

# Sex-dependent Depot Differences in Adipose Tissue Development and Function; Role of Sex Steroids

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Men and women are different in their fat mass and distribution pattern. The gynoid-type fat distribution, accumulation in lower-body, is considered to be protective while the android-type accumulation in upper-body, both in abdominal subcutaneous and visceral depots, is detrimental. Sex-dependent depot differences in adipose metabolic and endocrine functions are thought to contribute to the sexual disparity in fat distribution as well as its association with cardiometabolic risks. Although molecular details have not been completely elucidated, available evidence shows that sex steroid hormones are important factors governing sexual dimorphism in adipose tissue distribution and hence, risks for metabolic diseases. We will review sex-dependent heterogeneities in adipose tissue properties that can link their depot-specific biology to metabolic complications in men and women. In addition, we will also review how sex steroids regulate adipose tissue biology, both development and functional characteristics, with emphasis on their depot-dependent actions.

**Key words:** Estrogen, Androgen, Fat distribution, Adipocyte biology

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## INTRODUCTION

Obesity, defined as excess of adipose tissue, is increasing worldwide. Obese individuals have higher risk of developing multiple cardiometabolic diseases including insulin resistance, type 2 diabetes, cardiovascular diseases and several types of cancer. Mammals contain numerous anatomically distinct adipose depots and disparities in the developmental origin, metabolic and endocrine function and remodeling capacity of each depot have been appreciated. Over and above total adiposity *per se*, fat distribution pattern influences systemic metabolism and female-type fat distribution is considered to be healthier. Although factors that govern this sexual dimorphism are not completely elucidated, much attention has been paid to the role of sex steroid hormones, estrogen and androgens. In addition, both genetic (e.g. presence of the X and Y chromo-

some) and epigenetic events modulate the actions of sex steroids in adipose tissue through both central and peripheral actions.

## Adipose tissues

Different sizes and shades (white, brown and beige) of adipose tissues are present in multiple locations throughout an organism. White adipose tissues (WAT) are divided into subcutaneous adipose tissue (SAT) which underlies the skin and visceral adipose tissue (VAT) which is inside of intraabdominal cavity in association with the internal organs.<sup>1</sup> The major SATs in humans are the abdominal, gluteal and femoral and intraabdominal depots are omental (hangs off the stomach), mesenteric (associated with the intestine), epiploic (along the colon) and epicardial (around heart). Adult humans also contain brown adipose tissue (BAT) as well as inducible brown, brite/beige adipose tissues. Brown and brite/beige

beige compared to white adipocytes contain more mitochondria and express uncoupling protein 1 (UCP1) and hence, they are thermogenetically more active and dissipate energy as heat. Intense research is going on whether increasing BAT and brite/beige adipocytes has beneficial effects, improving metabolic complications and reducing body weight.<sup>2</sup> Each adipose depot differs in their cellular compositions and physiology which affect their metabolic and endocrine properties and their influence on systemic health.<sup>1</sup>

### Female type fat distribution is associated with lower risk of developing cardiometabolic diseases

It is well known that women and men differ in fatness. Women generally have higher fat mass than men; women with body mass indexes (BMI) of 20-25 kg/m<sup>2</sup> contain 25-30% body fat, while men with the same BMI have 10-20% body fat.<sup>3</sup> In addition, premenopausal women are characterized by less VAT and more fat accumulation in the lower body, gluteal-femoral region, whereas men accumulate more fat in the central regions, both abdominal subcutaneous and visceral fat (Fig. 1). The reasons why women have more fat mass and preferential accumulation in the femoral-gluteal region is not known, but one hypothesis posits that women reserve more energy in preparation for reproduction and lactation.<sup>4</sup> Nevertheless, these sexual-dimorphisms in fat distribution are thought to help explaining men having greater risks of developing cardiometabolic diseases than premenopausal women.<sup>5</sup>

In addition to the differences in total mass and distribution pattern in WAT, women have higher amount and activity of BAT than

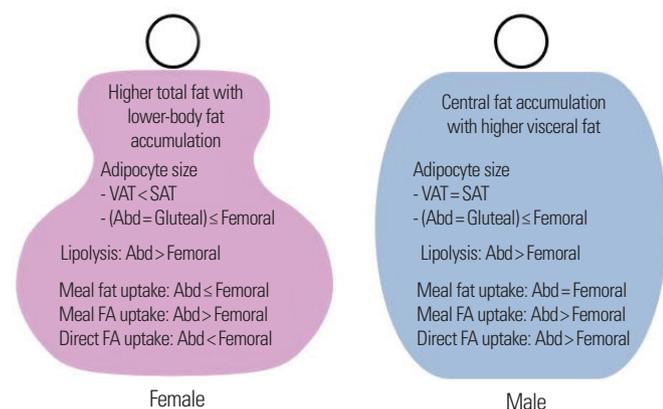
men.<sup>6</sup> Expression levels of mitochondrial genes and UCP1 in SAT are also reported to be higher in women than men<sup>7</sup>, suggesting that women have more brite/beige adipocytes in their SAT. In addition, women compared to men have higher potency to gain BAT-like properties in perirenal adipose tissue.<sup>8</sup> Women are more sensitive to cold<sup>8</sup> and this is likely to explain the higher beige and brown fat activity in women. Whether these sexual dimorphisms in brown and brite adipose tissue amount contribute the lower risk of metabolic diseases in women is not known.

### Depot-differences in adipose cellularity and remodeling capacity: potential sexual dimorphism in adipose expansion capacity

Adipocytes constitute most of the volume in adipose tissues and the size of a fat depot is determined by both the number and size of adipocytes. Adipocyte number is regulated by the balance of new fat cell formation (adipogenesis) and death of existing adipocytes and the size of adipocytes is determined by the amount of lipid stored, which in turn is regulated by the balance of storage and breakdown of lipid. Sex-dependent depot differences in the number and size of adipocytes have been reported (Fig. 1). Adipocyte size is greater in abdominal subcutaneous than visceral in women, while they are similar in size in men and extremely obese women.<sup>1</sup> Adipocyte size is generally bigger in femoral than abdominal and similar between abdominal and gluteal adipose tissues in both sexes.

Adipose tissue expansion during normal growth and in response to energy excess occurs by increasing the number (hyperplasia) and/or size (hypertrophy) of adipocytes. Hyperplastic growth is considered to be healthier while hypertrophic expansion leads to adipocyte death and high inflammation. Studies in rodent models suggest that sex-dependent depot differences in remodeling capacity through hyperplasia vs. hypertrophy impacts systemic metabolism. In response to energy excess, male gonadal depot grows mainly through hypertrophy, while female gonadal and subcutaneous depots in both sexes expand through both hypertrophy and hyperplasia.<sup>9,10</sup> As a result, adipocyte death and inflammation is much less in the gonadal depot from female compared to male mice and age-matched female mice are far less susceptible to insulin resistance caused by high fat diet-induced obesity.<sup>9,10</sup>

Similar to the results from rodent studies, a study indicates that



**Figure 1.** Sexual dimorphism in fat distribution and adipose tissue biology. VAT, visceral adipose tissue; SAT, subcutaneous adipose tissue; Abd, abdominal; FA, fatty acid.

hyperplasia is more predominant in subcutaneous than omental depot, while adipocyte hypertrophy is observed both in omental and subcutaneous depots of obese women.<sup>11</sup> Numbers of preadipocytes as well as their adipogenic capacity is reported to be higher in subcutaneous than omental human adipose tissues<sup>12</sup>, which may contribute to the hyperplasia in the subcutaneous depot. Higher adipocyte death and inflammation in visceral than subcutaneous depots have been reported in humans<sup>13,14</sup>, supporting the hypothesis that expansion through hyperplasia is healthier. Sex-dependent depot differences between visceral and subcutaneous depots in adipose expansion capacity and their contribution to systemic metabolism in humans are not known.

The number of adipocytes in both upper and lower body subcutaneous fat increases significantly by BMI category in women, but not in men.<sup>15</sup> Further, a recent study showed that fat cell size is bigger in men than women, while fat cell number is greater in women than men in the abdominal depot of non-obese subjects.<sup>16</sup> These results suggest that abdominal fat expansion is more hypertrophic and less hyperplastic in men than women in non-obese subjects. In addition, it has been reported that depot difference in the number of adipocytes between lower and upper body fat diminishes with increasing BMI in men but become more pronounced in women<sup>16</sup>, implying preferential hyperplastic expansion in the lower body depot of women with increasing degree of obesity. Similarly, Tchoukalova et al.<sup>17</sup> reported that premenopausal women compared to men have a greater number of early differentiated adipocytes specifically in the femoral than abdominal depot.

Only few studies have measured depot differences in adipocyte kinetics in vivo. The rates of adipose stem cell and adipocyte formation in vivo are reported to be higher in the femoral compared to abdominal depots<sup>18</sup> and after 8-week overfeeding, the rates of adipocyte formation is higher in the femoral than abdominal subcutaneous.<sup>19</sup> Thus, available data suggest that the rates of adipocyte generation are higher in the lower body than upper body subcutaneous depot. Whether these depot differences in adipocyte kinetics differ between women and men are not known.

### Role of sex steroid hormones in adipose tissue development

Sex-dependent differences in fat mass and distribution are observed as early as in puberty and age-related decline in sex steroids in humans is associated with more fat accumulation in central regions, indicating the importance of sex steroid hormones in adipose tissue development and distribution. Other factors are also important and Chen et al.<sup>20</sup> reported that the number of X chromosomes as well as Y chromosome, independent of sex steroid hormones, affects adiposity and metabolism in mice. The effects of sex steroids on adipocyte development and function have been investigated in both in vitro cell culture models and in vivo studies using gonadectomy and replacement/supplementation of hormones (Table 1). Sex steroids mediate their biological actions through their nuclear receptors, estrogen receptor (ER) and androgen receptor (AR), and knockout and overexpression transgenic models have been generated to understand the molecular details of

**Table 1.** Sex steroid regulation of adipose biology

	Estrogen	Androgen
Adipose development		
Proliferation of preadipocytes	↑ Greater effects in preadipocytes isolated from SAT vs. VAT and females vs. males	Inconsistent results; no effects at low concentration <sup>29</sup> or ↓ at high concentration <sup>33</sup>
Adipogenesis	In vitro, inconsistent results; ↑ <sup>28</sup> & ↓ <sup>30</sup> In vivo ↓ <sup>25,23</sup>	In vitro, ↓; effects are greater in preadipocytes from VAT vs. SAT <sup>34,35</sup> In vivo ↓ <sup>38,39,40</sup>
Adipose metabolic function		
Lipid uptake and storage	↓ Lipogenesis and LPL activity <sup>22</sup>	↓ Meal fat in VAT <sup>51</sup> and abdominal SAT <sup>49</sup> Inconsistent results; no effect <sup>49</sup> or ↓ FA storage in femoral <sup>53</sup>
Lipolysis	↑ Lipolysis <sup>22</sup>	Inconclusive
Adipose endocrine function		
Leptin	↑ Secretion from adipose tissues obtained from females, but not males <sup>57</sup>	↓ Gene expression and secretion <sup>58</sup>
Adiponectin	Inconsistent results <sup>60,61</sup>	↓ Secretion of total and HMW <sup>66</sup>

SAT, subcutaneous adipose tissue; VAT, visceral adipose tissue; LPL, lipoprotein lipase; FA, fatty acid; HMW, high molecular weight.

how estrogen and androgen regulate adipose tissue development in vivo. We briefly discuss the results from these studies with focus on depot-dependent effects.

### Effects of estrogen on adipose tissue development

Ovariectomy increases, while estrogen replacement after ovariectomy decreases, fat mass in rodents.<sup>21,22</sup> Impairment in estrogen signaling through knockout of ER $\alpha$ <sup>23</sup> also leads to obesity in both male and female mice. Further, decline in serum estrogen levels after menopause is associated with development of visceral obesity<sup>24</sup> whereas hormone replacement therapy in post-menopausal women reduces abdominal obesity.<sup>25</sup> These data suggest that estrogen is a negative regulator of fat mass in vivo. 2-week transdermal estradiol treatment in post-menopausal women increases preadipocyte differentiation in femoral but not in abdominal fat<sup>26</sup>, indicating depot-specific effects on adipocyte development. A recent study using a mouse model that can track adipogenesis in vivo showed that estrogen is one of the factors that contribute to the sex dependent depot-differences in adipocyte development.<sup>27</sup> Estrogen has been shown to consistently stimulate preadipocyte proliferation with greater effects in preadipocytes isolated from subcutaneous than intraabdominal depots and female than male adipose tissues<sup>28,29</sup>, indicating depot- and sex-dependent effects of estrogen on the proliferation of preadipocytes. Results from in vitro experiments testing the effects of estrogen on adipocyte differentiation are controversial and both suppression<sup>30</sup> and enhancement of adipogenesis by estrogen<sup>28</sup> have been reported. Estrogen can modulate fat mass and metabolism through its actions through the central nervous system<sup>31</sup> and hence, it is not clear whether estrogen regulates fat mass by directly affecting adipocyte development.

### Effects of androgens on adipose tissue development

Hypogonadism in men is associated with central obesity and increasing testosterone concentration in men leads to reduction in total fat mass<sup>32</sup>, suggesting that androgens are important regulators of fat mass as well as fat distribution in men. Dose dependent effects of androgens on the proliferation of preadipocytes, no effects at low concentration<sup>28</sup> and inhibitory at high concentrations<sup>33</sup>, are reported. Androgens have been shown to consistently inhibit adipogenesis in multiple cell types including human preadipocytes in vitro<sup>34,35</sup>

and hence, through this mechanism androgens could reduce fat mass. The expression levels of AR mRNA and androgen binding capacity are higher in intraabdominal than subcutaneous depots<sup>36,37</sup> and androgen-mediated suppression of adipogenesis is more pronounced in preadipocytes isolated from omental than subcutaneous adipose tissues.<sup>34,35</sup> Through this depot-specific inhibition of adipogenesis, androgens may preferentially reduce VAT. Mixed results are however, reported whether androgen treatment leads to preferential loss of abdominal or visceral adiposity in humans.<sup>38-40</sup> Duration of treatments and age of subjects vary between studies and further careful studies are needed to reach a conclusion.

While estrogen appears to have beneficial roles in the regulation of body fat and metabolic diseases in males both in rodents<sup>41</sup> and humans<sup>42</sup>, male sex steroids have opposite effects in females. Women with polycystic ovary syndrome exhibit hyperandrogenism concomitant with abdominal and visceral obesity.<sup>43,44</sup> In addition, treatment with an anabolic steroid with weak androgenic activity leads to visceral fat accumulation whereas treatment with an anti-androgen results in significant less subcutaneous fat loss in obese post-menopausal women.<sup>45</sup> Studies from rodent models also support that androgens have sex-dependent actions with unfavorable effects in females. Single pulse of testosterone given to female mice within 24 hr postnatal periods results in greater body weight and fat mass that are sustained throughout adult life<sup>46</sup>, suggesting that testosterone-mediated epigenetic programming during early life may lead to development of obesity in female mice in their later life. Further studies are needed to elucidate the mechanisms through which androgens regulate adiposity and metabolic diseases differentially between males and females.

### Sex-differences in adipose tissue function

Adipose tissue is highly active metabolic and endocrine organ. Adipocytes store excess energy as neutral lipid and release it as non-esterified fatty acids (FAs) and glycerol when body demands increase during starvation or exercise. Adipose tissue also secretes numerous peptide hormones, cytokines and bioactive metabolites, that are collectively called as adipokines. Sex differences in adipose metabolic and endocrine functions are beginning to be elucidated and thought to contribute to the sex-dependent differences in fat distribution as well as risks for developing cardiometabolic diseases.

### Sex-differences in adipose lipid metabolism

The net increases or decreases in adipocyte size and hence, fat mass is determined by the balance between uptake and storage of FAs into triacylglycerol (TAG) and breakdown of TAG and release through lipolysis. To address the mechanisms underlying sex differences in body fat distribution, Jensen's group measured *in vivo* lipid kinetics. Their studies showed that lower body fat has lower rates of lipolytic rates than upper body per unit mass of tissue in both sexes and women have higher rates of FA release from abdominal subcutaneous than men (Fig. 1).<sup>47</sup> Meal fat uptake is reported to be greater in lower body vs. upper body fat of lower-body obese women, while they are similar between the two depots of upper-body obese women and men.<sup>48</sup> Others show that meal FA uptake is greater in upper body than lower body SAT in both sexes.<sup>49,50</sup> These findings indicate that the meal fat storage may not explain sex-dependent body fat distribution. In women, however, the majority of FA is taken into subcutaneous fat, while in men, a significant portion is taken into visceral fat<sup>50</sup>, consistent with the fact that men storing fat more in visceral depots while females storing more in subcutaneous regions. Direct FA uptake is also greater in abdominal than femoral SAT in men, while it is higher in femoral than abdominal depot of women. Thus, direct FA uptake and possibly meal uptake rather than lipolytic rates may explain sex-dependent body fat distribution.

Estrogen deficiency in women resulted in a 10-20% increase in lipolysis whereas estrogen repletion does not affect adrenergic stimulated lipolysis.<sup>47</sup> Estrogen treatment in ovariectomized mice inhibits lipogenesis and increases adipocyte lipolysis, which in results leads to the reduction in adipocyte size.<sup>22</sup> Estrogen is also a potent suppressor of lipoprotein lipase (LPL) gene transcription. These results suggest that estrogen may decrease fat mass by reducing lipid accumulation. Whether estrogen plays a role in meal fat or direct FA uptake is not known.

The effects of testosterone on lipolytic rates have been inconclusive and reduction in abdominal subcutaneous and visceral lipolysis as well as no effects are reported.<sup>47</sup> Testosterone treatment decreases meal fat incorporation into TAG in VAT preferentially<sup>51</sup> and suppresses meal fat uptake into abdominal without affecting in femoral depots.<sup>49</sup> These studies provide evidence that testosterone may preferentially decreases TAG storage in central depots, which

could explain age-associated development of central adiposity in men. However, conflicting results are also reported. Testosterone supplementation for 2 years in elderly men increases meal fat storage into upper- vs. lower-body fat.<sup>52</sup> In addition, acute testosterone deficiency results in greater femoral adipose tissue meal FA storage rates, LPL activity, and acyl-CoA synthetase activity, indicating that testosterone plays a tonic role in restraining FA storage in femoral adipose tissue.<sup>53</sup>

### Sex differences in adipose endocrine function

Adipose tissue secretes multiple bioactive molecules. Several factors including leptin and adiponectin are derived from adipocytes while others especially proinflammatory cytokines are produced from mainly non-adipocytes present within stromal vascular fraction. We will discuss the two major adipokines, leptin and adiponectin, of which serum levels are known to be higher in women than men.

### Sex differences in leptin levels

Leptin is an endocrine hormone that regulates multiple aspects of physiology including energy homeostasis and immunity. Serum levels of leptin is higher in women than men and these sex differences in serum leptin levels begin even before birth, become more pronounced at later stages of sexual development and persist throughout life.<sup>54</sup> Sex difference is not due to the fact that women have higher amount of fat mass and women have higher leptin levels even after correcting differences in fat mass. Rather, sex steroid hormones appear to be important determinants of sexual dimorphism in leptin levels. In boys, testosterone levels are negatively correlated, while in girls, estradiol levels are positively correlated with serum leptin levels. Further, leptin concentrations in postmenopausal women are lower than in premenopausal women.<sup>55,56</sup> These data indicate that positive regulation by estrogen and negative regulation by androgens. Corroborating evidence has been obtained in *in vitro* studies using adipocyte culture models. Estrogen increases leptin secretion from adipose tissues from females but not those from males.<sup>57</sup> Conversely, both testosterone and dihydrotestosterone decrease leptin gene expression and secretion from human adipocytes.<sup>58</sup> Physiological significance of women having higher serum leptin levels is not known.

### Sex differences in adiponectin levels

Adiponectin is an anti-inflammatory adipokine and known to improve insulin sensitivity by suppressing hepatic glucose production and enhancing fatty acid oxidation in the liver and skeletal muscles. Men have lower adiponectin levels than BMI and age matched women.<sup>56</sup> Sexual dimorphism in circulating adiponectin levels develops during pubertal development in association with androgen levels<sup>59</sup>, indicating sex steroids regulation of adiponectin levels. Castration increases adiponectin levels and testosterone supplementation lowers serum adiponectin levels.<sup>56</sup> Men with hypogonadism have significantly higher serum levels of total adiponectin, which are reduced after testosterone replacement therapy.<sup>60</sup> In addition, testosterone decreases adiponectin secretion in cell culture models.<sup>56</sup> Controversial results are reported from ovariectomy and estrogen treatment on adiponectin levels both in vivo and in vitro.<sup>60,61</sup> Thus, low adiponectin levels in men vs. women appear to be predominantly mediated by male sex steroid hormones.

Adiponectin circulates as several oligomeric complexes, high, medium and low molecular weight forms and the ratio of high molecular weight (HMW) forms to total adiponectin are critical in determining insulin sensitivity and cardiovascular diseases. Sex differences in serum adiponectin are mainly due to the differences in its HMW form while the other two forms are not different.<sup>60</sup> This sexual dimorphism in HMW form of adiponectin is also mediated by androgens as testosterone selectively reduces the circulating levels of HMW adiponectin by inhibiting its secretion from adipocytes, while estrogen has no effect.<sup>56</sup> Selective reduction of HMW adiponectin in addition to total adiponectin by androgens might be one of the mechanisms that contribute to the higher risk of cardiometabolic diseases in males.

### CONCLUSION

Women and men differ in their fatness and fat distribution pattern. Women contain higher total fat mass, less visceral fat, and lower-body fat accumulation that can explain a significant portion of sexual dimorphism in cardiometabolic risks. Preferential fat accumulation in lower-body depot of women is thought to be explained by higher rate of direct meal fat uptake and FA uptake. In addition, available data suggest that subcutaneous vs. visceral and lower-body

vs. upper-body depots particularly in females expand preferentially through hyperplasia, which is considered to be healthier. It is well known that sex steroids are important regulators of adipose biology, both adipocyte development and function, although molecular details of their actions are not completely elucidated. Estrogen appears to be a negative regulator of adiposity in vivo which may be explained in part by estrogen-mediated lipid accumulation. Androgens are known to suppress adipogenesis and lipid accumulation in men and preferential reduction in visceral depots by androgen treatment in men are noted. Disparities in serum levels of the two major adipokines, leptin and adiponectin, also exist. Women have higher levels of leptin and adiponectin and these sexual dimorphisms are known to be mediated by sex steroid hormones. Further studies of sex-dependent depot differences in adipose biology and their relation to systemic health in humans are needed.

### CONFLICTS OF INTEREST

The authors declare no conflict of interest.

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