, protein kinase B; SNP, single nucleotide polymorphism; SGBS cells, Simpson–Golabi–Behmel syndrome cells.
levels (PPARG in WAT (SREBF1), suppression of LPL, as well as protein 1 (Acetyl CoA carboxylase-1 (ACACA)) and fatty acid synthase (FASN), sterol regulatory element-binding like acetyl-CoA carboxylase-1 (ACACA) and fatty acid synthase (FASN)). These effects are mediated by decreased transcript levels of genes that control lipid storage, such as SREBF1, suppression of LPL, and increased adipocyte lipolysis (D’Eon et al. 2005). Other studies support the role of E2 as a potent suppressor of LPL transcription, the enzyme that catalyzes the conversion of triglycerides to free fatty acids (Urabe et al. 1996), perhaps by an estrogen response element on the LPL promoter (Homma et al. 2000). In contrast, E2 has the opposing effect on hormone-sensitive lipase (HSL) in women (Palin et al. 2003). The administration of E2 to cultured subcutaneous adipocytes from women increases lipolysis in vitro by increasing HSL protein expression (Palin et al. 2003). Both aromatase-deficient (ArKO) and ERα-deficient (ERαKO) mice, which are E2-deficient models, show a phenotype similar to the pair-fed OVX mouse (Heine et al. 2000, Jones et al. 2001). In these mouse models, the increased WAT depots are not due to hyperphagia, but may be related to decreased energy expenditure (Heine et al. 2000, Jones et al. 2001). The role of ERβ in WAT lipolytic/lipogenic function remains dubious. Although ERβ is found in WAT, ERβ-deficient (ERβKO) mice do not demonstrate changes in adiposity or metabolic alterations when control fed (Walker & Korach 2004). However, when female ERβKO mice are high-fat fed (Foryst-Ludwig et al. 2008), they develop increased adiposity related to increased peroxisome proliferator-activated receptor gamma (PPARG) transcript levels (Foryst-Ludwig et al. 2008). PPARG, which is abundant in WAT, is a member of the nuclear receptor superfamily and is a transcription factor for a number of genes related to glucose and lipid metabolism (Evans et al. 2004).

Although sex- and depot-related differences in ERα and ERβ have been reported (Pedersen et al. 1991, Blouin et al. 2009), E2-deficient mouse models indicate that E2 is important for lipid homeostasis and adipocyte lipolytic/lipogenic activity in both sexes (Heine et al. 2000, Jones et al. 2001). A study that administered a potent anti-estrogen, acolbifene, to mice demonstrated that both males and females developed decreased WAT mass and circulating cholesterol (Lemieux et al. 2005). Although studies in rodents support similar WAT responses in males and females to direct E2 stimulation at classical ERs (Heine et al. 2000, Jones et al. 2001), other evidence supports a heterogenous response to E2, particularly in the context of obesity. Females have less visceral WAT and have increased adipocyte insulin sensitivity compared to males (Macotela et al. 2009). Additionally, women show increased sensitivity to catecholamine-induced lipolysis in the visceral compartment as compared to men due to an increased number of beta adrenergic receptors (Ramis et al. 2006). These factors provide substantial evidence as to why women are at a decreased risk for type II diabetes and metabolic syndrome compared to men (Gale & Gillespie 2001). In fact, estrogens counteract diet-induced increases in WAT mass in male mice to protect them against obesity (Dakin et al. 2015).

Menopause is the period of reproductive senescence in women characterized by lack of estrogens and acts a suitable clinical model for how lack of E2 affects adiposity in females (Broekmans et al. 2009). Both menopause and chemical castration of pre-menopausal women with gonadotropin-releasing hormone (GNRH) cause increased WAT accumulation, especially in the visceral compartment (Yamasaki et al. 2001, Keller et al. 2010) (Table 2). Although menopause is known to affect total body fat and body fat distribution, the cellular mechanisms underlying these findings are not well characterized (Zsakai et al. 2016). Yet, the accumulation of visceral WAT in post-menopausal women is readily treated with hormone replacement therapy (Ryan et al. 2002).

One recent study demonstrated that E2 treatment in post-menopausal women decreases the expression of key lipogenic genes such as stearoyl-CoA desaturase, FASN, acetyl coenzyme A carboxylase alpha and fatty acid desaturase 1 as well as the adipogenic gene, PPARG (Lundholm et al. 2008). Although E2 treatment has been shown to suppress in vivo LPL concentrations (Urabe et al. 1996), another study found no difference in LPL activity in pre- and post-menopausal women,

### Table 2

<table>
<thead>
<tr>
<th>Sex steroid concentration</th>
<th>Female</th>
<th>Male</th>
</tr>
</thead>
<tbody>
<tr>
<td>↑T ↓E2</td>
<td>PCOS: visceral obesity/android subcutaneous obesity</td>
<td>Normal physiology: android fat deposition</td>
</tr>
<tr>
<td>↑T ↓E2</td>
<td>Menopause: visceral obesity/android subcutaneous obesity</td>
<td>Hypogonadism: visceral obesity/android subcutaneous obesity</td>
</tr>
<tr>
<td>↑T ↑E2</td>
<td>Normal physiology: gynecoid fat deposition</td>
<td></td>
</tr>
</tbody>
</table>

T, testosterone; E2, estradiol.
indicating that increased WAT in post-menopausal women may be due to changes in adipocyte fatty acid storage factors and lipogenic enzymes alone (Santosa & Jensen 2013). Yet, other studies have found that post-menopausal women, in contrast to their younger counterparts, have higher LPL and basal lipolysis (Tchernof et al. 2004). Such conflicting findings demonstrate the overwhelming need for more well-designed and placebo-controlled studies to elucidate the mechanisms by which E2 affects lipolytic/lipogenic mechanisms across the female lifespan.

**Adipocyte differentiation**

Estrogens induce the proliferation of cells in estrogen-responsive female reproductive tissues, such as the endometrium (Pierro et al. 2001). However, the effects of estrogens on adipocyte differentiation, rather than proliferation, are more varied (Dieudonne et al. 2000,Anderson et al. 2001,Gupta et al. 2008, Rice et al. 2010). Pre-adipocytes express only ESR1, therefore, all estrogenic effects on adipogenesis are presumably mediated through the action of this receptor (Pallottini et al. 2008). As there are no WAT depot or gender differences in ESR1 distribution (Joyner et al. 2001), one would expect E2 to stimulate adipocyte differentiation in a dose-dependent manner irrespective of sex for both subcutaneous and visceral WAT compartments. However, a uniform relationship in both males and females between E2 dose and adipose tissue response is not supported by the literature (Table 1). In response to E2, female-derived pre-adipocytes undergo greater adipogenesis than male-derived pre-adipocytes (Anderson et al. 2001). Additionally, visceral pre-adipocytes convert to mature adipocytes more quickly than subcutaneous pre-adipocytes in both sexes (Anderson et al. 2001). Studies in rodents, on the other hand, have demonstrated no appreciable effect of E2 on adipogenesis of pre-adipocytes derived from visceral or subcutaneous WAT from males but a stimulatory effect of E2 on the adipogenesis of subcutaneous pre-adipocytes from females (Dieudonne et al. 2000). By contrast, E2 stimulation of GPER in murine 3T3-L1 immortalized pre-adipocyte cells inhibits adipogenesis (Zhu et al. 2013). Recent evidence also suggests a role for epigenetic modulation in the process of adipocyte differentiation. microRNA (miR) 125-a inhibits the expression of estrogen-related receptor α (ESRRA) (Ji et al. 2014), an orphan nuclear receptor that modulates the activity of ESR1 in some tissues (Stein & McDonnell 2006). Inhibition of miR-125a leads to the promotion of porcine pre-adipocyte differentiation, whereas overexpression leads to alterations in lipid metabolism within adipocytes (Ji et al. 2014).

Location of adipose tissue in men and women may direct its function. A recent study found that increased femoral adiposity in women was associated with adipocyte hyperplasia (i.e. increased adipogenesis), whereas increased femoral adiposity in men was associated with adipocyte hypertrophy (i.e. increased lipid storage) (Tchoukalova et al. 2008). Interestingly, the*in vitro* basal proliferation and differentiation of pre-adipocytes isolated from the same WAT depots in males and females are not different, which indicates it may be something in the microenvironment, rather than the inherent properties of the male and female pre-adipocytes, that affects differential adipogenesis between the sexes (Tchoukalova et al. 2010). These particular studies support the general rule that the estrogenic microenvironment in females stimulates adipogenesis, whereas a lack of androgens in the male microenvironment (i.e. hypogonadism) stimulates adipocyte lipogenesis.

**Insulin sensitivity**

Adipose tissue, along with liver and skeletal muscle, is intimately involved in glucose homeostasis and insulin sensitivity (Saltiel & Kahn 2001). There are marked sex differences in the relationship between insulin sensitivity and adipose tissue distribution (Varlamov et al. 2014). Interestingly, type II diabetes is more frequently diagnosed in younger and less obese men than women; yet, obesity, which is the main risk factor for the development of type II diabetes, occurs with increased prevalence in women (Kautzky-Willer et al. 2016). Although the relationship between sex and insulin sensitivity is well known, the role of WAT and sex steroids in that relationship is far less defined.

ERα is important to the regulation of insulin function in adipose tissue and glucose tolerance systemically in both sexes (Heine et al. 2000). Moreover, estrogens are not only important for female insulin sensitivity but also have been shown to protect glucose–insulin homeostasis in obese males (Dakin et al. 2015). Additionally, research in men with estrogen resistance due to a mutation in ER suggests that E2 alters carbohydrate and lipid metabolism (Smith et al. 1994). Studies using rodent models have further elucidated the relationship between E2 and insulin sensitivity (Tremblay et al. 2001, Ribas et al. 2010, Gorres et al. 2011, Manrique et al. 2012). ERαKO mice have decreased whole body glucose tolerance compared to wild-type mice, but the role of the adipose tissue ERα remains to be discerned (Ribas et al. 2010, Manrique et al. 2012). Interestingly, when female mice are O VX or sham treated and then high fat fed, GLUT4 and ESR1 proteins decrease in visceral WAT of both treatment groups (Gorres et al. 2011). This observation is in contrast to findings in high-fat fed male mice, which demonstrate a significant decrease in glucose metabolism in skeletal muscle rather than that in WAT (Tremblay et al. 2001). A more recent study in high-fat fed male rats found that although a high-fat diet modulated the ESR1 in the adipose tissue, ESR1 did...
not appear to be associated with whole body glucose tolerance (Metz et al. 2016). It is possible that there are species differences in relationship between ESR1 and insulin sensitivity, as studies in male rats and mice appear to conflict with respect to the effect of a high-fat diet on receptor expression in WAT. Such disparities underscore the importance of animal, human and cell culture-based studies of the effects of sex steroids on WAT function. Recent evidence suggests that ESR1 is a direct target of miR-222 with a specific site at the seed sequence of this miR (Rao et al. 2011). Furthermore, high E2 concentrations in visceral adipocytes upregulate miR-222 transcript levels and downregulate Esr1 and Glut4 transcript levels (Shi et al. 2014).

By contrast, Esr2 has been found to control Glut4 transcription via DNA methylation in induced murine adipocytes (Rüegg et al. 2011). In experiments using murine embryonic fibroblasts induced to become adipocytes, which originated from wild-type, ERβKO and ERαKO mice, it was discovered that hypermethylated CpG is part of a specificity protein 1 (Sp1) binding site and that Sp1 binding is impaired by methylation of this site, which leads to decreased GLUT4 expression (Rüegg et al. 2011). Recent evidence suggests that overexpression of miR-125a also leads to reduced mRNA expression of PPARG, LPL and adipocyte protein 2, which controls the expression of Esr2 (Ji et al. 2014). Although a direct relationship between miR-125a and E2 has not been established in adipocytes, miR-125a can be upregulated by E2 via Esr1 in hepatocytes, which protects them against non-alcoholic fatty liver disease through the inhibition of fatty acid synthesis (Zhang et al. 2015). Lastly, miR-125a is overexpressed in the WAT of rats that develop diabetes spontaneously (Herrera et al. 2009).

Molecular studies of the effects of E2 on adipocyte insulin signaling have primarily been conducted in 3T3-L1 cells. E2 modulates insulin signaling via ESR1 and stimulates the uptake of glucose into adipocytes via regulation of the tyrosine phosphorylation of insulin receptor substrate 1 (IRS-1) protein (Muraki et al. 2006). However, high concentrations of E2 (10^{-7}M) inhibited insulin signaling in adipocytes via modulation of IRS-1 phosphorylation at Ser1107 through a c-Jun NH2 terminal kinase-dependent pathway (Nagira et al. 2006). It also has been shown that E2 activates adenosine monophosphate-activated protein kinase (AMPK) through ER and activates protein kinase B (AKT) via AMPK even in the absence of insulin in cell culture (Kim et al. 2012).

Menopause not only results in an increased accumulation of WAT but also puts women at an increased risk of glucose intolerance and decreased insulin sensitivity (Thernomof & Poehlman 1998, Carr 2003). Moreover, estrogen replacement therapy (HRT) restores normal insulin sensitivity (Margolis et al. 2004) even if a post-menopausal woman still has a large amount of visceral WAT. Recent evidence suggests that HRT administered earlier in menopause improves insulin sensitivity but delivered 10 or more years after menopause decreases insulin sensitivity (Pereira et al. 2015). This shift in the metabolic benefits of HRT could be due to the downregulation of ER in an estrogen-deficient environment over time. Such evidence corroborates the critical role of E2 in the modulation of glucose homeostasis.

Adipokine production

Adipokines are cytokines secreted by adipose tissue, many of which regulate energy metabolism (Kwon & Pessin 2013). Although the effects of sex steroids on adipogenesis, lipogenesis and adipocyte metabolism have been studied extensively, the effects of sex steroids on adipokine production/release have been less explored with most research focused on leptin, adiponectin and resistin.

Leptin

Leptin is transcribed from the Ob gene and regulates body fat storage by control of appetite and activity levels via a hypothalamic feedback loop (Friedman & Halaas 1998). Circulating leptin is higher in women than that in men (Hickey et al. 1996), but there are also sex differences in leptin found in children (Hellström et al. 2000). Leptin concentrations are positively correlated with serum E2 levels in pre-menopausal women; however, the increase in WAT mass in post-menopausal women also increases circulating leptin concentrations (Hong et al. 2007). The aforementioned findings indicate that sex steroids and other sex-specific factors regulate leptin. Furthermore, leptin transcript expression is higher in the subcutaneous WAT of pre-menopausal women than that in post-menopausal women (Hong et al. 2007), presumably due to the higher E2 concentrations found in younger women. Similar support for the role of E2 in leptin secretion comes from experiments with rodent models wherein O VX rats demonstrate a 25% decrease in Ob transcript levels in visceral WAT (Machinal et al. 1999). By contrast, in the ArKO mouse serum leptin and leptin transcript levels are increased compared to wild type, and this phenomenon is reversed by E2 administration (Van Sinderen et al. 2015). Stimulation of visceral and subcutaneous adipocytes in cell culture with E2 result in an increase in Ob transcript levels and leptin secretion (Machinal et al. 1999). More recently, it has been discovered that in 3T3-L1 cells, E2 does not affect leptin secretion (Pektas et al. 2015). Certain leptin SNPs such as G-2548A (rs7799039) have demonstrated a relationship with serum leptin concentrations and obesity only in young females rather than males (Shahid et al. 2015). Such findings indicate a potential role for both E2 and gender in leptin action.
and regulation. The vast majority of evidence supports the premise that E2 increases leptin production and release from WAT, but that other factors besides E2 are related to the sexual dimorphism displayed by this adipokine (Machinal et al. 1999, Hellström et al. 2000, Hong et al. 2007).

**Adiponectin**

Adiponectin, which is exclusively produced by WAT (Scherer et al. 1995), improves insulin sensitivity and lipid metabolism (Gil-Campos et al. 2004). Additionally, its concentrations are inversely correlated with body mass index (BMI) (Arita et al. 1999). Recently, it has been discovered that marrow adipose tissue is the major source of adiponectin (Scheller et al. 2016); therefore, activity of sex steroids at the level of the marrow adipose depot may have important implications for adiponectin synthesis and secretion. Systemic adiponectin concentrations are higher in women than those in men, indicating a potential role for E2 in the modulation of this adipokine (Geer & Shen 2009). However, the relationship between E2 and adiponectin remains relatively inconclusive due to conflicting reports in the literature. OVX adult mice and anovulatory aged mice have increased serum adiponectin levels, but infant mice that undergo ovariectomy still experience a rise in adiponectin at the age of puberty (Combs et al. 2003). It is unclear why E2 and adiponectin in females rise in tandem at puberty but are indirectly correlated in adulthood (Böttner et al. 2004). Studies conducted in vitro on the effects of E2 on adiponectin secretion are equally conflicting. E2 causes decreased secretion of adiponectin in cell culture with murine 3T3-L1 cells (Pektaş et al. 2015), but has no effect on adiponectin secretion by the human SBGS adipocyte cell line (Horenburg et al. 2008). Further studies are needed to more conclusively determine the effects of E2 on adiponectin secretion. Several SNPs of the adiponectin gene (APIDOQ; rs6444174, rs1403697, rs7641507 and rs16861205) have been found to be associated with serum adiponectin levels in women but not men (Riestra et al. 2015). Additionally, one of those SNPs (rs6444174) was found to be associated directly with BMI and obesity (Riestra et al. 2015). Although a role of E2 in APIDOQ gene polymorphisms has not been elucidated, certainly there are differences in APIDOQ genetic determinants by gender and with respect to WAT mass.

**Resistin**

Resistin is an adipocyte-derived hormone in rodents but is secreted almost exclusively by immune and epithelial cells in primates, pigs and dogs (Park & Ahima 2013). Resistin causes insulin resistance in control mice when given exogenously, and anti-resistin antibody improves insulin sensitivity in obese mice (Steppan et al. 2001). Yet, the role resistin may have in obesity and diabetes in humans is controversial. Only a few studies have detected resistin mRNA and protein expression in adipose tissue of humans (McTernan et al. 2002, Chen et al. 2014). Several studies found that resistin induces lipolysis and suppresses adiponectin secretion in human visceral adipocytes (Ort et al. 2005, Chen et al. 2014), whereas in rodents, it inhibits insulin signaling, adipogenesis (Kim et al. 2004) and accelerates the fatty acid/triglyceride futile cycle (Ort et al. 2005). Still other studies have found positive correlations between resistin and obesity or insulin resistance (Kang et al. 2016) and others have found no relationship (Rea & Donnelly 2004). The effects of E2 on resistin secretion are as controversial as the biology of the hormone itself. E2 causes increased secretion of resistin in cell culture with 3T3-L1 cells (Pektaş et al. 2015); however, administration of E2 exogenously to female rats in diestrus causes decreased resistin in visceral WAT (Caja & Puerta 2007). In mice, experiments both in vitro and in vivo have demonstrated that E2 suppresses resistin transcript levels in WAT (Huang et al. 2005). In stark contrast to the other metabolic adipokines, in humans, resistin is higher in adult women but does not differ by sex throughout the stages of puberty (Martos-Moreno et al. 2006).

**Androgens**

**Lipolysis and lipogenesis**

Similar to ER, androgen receptor (AR) is expressed widely throughout the adipose tissue compartment, indicating that WAT adipocytes may display a particular sensitivity to androgens (Dieudonné et al. 1998). Androgens are well known to modulate the deposition of body fat in men (Blouin et al. 2008). As such, low plasma testosterone concentrations or hypogonadism in men is associated with android obesity and increased visceral WAT (Garaulet et al. 2000, Tsai et al. 2004) (Table 2). Testosterone administration to hypogonadal men decreases visceral WAT in a dose-dependent manner (Woodhouse et al. 2004), but has minimal effects on circulating lipids (Gruenewald & Matsumoto 2003). Although the evidence that androgens help modulate insulin homeostasis is strong, studies on the effects of androgens on lipolysis are more ambiguous. Testosterone has been shown to cause increased catecholamine lipolysis in the subcutaneous but not the visceral WAT of males and females possibly due to decreased β2 adrenergic receptors and decreased HSL and adenylate cyclase activity in this WAT compartment (Xu et al. 1991, Dicker et al. 2004) (Table 3). Yet, other studies both in men (Rebuffe-Scrive et al. 1991) and in vitro in human and rat adipocytes have found that testosterone (Xu et al. 1990) and dehydroepiandrosterone (DHEA) (Hernández-Morante et al. 2008) stimulate norepinephrine-stimulated lipolysis (Table 4). Evidence from subcutaneous WAT
demonstrates that androgen effects are mediated by AR as the AR antagonist, flutamide, significantly abolishes tissue responsiveness to dihydrotestosterone (DHT) (Anderson et al. 2002) (Table 4). In men and male rats, androgens also decrease lipogenesis in visceral WAT through decreased LPL activity and triglyceride uptake (Rebuffe-Schive et al. 1991, Li & Björntorp 1995). Similarly, a study that examined male AR-deficient mice (ARKO) demonstrated late-onset obesity and increased lipogenesis (Fan et al. 2005). When male mice with a conditional knock-down of AR in adipocytes (iARKO) are high-fat fed, they develop increased visceral WAT compared with control male mice, indicating a role for androgen action specifically in the visceral WAT of males (McInnes 2012). However, a study in male non-human primates castrated and supplemented with exogenous testosterone found no effects of testosterone on lipogenesis (Varlamov et al. 2012). It is important to note that studies with testosterone or DHEA cannot definitively determine if the effects seen are due to androgenic activity as these compounds are readily aromatizable to estrogens (Longcope et al. 1978). Further mechanistic studies, preferably utilizing a non- aromatizable androgen such as DHT, are warranted to completely elucidate the manner in which androgens affect lipogenesis in WAT of males.

Studies of the effects of androgens on WAT lipolytic/lipogenic activity in females are relatively scarce.

Table 3: Effects of testosterone on white adipose tissue adipocyte function of males and females.

<table>
<thead>
<tr>
<th>Steroid</th>
<th>Category of effect</th>
<th>Type of response</th>
<th>Males</th>
<th>Females</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Testosterone (T)</td>
<td>Lipolysis/lipogenesis</td>
<td>Decreased catecholamine lipolysis in subcutaneous WAT (human; mouse)</td>
<td>+</td>
<td>+</td>
<td>Xu et al. 1991, Dicker et al. 2004</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Suppresses HSL and adénylate cyclase activity (human; mouse)</td>
<td>+</td>
<td>+</td>
<td>Xu et al. 1991, Dicker et al. 2004</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Stimulation of visceral WAT lipogenesis (human)</td>
<td>-</td>
<td>+</td>
<td>Douchi et al. 1995</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Decreased lipolysis (NHP)</td>
<td>-</td>
<td>+</td>
<td>Varlamov et al. 2013</td>
</tr>
<tr>
<td>Adipocyte differentiation</td>
<td>Down-regulation of Plag1 and downstream Ppar with subsequent decreased adipogenesis (rat)</td>
<td>+</td>
<td>+</td>
<td>Mirowska et al. 2014</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>miR-375 transcript levels down-regulated with subsequent up-regulation of ADIPOR2 transcript levels and decreased adipogenesis (human)</td>
<td>?</td>
<td>?</td>
<td>Kraus et al. 2015</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Upregulates fatty acid synthase &amp; GLUT4 in WAT (NHP; human)</td>
<td>+</td>
<td>? (PCOS)</td>
<td>Rosenbaum et al. 1993, Varlamov et al. 2013</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Stimulates phosphorylation of PKCζ, which translocates glucose into adipocytes via GLUT4 in subcutaneous WAT (humans-lean)</td>
<td>?</td>
<td>+</td>
<td>Corbould et al. 2007</td>
</tr>
<tr>
<td>Leptin</td>
<td>Decrease transcript levels (SGBS cells; rats-SQ WAT only)</td>
<td>+</td>
<td>+</td>
<td>Machinal et al. 1999, Horenburg et al. 2008</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Increased transcript levels (NHP; rats-visceral WAT only)</td>
<td>+</td>
<td>-</td>
<td>Machinal et al. 1999, Varlamov et al. 2012</td>
</tr>
<tr>
<td>Adiponectin</td>
<td>Decreased secretion (3T3-L1 cells)</td>
<td>-</td>
<td>-</td>
<td>Nishizawa et al. 2002</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>No effect on secretion (SGBS cells)</td>
<td>-</td>
<td>-</td>
<td>Horenburg et al. 2008</td>
</tr>
<tr>
<td>T+E2</td>
<td>Lipolysis/lipogenesis</td>
<td>Inhibits adipogenesis (human-SQ)</td>
<td>+</td>
<td>+</td>
<td>Chazenbalk et al. 2013</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Increased insulin-stimulated fatty acid uptake (NHP)</td>
<td>-</td>
<td>+ (PCOS)</td>
<td>Yilmaz et al. 2009</td>
</tr>
</tbody>
</table>

WAT, white adipose tissue; NHP, non-human primate; HSL, hormone-sensitive lipase; Plag1, zinc finger transcript factor 1; ADIPOR2, adiponectin receptor 2; GLUT4, glucose transporter type 4; PKCζ, protein kinase C zeta; SGBS cells, Simpson–Golabi–Behmel syndrome cells; SQ, subcutaneous.
Female ArKO mice fed a high-fat diet develop increased body weight and body fat percentage similar to what was seen in control OVX mice. Additionally, DHT decreases subcutaneous WAT and cholesterol levels in OVX mice (Fagman et al. 2015). Similarly, female ArKO mice, which are estrogen deficient but still have low levels of circulating androgens, weigh more and have increased visceral WAT mass compared with controls (Van Sinderen et al. 2015). Such findings implicate the balance of estrogens and androgens in the control of adipose tissue lipogenesis in females. In contrast, in women with chronic androgen excess, such as PCOS patients, plasma testosterone levels are correlated with androgen levels and increased visceral WAT mass compared with controls (Van Sinderen et al. 2015). The increased WAT mass is associated with an increased risk for gonadal tissue and accessory sex gland cancers (Anderson et al. 2001).

Hyperandrogenemia dampens lipolysis and HSL activity in visceral WAT during the luteal phase of the ovarian cycle only (Varlamov et al. 2013). By comparison, testosterone has been found to increase fatty acid uptake during the follicular phase of the ovarian cycle (Varlamov et al. 2013). Thus, the relative balance of male and female sex hormones throughout the estrous cycle affects lipolysis and lipid storage in females (Varlamov et al. 2013). Overall, androgens appear to support increased visceral WAT mass in females through the inhibition of lipolysis and the stimulation of lipogenesis (Douchi et al. 1995, Seow et al. 2009, Varlamov et al. 2013). One exception to this idea is the increased lipolysis of visceral WAT by catecholamines in PCOS patients (Ek et al. 2002). This finding, which is unique to PCOS patients, may be due to a primary lipolytic defect in this patient population (Ek et al. 2002).

Adipocyte differentiation

In many cell types, androgens demonstrate significant mitogenic capabilities (Hartgens & Kuipers 2004). Although most studies have demonstrated that androgens suppress adipocyte differentiation (Table 3), some studies have demonstrated no appreciable effect of androgens on adipogenesis in either sex (Anderson et al. 2001). One of the mechanisms by which androgens modulate adipocyte differentiation is epigenetic control. Zinc-finger protein transcription factor (Plag1), a transcriptional factor for Pparg and fatty acid-binding protein 4 (Fabp4) whose downstream promoter is imprinted, experiences downregulated transcript levels in response to both testosterone and DHT (Mirowska et al. 2014). As PPARG is a pivotal regulator of adipogenesis and androgens inhibit ZAC1 leading to decreased PPARG transcript levels, WAT mass is reduced in intact as well as orchidectomized, testosterone-replaced rats (Mirowska et al. 2014). Increased WAT mass is associated with an increased risk for gonadal tissue and accessory sex gland cancers.
(Von Hae et al. 2004). Interestingly, miR-301a, which is found in pre-adipocytes, is induced in the case of prostate cancer to promote metastasis via downregulation of AR and increased TGF-β1 transcript levels (Xie et al. 2015). Pre-adipocytes recruited to cancerous prostate tissue modulate miR-301a function to control TGF-β1/Smad/matrix metalloproteinase 9 protein levels (Xie et al. 2015).

Many studies of the effects of androgens on adipocyte differentiation have utilized the weak androgen, DHEA. This choice of androgen treatment is likely due to the fact that circulating DHEA has a negative correlation with abdominal WAT accumulation in men (Couillard et al. 2000). A recent study using primary human pre-adipocytes found that DHEA had no effect on subcutaneous pre-adipocyte differentiation but decreased adipogenesis in omental pre-adipocytes (Rice et al. 2010). The 3T3-L1 pre-adipocyte cell line has been used extensively to assess the effects of androgens on adipogenesis and has shown varying responses. Some studies have found that DHEA reduces both the proliferation and differentiation of 3T3-L1 cells (Lea-Currie et al. 1998), whereas other studies have shown no effect of DHEA on 3T3-L1 adipogenesis (Rice et al. 2010). However, 3T3-L1 cells are losing favor for the study of pre-adipocyte and adipocyte cell behavior, as they may not recapitulate the physiology of primary cells. On the other hand, non-aromatizable DHT, which is a better estimate of true androgenic action, has been found to inhibit the adipogenic differentiation of mesenchymal stem cells and pre-adipocytes from both subcutaneous and omental compartments of men (Gupta et al. 2008). Androgen administration to women results in the reduction of late-stage differentiation of pre-adipocytes to adipocytes (Chazenbalk et al. 2013). These findings suggest that excess androgens (i.e., PCOS) would decrease the lipid storage capacity in subcutaneous abdominal WAT and promote lipotoxicity (Chazenbalk et al. 2013, Keller et al. 2014). This finding occurs apart from changes in insulin sensitivity, which affect lipogenesis, particularly in PCOS patients.

**Insulin sensitivity**

The role of androgens in glucose homeostasis is sexually dimorphic, with insulin resistance and obesity occurring in hyperandrogenemic (i.e. PCOS) females but in hypoandrogenic males (Navarro et al. 2015) (Table 2). Thus, it appears that androgens are necessary for the maintenance of metabolic homeostasis in men and are detrimental to the maintenance of metabolic homeostasis in women. To this end, it is well-established that treatment of prostate cancer in men with androgen ablation therapy results in hypogonadism coupled with increased BMI and a higher prevalence of metabolic syndrome (Braga-Basaria et al. 2006). Although such clinical evidence points to a strong effect of androgens on insulin sensitivity, the exact mechanisms underlying the role of WAT in this relationship are poorly understood. Non-human primate models have provided much of the knowledge base related to the effects of androgens on insulin sensitivity in WAT (Abbott et al. 2009, Abbott et al. 2010, Varlamov et al. 2012, 2013). Castration of male macaques does not induce insulin resistance but does result in the development of small white adipocytes in WAT (Varlamov et al. 2012). However, testosterone replacement in these animals improves WAT insulin sensitivity (Varlamov et al. 2012). In female macaques, treatment with exogenous testosterone increases insulin-stimulated fatty acid uptake under conditions of high E2 (i.e. menses) and upregulates fatty acid synthase and GLUT4 transcript levels in WAT under conditions of minimal E2 (i.e. luteal phase) (Varlamov et al. 2013).

PCOS patients show tissue-specific differences in insulin resistance with adipocytes demonstrating poor insulin sensitivity but normal responsiveness (Ciardi et al. 2009). PCOS patients are hyperandrogenemic and hyperinsulinemic and demonstrate decreased amounts of GLUT4 protein on adipocyte membranes (Rosenbaum et al. 1993), which may further contribute to hyperglycemia and insulin resistance in these patients. Additionally, within WAT of hyperandrogenized PCOS patients, the epigenetic control of gene sets related to diabetes and obesity suggests a role for androgens in the epigenetic modulation of adipocyte insulin sensitivity and metabolic status (Kokosar et al. 2016). Prenatally androgenized animals have been utilized extensively as models of PCOS (Abbott et al. 1998, Veiga-Lopez et al. 2011, Walters et al. 2012). The prenatally androgenized monkey exposed to testosterone in early gestation develops visceral obesity, hyperlipidemia and an increased propensity for type II diabetes throughout the life of the animal (Abbott et al. 2009). In contrast, studies in the prenatally androgenized sheep find that liver and skeletal muscle are insulin resistant but subcutaneous and visceral WAT are not insulin resistant in fetuses or adult sheep exposed to in utero testosterone (Lu et al. 2016). However, prenatally androgenized juvenile sheep present with insulin resistance that subsequently resolves by early adulthood (Cardoso et al. 2016). Furthermore, although prenatally androgenized sheep in early adulthood are not insulin resistant, they have a large amount of small-sized adipocytes in both the visceral and subcutaneous WAT (Cardoso et al. 2016), similar to what is seen in castrated male macaques (Varlamov et al. 2012). Such findings indicate that adaptation of WAT insulin sensitivity can occur over the life of a female. Varying species differences in the response of WAT to pre- and post-natal androgens indicate the need for further research on WAT physiology in human populations.

Unlike the relationship between WAT insulin sensitivity and E2, a paucity of mechanistic studies have investigated the effects of androgens on WAT
insulin sensitivity (Corbould 2007). In vitro testosterone treatment of differentiated, subcutaneous pre-adipocytes from lean women causes insulin resistance via insulin-stimulated phosphorylation of protein kinase Cζ (PKCζ), which initiates the translocation of glucose into the cell via GLUT4 (Corbould 2007). In men, five alpha reductase, which converts testosterone to DHT, has been found to modulate insulin sensitivity as inhibition of this enzyme in WAT causes increased body fat and decreased glucose uptake (Upreti et al. 2014).

Perhaps the most surprising relationship between WAT insulin sensitivity and androgens exists for the weak androgen DHEAS. Declining DHEAS concentrations with increasing age are inversely correlated with the WAT insulin sensitivity and androgens exists for the weak subcutaneous adipocytes of male rats (decreased human subcutaneous WAT in culture, but aromatase, the other hand, DHEA increases leptin secretion from the cell via GLUT4 (Corbould 2007)). In vitro DHT stimulation results in decreased leptin protein quantities in human SGBS adipocytes (et al. 2004). Additional studies using 3T3-L1 cells have shown a decrease in adiponectin secretion in testosteronetreated cells (Nishizawa et al. 2002). Therefore, it is possible that the effects of androgens on leptin production/secretion are intimately related to the concentration of both androgens and estrogens in the body in addition to some other sex-specific factor besides sex steroids.

**Adipokine production**

**Leptin**

The effects of androgens on leptin production and secretion are variable (Tables 2 and 3). Castration results in a depot-specific modulation of Ob transcript levels, with transcript levels increasing in visceral WAT but decreasing in subcutaneous WAT (Machinal et al. 1999) and causes decreased circulating leptin levels (Yao et al. 2011). In vitro DHT stimulation results in decreased Ob transcript levels in both visceral and subcutaneous adipocytes of male rats (Machinal et al. 1999). Testosterone has also been shown to decrease leptin protein quantities in human SGBS adipocytes in vitro (Horenburg et al. 2008). However, in vivo studies in primate models have found that androgens correspond with increased transcript expression of leptin in WAT of male macaques (Varlamov et al. 2012). On the other hand, DHEA increases leptin secretion from human subcutaneous WAT in culture, but aromatase inhibitors block the secretion (Machinal-Quélin et al. 2002). This finding indicates that the increased secretion of leptin in this case is due to the conversion of DHEA to E2. Similarly, in pre-menopausal women, serum DHEAS concentration is directly correlated with leptin transcript levels in visceral WAT presumably also due to its aromatization to E2 (Fajardo et al. 2004). Some studies in PCOS patients determined that adipocyte secretion of leptin and other adipokines was related to adiposity rather than systemic androgen concentration (Lecke et al. 2011). Yet, other studies of PCOS patients found that circulating leptin concentrations were higher in PCOS patients irrespective of obesity (Pusalkar et al. 2010). It is possible that the effects of androgens on leptin production/secretion are intimately related to the concentration of both androgens and estrogens in the body in addition to some other sex-specific factor besides sex steroids.

**Adiponectin**

The effects of androgens have been minimally studied with respect to the production or secretion of adiponectin (Tables 3 and 4). Adiponectin levels decrease in response to increasing production of androgen levels at puberty in boys (Böttner et al. 2004). Application of male serum to human SGBS adipocytes results in decreased adiponectin expression, but treatment of these same cells with pure testosterone does not affect adiponectin expression or secretion (Horenburg et al. 2008). However, studies using 3T3-L1 cells have shown a decrease in adiponectin secretion in testosteronetreated cells (Nishizawa et al. 2002). Therefore, it is possible that the cell lines in which in vitro studies were conducted do not recapitulate in vivo physiology. In vivo studies demonstrate a definitive role for testosterone in adiponectin regulation. Castration (Nishizawa et al. 2002) and hypogonadism (Lanfranco et al. 2004) cause increases in serum adiponectin concentrations, yet treatment with testosterone replacement therapy decreases adiponectin concentrations. Additionally, miR-375 transcript levels are downregulated in response to androgens (Kraus et al. 2015). In response to suppressed miR-375 activity, adiponectin receptor 2 transcript levels are upregulated, and there is decreased conversion of pre-adipocytes to adipocytes (Kraus et al. 2015). Therefore, androgen-regulated miR-375 plays an important role in adipokine function and adipogenesis.

**Resistin**

Surprisingly, the effects of androgens on resistin have been little studied in male rodents, which would be the ideal model for their assessment. Castration of mice induces a decrease in serum resistin concentrations in control and high-fat-fed mice, but only induces a decrease in serum resistin concentrations when mice are
high-fat fed (Floryk et al. 2011). Such findings indicate that although androgens are related to the modulation of resistin secretion, they are not the only factors to modulate this adipokine. Similarly, testosterone replacement therapy for hypogonadal men does not affect resistin concentrations in circulation (Kapoor et al. 2007). Resistin has been examined cursory in the PCOS research field to date, which is surprising given its potential role in the modulation of insulin sensitivity (Steppan et al. 2001, Kim et al. 2004). Furthermore, reports in the literature regarding how resistin might differ between PCOS patients and normal women are not in agreement. Some studies found no difference in circulating resistin concentrations in PCOS patients (lean or obese) and normal women (Panidis et al. 2004, Svendsen et al. 2012). However, other studies found that resistin concentrations (Yilmaz et al. 2009) and transcript levels in adipocytes were higher in PCOS patients than those in controls (Seow et al. 2004).

Conclusion
The role of sex steroids in WAT function is complex and multifaceted. Both the concentrations of androgens and estrogens in circulation as well as other sex-specific factors appear to affect the sexually dimorphic modulation of WAT function. Utilization of clinical data, diverse animal models and in vitro cell culture studies has increased our knowledge of the relationship between sex steroids and WAT function. The differential effects of menopause, hypogonadism and PCOS on the modulation of WAT metabolism. Interestingly, high-fat diet appears to interact with sex steroids to apply an additional layer of control over adipose tissue function (Floryk et al. 2011, Gorres et al. 2011, McInnes 2012, Metz et al. 2016). As the obesity rate continues to rise worldwide, disproportionately affecting women (Flegal et al. 2016), it is likely an increased importance will be placed upon understanding the mechanistic control of WAT function. In particular, it is not clear whether or how androgens promote insulin resistance in females. Furthermore, whether hyperandrogenemia and high-fat diet-induced obesity interact synergistically to cause insulin resistance in WAT of females is also unknown. Future research in this area should focus on the effects of androgens on insulin signaling and glucose homeostasis in WAT of females, particularly of obese females. Additionally, examination of how the ratio of androgens and estrogens in males and females may affect WAT function is warranted. Without fully understanding the effects of sex steroids on WAT metabolism, differentiation and endocrine function, the development of new therapies for the control of obesity-associated diseases like hypogonadism and PCOS will remain difficult.

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

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