Chapter 1A

General Introduction

Sex Difference in Adipose Tissue Biology: Gonadal and Adrenal Steroid Perspectives

Manuscript in preparation

1

1. Introduction

Excessive or abnormal fat accumulation in the body causes overweight and obesity. The latest report by the World Health Organization revealed that the prevalence of obesity worldwide has almost tripled from 1975 to 2016 and that 39% of adults were overweight and 13% or over 650 million were obese in 2016 (1). Obesity is a risk for many noncommunicable diseases such as insulin resistance and type 2 diabetes mellitus, cardiovascular diseases, hypertension, and also some types of cancer (1,2). Studies have demonstrated that chronic obesity leads to adipose tissue dysfunction and systemic low-grade inflammation. Intriguingly, this obesity-induced inflammation likely causes disrupted energy homeostasis, resulting in insulin resistance and other metabolic diseases, such as cardiovascular diseases and hypertension (1,3)

Although women in most ethnic groups have a higher prevalence of obesity than men, women in their reproductive age have a lower incidence of metabolic diseases than men. This sex difference is attenuated after women become postmenopausal (4-7). The relatively higher metabolic risk of men and postmenopausal women is correlated with the distribution of adipose tissue to the visceral part of the body, called visceral obesity. In other words, the subcutaneous fat accumulation pattern of premenopausal women is associated with a lower metabolic risk (8,9). Hence, it is important to study the mechanisms that contribute to the sexual dimorphism in adipose tissue biology since it may lead to a better sex-specific treatment option for combating this obesity pandemic. Moreover, insight about how the sex differences in adipose tissue distribution is regulated might reveal novel treatment modalities to combat obesity and the associated metabolic complications.

Differences in gonadal and adrenal steroid levels and responses to those steroids play an important role in the sexually dimorphic pathophysiology of obesity and metabolic diseases. It is evident that males are more prone to metabolic consequences of chronic stress than females (10,11). As a response to stress, glucocorticoid (GC) is synthesized and secreted under the control of the hypothalamic-pituitary-adrenal (HPA) axis (12). The hypothalamus and the anterior pituitary also regulate the sex steroid synthesis of the gonads, termed the hypothalamic-pituitary-gonadal (HPG) axis (13,14). The HPA and HPG axes are interconnected at many levels which could partly account for the sex difference in metabolic alterations. For example, sex steroids interfere with the negative feedback of the HPA axis and with the expression and function of the GC receptor (GR) (11). On the other hand, administration of corticosterone in female mice to reach maximal concentrations that occur in response to stress suppressed estradiol-induced LH secretion and completely blocked the ovarian

cycle (15). Activation of the HPA axis in male rats by repeated immersion in cold water (chronic stress) gradually reduced serum testosterone levels and disrupted spermatogenesis (16).

Other mechanisms could also contribute to sex difference in obesity and adipose tissue metabolism, such as epigenetic modifications, e.g. DNA methylation, histone modifications, small single-stranded non-coding RNA [microRNA (miRNA)] interference; the presence of sex chromosomes (X- and Y-chromosomes in males, only X-chromosomes in females); and perinatal and pubertal development of the brain, known as the organizational effect of sex steroids (5,17,18). However, these topics are outside the focus of this chapter that will focus on the effects of sex steroids after puberty, the principal difference between males and females at reproductive age. The majority of studies in humans and rodents on the effects of sex steroids on metabolism and energy homeostasis have focused on the effects of estrogens and androgens in both sexes (19,20). The contribution of progesterone, another female sex hormone crucial for the luteal phase of the reproductive cycle and during pregnancy, to sex differences in energy metabolism has barely been investigated.

This chapter will provide an overview of sex differences in adipose tissue function and distribution, and the roles of gonadal and adrenal steroids therein. In addition, this chapter will touch upon the mechanisms by which gonadal and adrenal steroid hormones affect adipose tissue function and distribution in various clinical conditions associated with disturbances in gonadal and adrenal steroids.

2. Adipose tissue function, characteristics, and distribution

For a long time, adipose tissue had been considered only a passive organ for storing excess calories as triglycerides (TGs) and providing energy-rich substrates to the body when needed. However, it is currently known that adipose tissue is not merely a passive bystander, but secretes a plethora of bioactive products, termed adipokines, which regulate whole-body homeostasis and reflect its adaptation in physiological and pathological states (21). This section will discuss the types and distribution of adipose tissue as well as its metabolic and secretory functions, with a focus on sex differences in these properties. Other auxiliary functions of adipose tissue, which will not be discussed in detail, include cushioning mechanical stress such as in palms, buttocks, and soles, and providing thermal insulation for the body since thermal conductivity of adipose tissue is only about 40–50% of that of lean tissues (22).

2.1 Types and distribution of adipose tissues

Adipose tissue has traditionally been categorized into two types which serve opposite roles: lipid-storing white adipose tissue (WAT) and thermogenic lipid-burning brown adipose tissue (BAT). Many studies have demonstrated a high degree of plasticity in adipose tissues. WAT can become brown-like by, for example, chronic exposure to cold or sustained adrenergic stimulation. This process is called browning and the resulting adipose tissue is called beige (or brite [brown-in-white]) adipose tissue (23,24). In contrast, BAT can be 'whitened' by multiple factors, e.g. acclimation to chronic warm temperatures and β-adrenergic signaling impairment (25).

To store excess energy in WAT, adipose tissue can expand in size (enlargement of existing adipocytes, called hypertrophy) and/or increase cell number (forming new adipocytes from the resident progenitor cells, called hyperplasia). Hypertrophic expansion is considered detrimental since the enlarged adipocytes reach a limit of oxygen diffusion, resulting in hypoxia, inflammation, fibrosis, adipose tissue dysfunction, and subsequently insulin resistance (26-29). Hyperplastic expansion is considered a healthy adaptation and occurs together with proper angiogenesis – formation of new vasculature to supply the growing adipocytes (21,30). Adipogenesis and angiogenesis are reciprocally regulated by peroxisome proliferator-activated receptor-γ (PPARγ) and vascular endothelial growth factor (VEGF). Reducing PPARγ activity by a dominant-negative PPARγ results in reduced preadipocyte differentiation and inhibits angiogenesis and an immunologic inhibition of VEGF blocks vessel formation and inhibits adipocyte differentiation (31), showing that these two processes are reciprocally regulated.

Concerning body fat distribution, WAT can generally be divided into two anatomical depots: subcutaneous depots, e.g. abdominal subcutaneous and gluteofemoral depots for humans or anterior (axillary) and posterior (inguinal) subcutaneous depots for rodents; and visceral depots, e.g. omental, mesenteric, gonadal, and retroperitoneal depots (32,33). Depot difference in WAT expansion is evident in rodent and human studies. In mice fed a high-fat diet (HFD), subcutaneous WAT expands more significantly by hyperplasia while visceral WAT shows a greater hypertrophic expansion. Indeed, adipose progenitor cells, responsible for adipogenesis, are more abundant in the subcutaneous depot than the visceral depot of mice (34). Likewise, adipocyte progenitor cells isolated from a subcutaneous depot of mice differentiated better by a standard *in vitro* culture protocol and express higher levels of pro-adipogenic genes and lower levels of anti-adipogenic genes than cells isolated from a murine visceral depot (35).

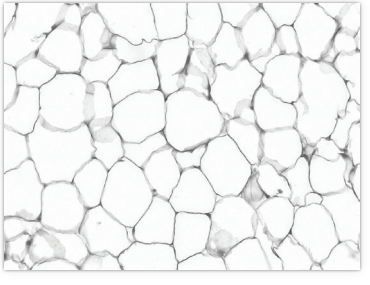
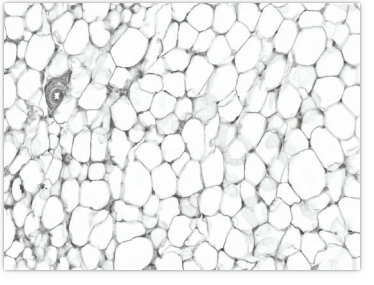
Also in humans, obese subjects have more preadipocytes in their abdominal subcutaneous WAT than in their omental visceral WAT. Isolated preadipocytes from the subcutaneous depot also differentiated more efficiently into mature adipocytes and expressed higher levels of adipogenic transcription factors than those isolated from the visceral depot (36,37). In morbidly obese subjects [body mass index (BMI) > 40 kg/m^2], the amount of visceral fat in insulin-resistant individuals is negatively correlated with insulin sensitivity: insulin-resistant obese subjects have significantly higher waist circumference and visceral fat accumulation than insulin-sensitive obese persons (27). Overall, visceral fat accumulation is associated with an increased risk of metabolic complications of obesity and clinical practice guidelines support measurement of waist circumference (a reasonable proxy for visceral obesity) as an indicator for the risk to develop metabolic diseases (38-41). A brief summary of adipose tissue expansion and its adaptive mechanisms is presented in **Table 1**. Of note, some characteristics will be discussed in following sections of this chapter.

2.1.1 Sex difference in adipose tissue distribution

Throughout life, women typically have a higher percentage of body fat than men at an equivalent BMI (42-44). Body composition also shows many sex-dependent characteristics including regional distribution. Women tend to accumulate body fat around hips and thighs, called a gynoid or pear-shaped pattern, whereas men accumulate fat around the abdomen, termed an android or apple-shaped pattern (8,9,44). Subsequently, women and female rodents have relatively more subcutaneous WAT and less visceral WAT than age-matched men and male rodents (19,45-47). One of the possible mechanisms accounting for this sex difference is that women have a higher activity of lipoprotein lipase (LPL) in subcutaneous WAT than men (48). LPL is the enzyme involved in hydrolysis of TGs to yield fatty acids that can be taken up by adipocytes. Upon uptake, the fatty acids will be re-esterified with glycerol into TGs and stored in the adipocyte (see section 2.3 for more detail). The sex-dependent pattern of fat distribution is apparent after pubertal development, indicating a role of sex steroids in fat accumulation (49). Moreover, this sex-dependent fat accumulation diminishes at an older age; in other words, women gain more visceral fat, and thus their body fat distribution becomes android-like, after menopause (50,51).

Inflammation in adipose tissues typically precedes systemic metabolic inflammation and occurs when proper adipogenesis is limited (52). This is especially the case in visceral depots, the predominant fat depot of males. A mouse study demonstrated that female mice had a higher ratio of adipose progenitor cells to adipocytes in gonadal and inguinal WATs than male mice (53).

Table 1 WAT expansion and relevant characteristics

Characteristics	Mode of WAT Expansion	
	Hypertrophy	**Hyperplasia**
Adipocyte adaptation	Increased cell size/volume	Increased cell number
Adipose depot size	Enlarged	Enlarged
Storage type	Metabolically detrimental	Metabolically healthy
Main anatomical sites	Visceral depots	Subcutaneous depots
Histological appearance (similar magnification)		
Blood flow	Relatively insufficient	Sufficient due to angiogenesis
Hypoxic/necrotic adipocytes	Present	Absent or minimal
Major immune cell infiltration	Pro-inflammatory cells	Anti-inflammatory cells
Inflammatory cytokine secretion	High	Low
Adiponectin production	Highly decreased	Increased
Leptin production	Highly increased	Relatively decreased
Adiponectin/leptin ratio	Low	High
Response to insulin	Insulin resistant	Insulin sensitive

Fed a HFD for 14 weeks, males expanded the gonadal depot only by hypertrophic expansion accompanied by an accumulation of macrophages and other immune cells, whereas females expanded the gonadal depots by both hyperplasia and hypertrophy. This difference coincided with more weight gain and a worse metabolic profile in males than in females after the HFD challenge (53). Other studies also confirmed that females had less intra-abdominal fat accumulation than males although both sexes consumed an equal amount of HFD. These sex-differential effects were absent when the female sex steroids were depleted by ovariectomy. Treatment with 17β-estradiol (E2) in the HFD-fed ovariectomized mice restored the visceral deposition to the amount of non-ovariectomized females.In addition, gonadal adipocytes were larger in males and ovariectomized females than in non-ovariectomized females and E2-treated ovariectomized females, suggesting protective properties of E2 on hypertrophic expansion (54, 55). Another study in male and female mice demonstrated that an obesogenic (high-fat and high-sugar) diet resulted in greater diet-induced obesity, higher mesenteric fat accumulation, and a worse metabolic profile in males. Intriguingly, E2 treatment feminized and attenuated the diet-induced disturbances of male mice (56). Altogether, these data in humans and rodents suggest direct effects of sex steroids on adipose tissues, which will be discussed in more detail in section 3.

2.2 Adipogenic programming

Adipogenesis is a complex process in which multipotent mesenchymal stem cells commit to the adipogenic lineage after which these preadipocytes differentiate into lipid-containing mature adipocytes (**Figure 1**). White adipocytes and classical brown adipocytes (as found in interscapular BAT depots in rodents and human infants) are derived from different progenitor lineages. Brown adipocytes and skeletal myoblasts arise from the paired box 7 (Pax7)- and myogenic factor 5 (Myf5)-expressing progenitors. The transcriptional regulator PR domain-containing 16 (Prdm16) controls the differentiation towards brown adipocytes, whereas myogenic factors, i.e. Myf5 and the myogenic differentiation (MyoD), repress Prdm16 and promote myoblast differentiation (57,58). White and beige adipocytes are derived from Pax7- and Myf5-negative progenitors, but activation of the transcription factor early B-cell factor 2 (Ebf2) commits the progenitor cells to a beige adipocyte lineage. Of note, *Prdm16* is an Ebf2-target gene and brown adipocytes also express Ebf2 (59). On the contrary, the transcriptional regulator zinc finger protein (Zfp423) is critical for white preadipocyte commitment since Zfp423 can bind and repress Ebf2 transcriptional activity, allowing preadipocytes to differentiate to white adipocytes (60).

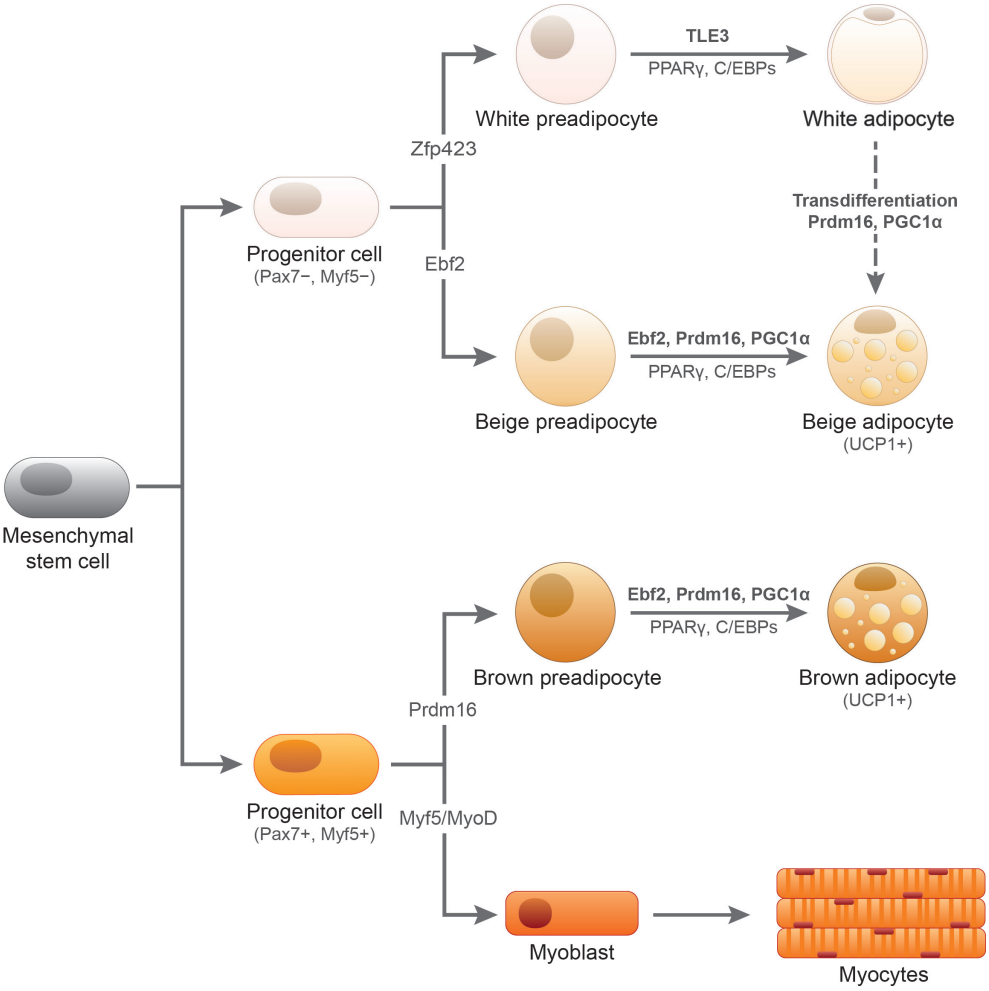


Figure 1 Adipogenesis and progenitor lineage

Classical white and brown adipocytes are derived from different progenitors. Adipogenic programming is modulated by distinct transcription factors, listed by the arrows in the diagram.

The general program of differentiation into mature lipid-containing adipocytes is regulated by the transcription factor PPARγ (the master regulator of adipogenesis) and the transcription co-activators CCAAT/enhancer-binding protein α and β (C/EBPα and C/EBPβ) (61). White preadipocyte differentiation is promoted by the transducin-like enhancer of split 3 (TLE3), whereas beige and brown preadipocyte differentiation is promoted by Prdm16 (62). During differentiation, committed preadipocytes arrest in growth, accumulate lipids, and form functional insulin-responsive mature adipocytes. Early differentiated adipocytes express PPARγ, C/EBPα or C/EBPβ, fatty acid binding protein 4 (FABP4, also known as adipocyte protein 2 [aP2]), and the insulin-responsive glucose transporter 4 (GLUT4) (30). Continuous activation of PPARγ promotes terminal differentiation by inducing a variety of differentiation-dependent target genes. Mature adipocytes then express the genes important for adipose tissue function, such as LPL, adipose triglyceride lipase (ATGL), hormone-sensitive lipase (HSL), perilipin, adiponectin, and leptin, as well as all of the early differentiation markers (30,63).

The key regulator important for brown and beige adipocyte differentiation is the transcriptional coactivator protein PPARγ coactivator 1α (PGC1α). PGC1α co-activates PPARγ and other transcriptional factors to initiate a broad program of mitochondrial biogenesis, including the induction of expression of the gene encoding the thermogenic uncoupling protein 1 (UCP1), a protein in the inner mitochondrial membrane that allows protons to leak independently of ATP synthesis and dissipate energy of substrate oxidation as heat, a unique function of brown and beige adipocytes (64,65). The thermogenic function of BAT is discussed in more detail in section 2.5. Upon prolonged cold exposure or β-adrenergic stimulation, white adipocytes can also transdifferentiate into beige adipocytes by upregulating Prdm16 and PGC1α (61,66), and thereby having a beneficial contribution to energy metabolism.

2.3 Lipid metabolism in adipose tissue

As an energy reservoir in postprandial or positive energy balance conditions, WAT depots store excess nutrient calories as TG by uptake of fatty acids from plasma or *de novo* lipogenesis. In prolonged fasting or high energy demand conditions, on the other hand, white adipocytes lipolyze the stored TGs to supply fatty acids and glycerol as energy substrates to the circulation to be used by other tissues (67). The balance in lipid storage and breakdown determines adipose tissue mass. Principle enzymes and substrates for lipid metabolism in white adipocytes are illustrated in **Figure 2**.

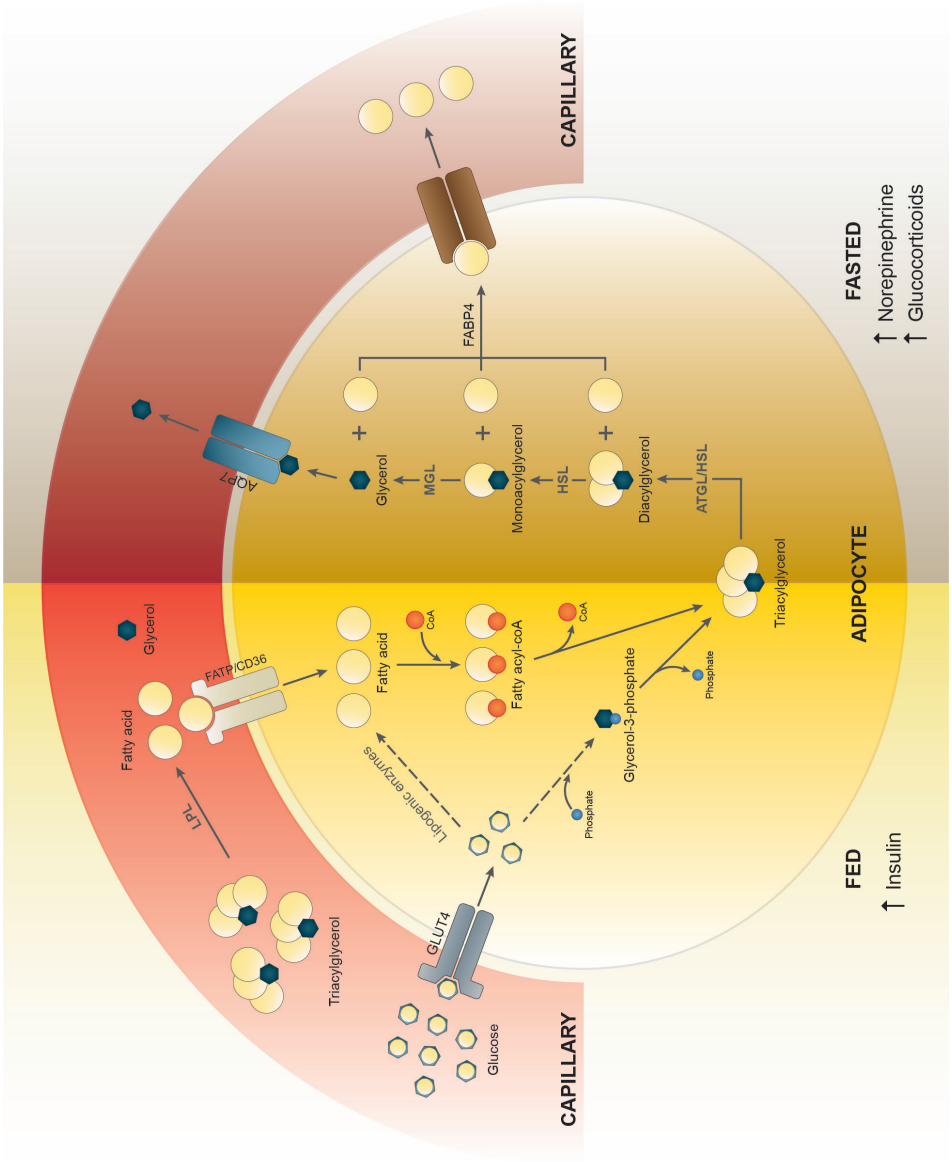


Figure 2 Lipid metabolism in white adipocytes

During physiological substrate-rich conditions, e.g. after meals, an increase in plasma insulin levels not only promotes glucose uptake but also stimulates fatty acid uptake and lipogenesis in white adipocytes (68). For the uptake, adipocytes obtain fatty acids from two TG-rich lipoproteins, namely chylomicrons (enterocyte-derived lipoproteins that transport exogenous [dietary] fats) and very low-density lipoproteins (VLDL, endogenous lipoproteins secreted by the liver). The fatty acids are hydrolyzed from the TG-rich lipoproteins by LPL after which they can be taken up through fatty acid transport proteins (FATP) or fatty acid translocase (FAT, also known as CD36). In the adipocytes, these fatty acids are activated to fatty acyl-CoAs and sequentially esterified with glycerol-3-phosphate to form TGs that are subsequently packed and stored in lipid droplets (67-69).

De novo lipogenesis contributes only marginally to the TG content of adipocytes. This pathway is less active in adipocytes than in the liver and is less important in humans than in rodents (68,70). An increase in intracellular substrate concentrations, for example by insulin stimulation, stimulates *de novo* lipogenesis in adipocytes by inducing the transcription factors carbohydrate response element-binding protein (ChREBP) and sterol regulatory element-binding protein 1c (SREBP1c), which subsequently induce many lipogenic genes such as those that encode for acetyl-CoA carboxylase 1 (ACC1), fatty acid synthase (FASN), and stearoyl-CoA desaturase 1 (SCD1) (71-73).

When the body lacks energy, e.g. upon prolonged fasting, or requires extra metabolic supplies, e.g. during exercise, lipolysis in adipocytes is induced. In this process that involves multiple lipases, a complete breakdown of one molecule of TG generates three fatty acid molecules and one molecule of glycerol as substrates for direct utilization in other tissues or for gluconeogenesis in the liver. The first fatty acid is cleaved from TG by ATGL or, to a minor extent, by HSL. The remaining diacylglycerol is hydrolyzed into monoacylglycerol and a second fatty acid by HSL. Monoacylglycerol lipase (MGL) cleaves the remaining monoacylglycerol into a third fatty acid and glycerol (67,74). Likely various transporters such as FABP4 facilitate the export of fatty acids, while aquaporin 7 (AQP7) facilitates the transport of glycerol out of adipocytes (75,76).

At basal conditions, proteins such as perilipins coat the surface of lipid droplets preventing them from lipase action (67,77). When lipolysis is required, systemic cues such as the sympathetic nervous system and the HPA axis can regulate lipase activities in many ways. The sympathetic outflow products catecholamines bind to β-adrenergic receptors and initiate signaling cascades to inactivate the protecting effect of perilipins and promote the translocation of HSL to the lipid droplets. GCs, produced in response to activation of the HPA axis, promote lipolysis in adipocytes by inducing the transcription of the lipolytic enzymes ATGL and HSL (67,74,77,78).

2.4 Secretory function of adipose tissue

Besides the well-known function as an energy reservoir, adipocytes produce and secrete various adipokines that function as autocrine, paracrine, and endocrine signaling molecules. Various systemic functions of adipokines are known, such as regulation of systemic energy homeostasis, endothelial function, insulin sensitivity, and inflammation. More than 600 adipokines have been discovered, including adiponectin, adipsin, apelin, bone morphogenetic protein 4 (BMP4), BMP7, dipeptidyl peptidase 4 (DPP4), fibroblast growth factor 21 (FGF21), interleukin-1β (IL-1β), IL-6, leptin, lipocalin 2, omentin, resistin [for rodents], retinol binding protein 4 (RBP4), tumor necrosis factor α (TNFα), and visfatin (79-81). Leptin and adiponectin are the two most studied adipokines which regulate feeding behavior and whole-body insulin sensitivity and will therefore be discussed in more detail below.

2.4.1 Leptin

Leptin was the first identified adipokine in 1994 (82), but its physiological roles had already been known since 1950, when an autosomal inherited mutation was identified in an obese mouse strain (83). This mutation, and later the gene, was originally called obese (*ob*). Another gene mutation, which turned out to encode the leptin receptor, was identified in 1966 in an obese and early-onset diabetic (*db*) mouse strain (84). The leptin-deficient *ob/ob* mice and the leptin receptor-deficient *db/db* mice are among the most used animal models of obesity and metabolic diseases since the shared phenotypes of these two models include profound obesity, hyperphagia, reduced energy expenditure, hyperglycemia and hyperinsulinemia (which leads to insulin resistance or diabetes depending on age and strain), and hyperlipidemia (85).

Leptin is a 167-amino acid protein produced mainly in adipose tissues, with WAT producing significantly more leptin than BAT. *Lep* mRNA expression is however detected in many other tissues as well, including skeletal muscles, stomach, placenta, and ovaries (86). Under healthy physiological conditions, excess nutrient calories promote an expansion of adipose tissues which induces leptin production and secretion. Thus plasma leptin concentrations reflect energy status and total fat mass. Leptin binds to the leptin receptors on neurons in various brain regions, including energy-control centers in the hypothalamus. Here, leptin promotes satiety signals to reduce food intake and accelerate energy expenditure in peripheral tissues through sympathetic nervous system activation. In addition, leptin also regulates lipid metabolism in WAT since sympathetic activation induces lipolysis and reduces *de novo* lipogenesis. While plasma leptin concentrations are positively correlated with the total amount of body fat, the leptin-induced inhibition of food intake is blunted in many obese subjects, indicating that obesity may reflect a leptin-resistant state (87-90).

2.4.2 Adiponectin

Adiponectin, a 247-amino acid adipokine that has a higher plasma level (μg/mL) than other conventional factors, e.g. insulin and leptin (ng/mL), was discovered in the mid 90's by many research groups giving this protein different names: Acrp30 (adipocyte complement-related protein of 30 kDa), AdipoQ, apM1 (adipocyte most abundant gene transcript 1), and GBP28 (gelatin-binding protein of 28 kDa) (91-94). Adiponectin is generally accepted as an adipocyte-specific marker produced by BAT and WAT (95,96). However, *Adipoq* mRNA expression has been detected beyond adipose tissue depots under some specific conditions, e.g. lipopolysaccharide-induced muscle inflammation (97,98).

Adiponectin displays many metabolically favorable effects and circulating adiponectin concentrations decline with increasing BMI, increasing waist circumference, and insulin resistance (89,99,100). Within morbidly obese individuals (BMI > 40 kg/m^2), the insulin-resistant group exhibits lower serum adiponectin levels than the insulin-sensitive group. Thus, lower circulating adiponectin concentration together with adipose tissue inflammation appears a good predictor of insulin resistance (27). Adiponectin is also associated with an hyperplastic expansion of adipose tissue (99). Since plasma leptin concentrations increase and adiponectin concentrations decrease in obese subjects, the adiponectin/leptin ratio is considered a reliable indicator for assessing subclinical insulin resistance, metabolic disorders, and adipocyte dysfunction (95,99,101,102).

Another unique characteristic of secreted adiponectin is that it circulates in higher-order complex forms: the high-molecular-weight (HMW) form consisting of 12–18 adiponectin molecules; the low-molecular-weight (LMW) hexamer form; and the trimer form. Distribution of adiponectin complexes contributes to distinct biological effects and HMW adiponectin is considered the active form of this adipokine. As a result, the HMW/total adiponectin ratio is considered another predictor for insulin sensitivity (103,104).

2.4.3 Extragonadal steroid synthesis

In addition to adipokines, WAT depots are an important source of extragonadal estrogen biosynthesis, through aromatization of intracellular androgens by the enzyme aromatase (CYP19A1) (105). Aromatase can convert testosterone (the main circulating male sex hormone) to E2 (the main circulating female sex hormone), or androstenedione (a weak androgen) to estrone (a weak estrogen). The aromatization of androgens in adipose tissues contributes substantially to circulating estrogen levels, especially in obese men and postmenopausal women (106,107). Moreover, aromatization might contribute to local effects of sex

steroids on adipose tissue. An imbalance in the testosterone/E2 ratio in obese men causes metabolic syndrome and hypogonadism, which will be discussed further in section 3.3.

GCs (cortisol for humans or corticosterone for rodents) can be converted from their inactive 11-keto steroid precursors (cortisone or 11-dehydrocorticosterone, respectively) by the reductase activity of the enzyme 11β-hydroxysteroid dehydrogenase type 1 (11β-HSD1) in adipose tissues. The active GCs act locally to promote preadipocyte differentiation, adipocyte hypertrophic expansion, and hence adipose tissue and systemic insulin resistance, rather than contributing to an increase in circulating GC levels (108). Interestingly, 11β-HSD1 reductase activity is higher in preadipocytes from visceral depots than in those from subcutaneous depots of male mice, supporting a role of local activation of GCs in visceral obesity (109).

2.5 Thermogenic function of BAT

BAT is a thermogenic organ that dissipates nutrient energy as heat through its classical mitochondrial protein UCP1. It was originally recognized that BAT was present and actively functioning only in human infants, small mammals, and hibernating animals to maintain body temperature without thermogenic shivering of skeletal muscles. Likewise, the concept has been that BAT in humans regresses within the first years of life, resulting in an absence of BAT in adults (110). Although the existence of activated BAT in healthy adults had been suggested in the positron emission tomography/computed tomography (PET/CT) imaging since 2002 (111), direct evidence of cold-activated glucose uptake in BAT together with UCP1-immunoreactive brown adipocytes was confirmed in 2009 (112-115). After the rediscovery of active BAT in adults, BAT has gained much attention from researchers as activating BAT is suggested a promising tool to combat the obesity pandemic.

Under physiological conditions, exposure to low ambient temperatures stimulates cutaneous thermoreceptors to transmit sensory signals to the thermocenter preoptic area of the hypothalamus. Subsequently, the hypothalamic network provides signals to stimulate the sympathetic premotor neurons that hence activate 1) cutaneous vasoconstriction (to reduce heat loss), 2) non-shivering thermogenesis in BAT, and 3) shivering in skeletal muscles (116). In humans, repeated exposure to cold (cold acclimation), e.g. 2 hours/day and 5 days/week for 4 weeks, increases BAT mass and the oxidative capacity of BAT. Furthermore, the prevalence of BAT detected by PET/CT imaging is higher during winter than other seasons and negatively correlates with outdoor temperatures (117,118).

Cold exposure induces sympathetic nerves in BAT to secrete norepinephrine that stimulates BAT thermogenesis through β-adrenergic receptors (β-ADR). Although all three types of β-ADRs (β_1, β_2, and β_3) are expressed in human and murine BAT, to date only treatment with β_3-ADR agonists, but not with non-specific β-ADR agonists, has resulted in increased BAT activity, suggesting that β_3-ADR mediates BAT thermogenesis (119-121). A single dose of propranolol (a competitive non-selective β-ADR antagonist) given to patients with a strong BAT activity signal in PET/CT imaging suppressed BAT activity in most cases (122,123). Since propranolol binds β_1- and β_2-ADRs with high affinity but has a lower binding affinity for β_3-ADR (124), the suppression of BAT activity after administration of propranolol may indicate less specificity in β-ADR subtypes regulating human BAT activity.

The acute adrenergic response of BAT enhances lipolysis through the activation of ATGL and HSL, and the fatty acids released in this process stimulate UCP1 activity (125). In addition, cold exposure stimulates the uptake of glucose and fatty acids in BAT, resulting in a decrease in circulating glucose levels and also reductions in plasma free fatty acid and TG concentrations (126). After the fatty acids are imported into brown adipocytes, they are most likely first esterified into TG and incorporated into lipid droplets after which they are hydrolyzed to be metabolized for uncoupling thermogenesis (126). This was confirmed by the presence of significantly defective BAT thermogenesis in ATGL-deficient mice upon an acute cold exposure (127), underscoring the need of intracellular TG. **Figure 3** illustrates the current hypothesis on lipid metabolism in brown adipocytes for UCP1 thermogenesis.

Interestingly, not only the transcription of genes and activation of enzymes involved in substrate turnover are upregulated, UCP1 transcription and protein abundance in BAT are also increased upon cold exposure, called adaptive thermogenesis (**Figure 3**) (126,128-130). Prolonged cold exposure also induces browning of inguinal WAT in mice, with subsequent upregulation of *Ucp1* mRNA and protein expression (59,66,128). This inducible browning process was observed in all WAT depots, but was much more pronounced in subcutaneous depots than in visceral depots (131).

2.5.1 Sex difference in BAT abundance and function

In general, research has revealed that women and female rodents have a greater BAT mass and/or higher prevalence of metabolically active BAT and have greater inducible browning of their WAT depots than men and male rodents (132,133). A retrospective study determining the prevalence of BAT by PET/CT imaging found that BAT was detectable in 328 out of 4,842 (6.8%)

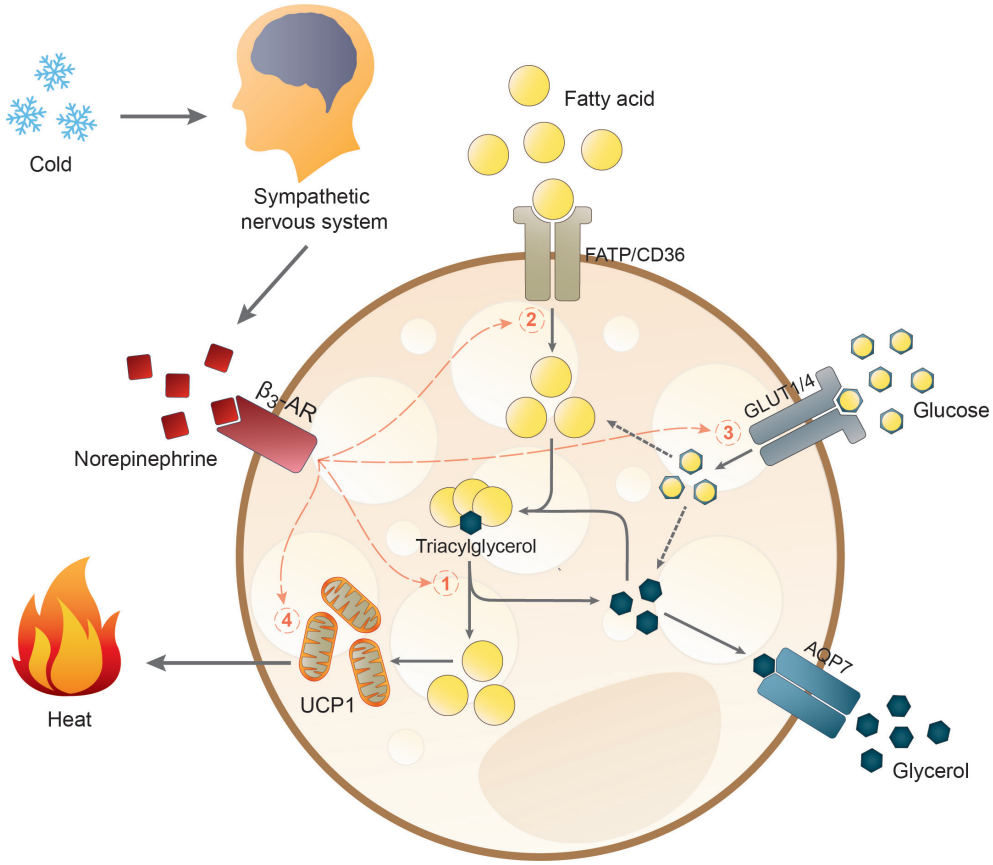


Figure 3 Activation of BAT thermogenesis and lipid metabolism in brown adipocytes

Acute thermogenic responses by β_3-adrenergic stimulation include activation of (1) intracellular lipolysis, (2) fatty acid uptake, and (3) glucose uptake. Altogether, these processes increase intracellular free fatty acid availability for mitochondrial UCP1 thermogenesis. Prolonged cold exposure also induces adaptive thermogenesis by (4) upregulating *Ucp1* mRNA expression.

participants and significant determinants for BAT activity included, besides low outdoor temperatures (seasonal variation), young age, low-to-normal BMI, absence of diabetes, and female sex (118). Of note, the influence of sex on BAT prevalence declined with age (118). Also in rodents, female rats have a higher BAT mass (relative to body mass), higher total and mitochondrial protein content in BAT with larger mitochondria with more cristae, and higher BAT UCP1 protein expression than male rats under normal housing conditions at 22°C and *ad libitum* fed with chow diet (134,135). Circulating sex steroids are likely one of the most important regulators of BAT differentiation and activity (136). In cultured brown adipocytes isolated from BAT of mice or rats, treatment with E2 or progesterone stimulated while testosterone inhibited mitochondrial biogenesis signaling and brown adipocyte differentiation (137,138).

Under caloric restriction conditions (e.g. 60% caloric intake of the *ad libitum* fed animals for 100 days), female rats showed a greater deactivation of BAT thermogenesis to reduce energy expenditure than male rats, and hence females are better able to protect other metabolically active organs, advantageous for survival in food-limited conditions, than males (139). When fed a HFD for 8 weeks, female rats maintained a higher expression of proteins involved in thermogenesis (e.g. UCP1, PGC1α) and fat oxidation, and a lower expression of proteins involved in fat synthesis (e.g. FASN, ACC1) in BAT than male rats, again suggestive for increased protective adaptations of female BAT, also under energy-excess conditions (140). Another study, in which rats had been exposed to a high-fat, high-sugar diet for 100 days followed by a chow diet for 70 days, confirmed that female rats had a higher BAT mass (relative to body mass), higher total and mitochondrial protein in BAT, higher *Ucp1* and *Adrb3* (β_3-ADR) mRNA expression, and a greater weight loss during the chow diet state than male rats. This study confirms a higher functional capacity of BAT in females during an overweight state (134).

3. Effects of sex and stress steroids on adipose tissues

This section will review the effects of sex- and stress-steroids on the distribution and function of adipose tissues. Such effects have mainly been addressed by administration of the biological hormones or their agonists/antagonists to humans and rodents, as wells as *ex vivo* treatment of adipose tissue-derived cells and stable adipocyte cell lines. Also, the removal of the sex steroid producing gonads (gonadectomy) and animals deficient in specific steroid receptors are commonly used methods and models to study the role of sex steroids. A brief summary of the effects of sex- and stress-steroids on adipose tissues is presented in **Table 2** and will be discussed in more detail below.

Table 2 Sex- and stress-steroid actions in fat distribution and adipokine production

Properties	Estrogens	Progestogens	Androgens	Glucocorticoids
Major circulating hormone	E2	Progesterone	Testosterone	Cortisol (humans) or corticosterone (rodents)
Main functioning nuclear receptor in adipose tissue	ERα	PR	AR and ERα (for testosterone-derived E2)	GR
Other receptors expressed in adipose tissue	ERβ, GPER	PAQR, PGRMC	–	MR
Fat distribution and adipogenesis	Promote subcutaneous fat and reduce visceral fat deposition	For males: no data For females: likely promote fat accumulation, but effects on fat distribution remain controversial	For males: reduce fat mass in both visceral and subcutaneous depots (for eugonadism) For females: promote visceral fat and reduce subcutaneous fat deposition	Promote visceral fat and reduce subcutaneous fat deposition Crucial for initial steps of adipogenesis
Leptin production	Stimulate	Controversial	Inhibit	Stimulate
Adiponectin production	Likely inhibit, but increased adiponectin/leptin ratio	Controversial	Inhibit	Controversial
Whole-body insulin sensitivity	Increase	For males: no data For females: likely decrease	For males: likely increase (for eugonadism) For females: decrease	Decrease

Abbreviations: AR, androgen receptor; E2, 17β-estradiol; ER, estrogen receptor; GPER, G protein-coupled ER; GR, glucocorticoid receptor; MR, mineralocorticoid receptor; PAQR, progestin and adipoQ receptor; PGRMC, PR membrane component; PR, progesterone receptor.

3.1 Estrogens

Estrogens are female sex hormones, the effects of which at target organs are classically mediated by the nuclear estrogen receptors (ERs): ERα and ERβ. Upon binding to ER, the estrogen-ER complex interacts with estrogen response elements and other transcription factors, and hence stimulates or inhibits target gene expression (19). Estrogens can also bind to membrane-associated ERs, such as the G protein-coupled ER (GPER, formerly known as GPR30), and initiate rapid non-genomic actions (141). In premenopausal women, E2 is the main circulating estrogen produced by ovaries during the menstrual cycle.

Estrogen deficiency after menopause is associated with many metabolic risks, such as obesity, metabolic syndrome, type 2 diabetes mellitus, and cardiovascular diseases. Postmenopausal estrogen therapy has beneficial effects against these metabolic risks. However, individualized treatment formula, route of administration, dosage, and duration should be considered because thromboembolism, a major harmful consequence of the hormone treatment, needs to be monitored and evaluated (19,142,143). Estrogens also regulate energy homeostasis via the central nervous system, mainly through activation of ERα in many brain regions, which is beyond the scope of this chapter. The net central effect of estrogens is towards a negative energy balance by inhibiting feeding behavior and promoting energy expenditure through sympathetic nervous system activation, and hence BAT thermogenesis [see for a comprehensive review (144)].

Concerning adipose tissue expansion and distribution, estrogens promote the subcutaneous gluteofemoral or gynoid/pear-shaped fat distribution, which is associated with low metabolic risks. Postmenopausal women shift towards the visceral or central/android/apple-shaped fat distribution and increase their body weight and fat mass. Hormone replacement therapy in early postmenopausal women counteracts weight gain, prevents the central fat distribution, and increases gluteofemoral fat accumulation (145,146). Interestingly, administration of E2, in combination with an antiandrogen, for gender-affirming hormone therapy in transwomen (male-to-female transgender persons) resulted in a marked increase in subcutaneous fat deposition at the abdominal area, hip, and thigh, but only a slight increase in visceral fat deposition (147).

Studies in rodents also confirm this effect of estrogens on fat distribution. Female rats have more subcutaneous fat and less visceral fat than male rats. Ovariectomy increased visceral fat deposition in females and E2 treatment of ovariectomized females reversed the fat distribution to that of ovary-intact females. In addition, E2 treatment of castrated males also increased the subcutaneous fat percentage (148). Male and female mice with estrogen deficiency such

as aromatase knockout (ArKO) mice, which cannot synthesize endogenous estrogens due to targeted disruption of the aromatase gene, had heavier gonadal and infrarenal fat pads than their wild-type (WT) littermates. E2 replacement in female ArKO mice restored the fat pad mass to those of WT animals (149).

To study whether ERα or ERβ is involved in the effects of estrogens on adipose tissues, studies with ERα knockout (ERαKO) male and female mice showed that these animals had an increased WAT mass, especially the gonadal WAT (150). Studies comparing ERαKO, ERβ knockout (ERβKO), and double ERs knockout (ERαβKO) mice showed that ERαKO and ERαβKO, but not ERβKO females had increased overall fat mass and circulating leptin levels. In addition, treatment of ovariectomized females with E2 resulted in a reduction in gonadal fat mass in WT and ERβKO, but not in ERαKO or ERαβKO mice (151,152). Adipocyte-specific deletion of ERα led to visceral obesity and adipose tissue inflammation in both sexes of mice, but more severe metabolic disturbances were observed in male mice (153). These animal studies underscore that the fat-reducing effect of estrogens is mediated through ERα, which is in accordance with a finding in humans that adipose tissues of obese women had lower ERα mRNA levels than those of non-obese women (154).

Recent studies revealed that GPER may also regulate adipose tissue function and expansion, but the findings are still contradictory and warrant further studies. One study found that female but not male GPER knockout (GPERKO) mice were protected from HFD-induced obesity without significant changes in food intake and energy expenditure. GPER deficiency did not affect the metabolic phenotypes of chow-fed male and female mice (155). In contrast, another study found that chow-fed GPERKO males had increased visceral and subcutaneous fat mass, elevated circulating proinflammatory cytokine levels, and reduced adiponectin levels, without changes in food consumption or physical activity (156).

Regarding adipokine production, plasma adiponectin levels are in general higher in women than in men (157). Postmenopausal women, nevertheless, have higher circulating total and HMW adiponectin levels than premenopausal women (158,159). Total and HMW adiponectin concentrations were negatively correlated with E2 levels and insulin resistance status (158). Although estrogen status alone cannot fully explain adiponectin levels in all mentioned conditions, a higher adiponectin level remains a significant determinant for lower risk of insulin resistance in postmenopausal women, confirming the anti-diabetic effect of adiponectin (159). Gender-affirming hormone therapy in transwomen not only increased total fat mass but also serum leptin and adiponectin concentrations (160,161). In addition, *in vitro* stimulation of WAT explants or isolated adipocytes from subcutaneous WAT of women confirmed a direct stimulatory effect of E2 on leptin secretion and on *LEP* mRNA expression (162). Likewise,

E2 directly reduced adiponectin production and secretion in cultured 3T3-L1 adipocytes (163).

Also female mice have higher plasma adiponectin levels, greater HMW/total adiponectin ratio, and better insulin sensitivity than male mice (104,163). Ovariectomy increases while E2 treatment in ovariectomized mice decreases plasma adiponectin levels (163). Another study found that E2 treatment reduced body weight and abdominal fat mass and attenuated insulin resistance of female mice with HFD-induced obesity. Circulating leptin levels were elevated while adiponectin levels were unchanged by HFD, yet E2 treatment reduced the circulating levels of both leptin and adiponectin. Most likely, this is mediated through a different mechanism since E2 treatment did not affect *Adipoq* mRNA expression in WAT of the HFD-fed females while it reduced *Lep* mRNA expression (164).

The finding that E2-treated obese mice had improved insulin sensitivity but decreased circulating adiponectin levels is contradictory to a general observation that adiponectin levels decline in obese and insulin-resistant state, whereas leptin levels increase proportionally to fat mass and correlate with insulin resistance (81). Another study in ovariectomized mice found that E2 treatment reduced fat mass, adipocyte size, and serum leptin and adiponectin levels, but increased the adiponectin/leptin ratio which was in parallel with an improved glucose tolerance (165). This discrepancy in adiponectin levels and insulin sensitivity in each estrogen condition is likely caused by physiological adaptations of adipose tissue, modes of adipose tissue expansion, or alterations of circulating adiponectin isoforms, which require further investigation to draw a firm conclusion.

3.2 Progestogens

Progestogens are steroid hormones synthesized in ovaries, adrenal glands, and the placenta. Progesterone, the natural endogenous progestogen, is essential for the development of female reproductive organs in the luteal phase of female reproductive cycle, when it prepares the endometrium for possible implantation of a fertilized egg, and for maintenance of pregnancy. The classical signaling pathway of progestogens is through binding to the nuclear progesterone receptors (PRs), subsequently interacting with progesterone response elements, and initiating transcription of PR target genes. In addition, progestogens can also signal through non-classical pathways by binding to other receptors, e.g. membrane-associated receptors (mPRs), which belong to the progestin and adipoQ receptor (PAQR) family, and the PR membrane component (PGRMC) 1 and 2 (166). Furthermore, progesterone can be converted into the neuroactive metabolite allopregnanolone which acts through the membrane-associated GABA type A ($GABA_A$) receptor (166).

Clinical observations propose a lipogenic effect of progestogens. A longitudinal study in pregnant women found that weight gain during pregnancy was positively correlated with plasma progesterone levels but not with E2 levels or amount of dietary intake (167). Women using depot-medroxyprogesterone acetate (DMPA), a progestogen-only injectable contraceptive, gained more weight than women without hormonal contraceptives. This weight gain was contributed to an increase in fat mass with a more central distribution (168). Of note, although DMPA has the highest affinity to PR, it can also bind to GR and AR albeit with low affinity, and thus DMPA also has some androgenic and glucocorticoid effects (169). Furthermore, progestogen-only contraceptive users usually have elevated plasma insulin concentrations after a glucose challenge, suggesting progestogen-induced insulin resistance (170).

Progesterone administration to rats had no effect on body weight and fat mass of male rats, but led to increased body weight and inguinal fat mass in female rats without affecting the intra-abdominal fat mass. Activity and mRNA expression of lipogenic enzymes were also upregulated in the inguinal WAT of female rats (171,172). An *in vitro* progesterone treatment of rat adipocytes, obtained from parametrial fat pads of females, confirmed the lipogenic effects of progesterone since it dose-dependently upregulated *Srebp1c* and *Fasn* mRNA expression (173). However, an *in vitro* study using cultured adipocytes isolated from subcutaneous and omental WATs of women, showed inconsistent effects of progesterone on adipocyte differentiation and lipid accumulation (174).

Concerning adipokine production, progesterone administration in rats resulted in upregulated *Lep* mRNA but downregulated *Adipoq* mRNA expression in inguinal WAT of females. The effect of progesterone on the adipokine mRNA expression was abolished when mifepristone (RU486, a potent PR and GR antagonist) was co-administered, suggesting a PR-regulated mechanism. Of interest, progesterone did not alter *Lep* and *Adipoq* mRNA expression in retroperitoneal WAT of females or in inguinal, epididymal, and retroperitoneal WATs of males, and did not affect circulating levels of leptin and adiponectin in both sexes (171). However, prolonged treatment with progesterone increased adiponectin production but did not affect leptin production in 3T3-L1 adipocytes (175). Hence, these depot- and sex-specific effects of progesterone on adipokine production warrant further investigation, especially since this female sex steroid is less well studied than the other major hormone, E2.

3.3 Androgens

Androgens are male sex hormones which are not only required for male reproductive system development and secondary male sex characteristics, but are also involved in energy/metabolic homeostasis. Testosterone is the main

circulating androgen for adult men and male rodents, synthesized in testes. At target organs, testosterone can be converted to E2 by the enzyme aromatase, e.g. in WAT, or to the more potent androgen derivative dihydrotestosterone (DHT) by the enzyme 5α-reductase in, for example, male reproductive organs, hair follicles, liver, and many brain regions. Principal actions of testosterone and DHT are mediated by the androgen receptor (AR). Apart from the gonads, adrenal glands also produce androgen precursors, so-called adrenal androgens, i.e. dehydroepiandrosterone (DHEA), DHEA sulfate, and androstenedione, which are found in plasma of both sexes after the adrenarche, i.e. maturation of adrenal steroidogenesis (20,176).

Male hypogonadism, i.e., men with low testosterone levels, is associated with visceral obesity, metabolic syndrome, and an increased incidence of type 2 diabetes mellitus (177,178). Testosterone replacement therapy for male hypogonadism reduces fat mass and improves the metabolic profile (177). In contrast, androgen deprivation therapy (so-called chemical castration) for patients with prostate cancer leads to increases in body weight, total fat mass, and insulin resistance (179,180). The mentioned studies suggest that androgen deficiency in men contributes to obesity and the metabolic syndrome. On the other hand, obesity itself is also considered a cause of male hypogonadism since leptin and obesity-induced proinflammatory cytokines have been shown to suppress the HPG axis and testicular testosterone production (181). Nutritional intervention for weight reduction or bariatric surgery (surgical treatment for severely obese patients) leads to increased plasma testosterone concentrations (182). Therefore, it should be concluded that obesity and testosterone deficiency are bidirectionally regulated.

Regarding fat distribution, testosterone treatment as a gender-affirming hormonal therapy for transmen (female-to-male transgender persons) resulted in a reduced subcutaneous fat deposition and a slight increase in visceral fat accumulation, resembling the male fat distribution pattern (147). In healthy men, testosterone administration dose-dependently affects total fat mass. Low serum testosterone concentrations increase fat mass while supra-physiological concentrations reduce fat mass, but the changes in fat mass are evenly distributed between the visceral and subcutaneous depots (183). In men with hypogonadism, treatment with testosterone alone or in combination with finasteride (a 5α-reductase inhibitor for blocking peripheral conversion of testosterone to DHT) both resulted in an increase in lean body mass; decreases in waist circumference and total fat mass; improvement of physical performance; and decreased levels of leptin, insulin, inflammatory markers, and plasma lipid concentrations (184,185). These studies suggest a beneficial effect of testosterone on the metabolic profile in men, including a decrease in visceral adiposity.

However, a study in abdominally obese men found that testosterone decreased whereas DHT increased visceral fat mass, while subcutaneous fat mass was unaffected by either treatment. Testosterone but not DHT increased glucose disposal under fixed hyperinsulinemic conditions, decreased fasting plasma glucose levels, and improved plasma lipid profiles, suggesting a complex mechanism rather than only a direct activation of AR (186). These studies are suggestive for (local) effects of E2 because DHT cannot be converted into E2 whereas testosterone can. Indeed, testosterone treatment to hypogonadal men led to increased plasma concentrations of both testosterone and E2. Moreover, this treatment restored the reduced mRNA expression of *AR*, *ER*α, and *CYP19A1* in adipose tissue of hypogonadal men to those in eugonadal men (187). Importantly, an elegant study in healthy men revealed that the fat mass reducing effect of testosterone disappeared when an aromatase inhibitor was co-administered with testosterone. This co-administration resulted in a greater total fat mass than at baseline. In other words, estrogen deficiency may in fact be the main cause of increased body fat in hypogonadal and obese men (188). These studies illustrate the complex hormonal regulation of adipose tissue function and a potential estrogenic effect of testosterone through ERα in male adipose tissues.

Studies in male mice demonstrate some contradictory effects of castration on fat masses. For example, one study showed that castration led to an increased gonadal fat mass (189), whereas other studies showed no significant effect of castration on retroperitoneal, gonadal, and subcutaneous fat masses (47,190). Surprisingly, specific activation of AR by DHT and combined treatment of testosterone and an aromatase inhibitor in castrated mice resulted in an increased retroperitoneal fat mass, but neither testosterone treatment alone nor E2 treatment negatively affected the fat mass (190). However, the AR knockout (ARKO) mouse model revealed that ARKO males have increased fat mass, elevated serum leptin levels, and reduced physical activity, but the effects on insulin sensitivity, leptin sensitivity, adiponectin levels, and food intake were inconsistent (191-193). A possible explanation of the inconsistent phenotypes is the markedly reduced testosterone level and thus aromatase-synthesized E2 in ARKO mice because AR is crucial for testicular testosterone production and ARKO males had atrophic testes. In fact, male mice with adipose-specific AR deficiency showed normal body weight and adiposity with unaffected serum testosterone and E2 levels, but an increase in intra-adipose E2 levels and hyperleptinemia, yet without leptin resistance. The increased leptin production is likely E2-driven (194).

Concerning adipokine synthesis, androgen deprivation in healthy men induced by gonadotropin-releasing hormone (GnRH) antagonist treatment led

to elevated plasma leptin and adiponectin concentrations, whereas testosterone administration suppressed the plasma concentrations of both adipokines (195). Likewise, testosterone treatment in transmen also resulted in reduced serum concentrations of leptin and adiponectin (160,161). *In vitro* stimulation of WAT explants or isolated adipocytes from subcutaneous WAT of men confirmed that DHT inhibited *LEP* mRNA expression and decreased leptin secretion. Moreover, the inhibitory effect of DHT on leptin synthesis was attenuated upon cotreatment with an AR antagonist, suggesting an AR-mediated mechanism (162). In male mice, castration resulted in elevated levels of total and HMW adiponectin, whereas testosterone treatment in castrated males reduced the circulating adiponectin levels. In addition, testosterone or DHT treatment in cultured adipocytes confirmed a direct inhibitory effect of testosterone on adiponectin secretion (196,197).

In contrast to men, hyperandrogenism in women, e.g. women with polycystic ovary syndrome (PCOS), is associated with increased total fat mass and insulin resistance, but without a difference in regional fat distribution compared to BMI-matched female controls (198). Another study however showed that women with PCOS had increased visceral fat accumulation and decreased insulin sensitivity, features which were also observed in normal-weight PCOS subjects (199). Interestingly, recent studies revealed that the reduced insulin sensitivity of PCOS women was associated with low serum adiponectin levels, hypertrophic morphology of adipocytes, and an increased waist/hip ratio, but not with androgen excess (200,201). Also in mice, continuous administration of DHT in prepubertal females resulted in a disturbed metabolic phenotype that included increased body weight, enlarged adipocytes in gonadal and inguinal WAT depots, impaired glucose tolerance, elevated leptin levels, and reduced adiponectin levels (202). Intriguingly, female mice with global loss of AR signaling were protected against the ovarian and metabolic consequences of DHT treatment, indicating that AR-mediated androgen actions are crucial for the pathogenesis of PCOS in the rodent DHT model (203).

3.4 Glucocorticoids

GCs are synthesized in adrenal glands under the control of the HPA axis, as a crucial stress response mechanism. GCs regulate energy substrate metabolism in many aspects, namely by inducing hepatic gluconeogenesis, reducing glucose uptake in skeletal muscles and adipose tissues, promoting lipolysis in adipose tissues, inhibiting insulin secretion from pancreatic β cells, and stimulating glucagon secretion from pancreatic α cells (204). Endogenous GC synthesis is different among mammal species. Due to a lack of the steroidogenic enzyme CYP17A1 (functioning as 17α-hydroxylase or 17,20-lyase) in adrenal

glands of mice and rats, corticosterone is the main endogenous GC for rodents, whereas cortisol is the main endogenous GC for humans (12,205). At target tissues, GCs bind the GR or the mineralocorticoid receptor (MR), depending on their expression profile and function in each target tissue.

Synthetic GCs, such as dexamethasone, prednisolone, and hydrocortisone, are commonly prescribed medications due to their immunosuppressive properties. Weight gain is one of the most common side effects of synthetic GCs with a prevalence of 70% based on a self-reported population-based study, or at a hazard ratio of 2.4 based on an outpatient rheumatology clinic study (206,207). In patients with Cushing syndrome (clinical manifestation of pathological hypercortisolism), overweight/obesity is the most prevalent phenotype, present in 57–100% of patients, with a preferential pattern towards visceral rather than subcutaneous fat accumulation (208). The GC-induced visceral obesity can be explained by several mechanisms, especially with respect to the effects of GCs on WAT. First, *GR* mRNA expression levels and the binding capacity for GC in WAT homogenates are higher in omental tissues than in abdominal subcutaneous tissues (48,209). Second, intra-adipose cortisol levels are higher in omental WAT than in subcutaneous WAT, independent of serum cortisol level. This can be explained by an increased turnover rate and local generation of GC in adipose tissues, controlled by the 11β-HSD enzymes (209,210). The reductase 11β-HSD1 activates cortisone to cortisol, whereas the hydrogenase 11β-HSD2 deactivates cortisol to cortisone. Both enzymes are present and functioning in WAT, but only the reductase activity and *HSD11B1* mRNA expression are positively correlated with adipocyte size and total fat mass. Actually, GC treatment indirectly promotes the reductase activity by providing the cofactor NADPH for 11β-HSD1 activity in adipocytes from omental depots only. Direct effects on *HSD11B1* mRNA expression or 11β-HSD1 protein levels, however, remain inconclusive (209,210). Third, LPL activity was higher while norepinephrine-induced lipolysis was lower in abdominal WAT of Cushing patients than in those of non-Cushing obese subjects, suggesting GC-induced lipid accumulation in the visceral depot. Of note, lipogenic and lipolytic activities in femoral WAT of Cushing patients were unaffected (211).

Concerning direct effects of GCs on lipid metabolism, dexamethasone (a potent GR agonist with a very weak binding to the MR) treatment of rat adipocytes directly stimulated lipolysis in a dose-dependent manner and this effect was abolished by RU486 (a potent GR and PR antagonist). Likewise, dexamethasone treatment of rats resulted in increased plasma levels of glycerol and free fatty acids, confirming the lipolytic effect of GCs. Epididymal fat of dexamethasone-treated mice also showed higher lipolytic activity and greater levels of HSL and ATGL mRNA expression and protein content (212). An *in*

vitro stimulation of 3T3-L1 adipocytes with corticosterone confirmed the direct lipolytic effect of GCs and the GC-induced basal lipolysis in adipocytes that had been chronically exposed to GCs (213).

However, the direct lipolytic effect of GCs alone cannot explain the increase in visceral fat accumulation in Cushing patients. Indeed, GCs have another crucial function in adipose tissues, namely promoting adipogenesis. Corticosterone treatment in rats showed that corticosterone increased in fat mass and number of adipocytes in the visceral depot but not the subcutaneous depot, indicating depot-dependent adipogenic recruitment (213). Of interest, among many compounds used in a standard cocktail for 3T3-L1 adipocyte differentiation, dexamethasone is the most crucial compound in the initial stage of differentiation, as without it adipogenesis is not induced (214). Likewise, in human preadipocytes obtained from abdominal subcutaneous adipose tissues of healthy subjects, knockdown of GR by small interfering RNA (siRNA) completely blocked the adipogenic action of cortisol, whereas knockdown of MR did not affect the differentiation (215). Hence, all studies suggest that GR plays a more important role than MR for the adipogenic action of GCs.

Regarding adipokine production, plasma leptin levels were elevated in patients with Cushing syndrome compared to non-obese subjects or obese subjects without endocrine diseases. Curative resection of adrenal tumors reduced plasma leptin levels while a dexamethasone challenge in healthy individuals increased plasma leptin levels (216). Cortisol treatment of cultured human adipocytes also resulted in increased *LEP* mRNA expression and leptin secretion. A siRNA knockdown of the gene encoding GR but not MR reduced the stimulatory effect of cortisol on leptin production, showing that GR is also the receptor involved in the stimulatory effect of GCs on leptin production (215). Likewise, dexamethasone treatment in cultured rat adipocytes also led to an upregulated *Lep* mRNA expression (217).

The effects of GCs on adiponectin are different. Non-obese Cushing patients had a lower plasma adiponectin concentration than non-obese control subjects, but obese Cushing patients had the same low level of plasma adiponectin concentration as the obese control subjects. Hydrocortisone injection in healthy individuals confirmed the inhibitory effect of GCs on adiponectin production (218). Also in cultured human subcutaneous adipocytes, dexamethasone directly suppressed adiponectin secretion (219). In contrast, another study found that cortisol treatment of cultured human adipocytes isolated from abdominal subcutaneous depots induced *ADIPOQ* mRNA expression and adiponectin secretion. Knockdown of GR by siRNA reduced the stimulatory effect of cortisol on adiponectin production, whereas knockdown of MR had no effect (215). The contradictory effect of GC on adiponectin production warrant further studies.

The HPA axis has been shown to exhibit sexually dimorphic regulation and activity. For example, many stress-induced psychiatric disorders, such as depressive and anxiety disorders, are more prevalent and severe in women than in men (220). Also in rodent studies, female rats have higher baseline levels and higher stress-induced levels of corticosterone and adrenocorticotropic hormone (ACTH; the pituitary hormone that stimulates the adrenal glands to synthesize and secrete GCs) than male rats (221,222). However, there are only a limited number of studies addressing sex differences in GC-induced alterations in adipose tissue function. In male mice, cotreatment of corticosterone and DHT potentiated, whereas cotreatment of corticosterone and the AR-antagonist enzalutamide attenuated GR responses in WAT (223). Another recent study showed that the GC-induced obesity and hypertrophic expansion of visceral fat depots in ovariectomized rats were attenuated by E2 treatment (224). Further studies are required to elucidate if the GC-induced metabolic derangements, including effects on adipose tissues, are sex-dependent.

4. Conclusion

Males and females display differences in adipose tissue distribution and functions that contribute to differences in the risk to develop obesity and obesity-related comorbidities. Differences in sex steroid hormone levels contribute significantly to these differences. However, studies in human and animal models also revealed that sex steroids have sex-dependent effects on adipose tissues. Recent studies showing that sex steroid hormone levels influence the metabolic effects of glucocorticoids, underline the need for more studies to gain a full understanding of the molecular mechanisms regulating these sex-dependent effects. Additional studies are also needed since sex-dependent effects of sex steroids and glucocorticoids have largely been overlooked with respect to their clinical implications, for instance, sex differences in the effects of pharmacological GCs. Better understanding of sex-dependent regulation of adipose tissues will facilitate the development of novel sex-specific therapeutic strategies to combat the obesity pandemic.

References

1. World Health Organization. Obesity and overweight - Fact sheets. 2018; https://www.who.int/en/news-room/fact-sheets/detail/obesity-and-overweight. Accessed 13 June, 2019.
2. Pearson-Stuttard J, Zhou B, Kontis V, Bentham J, Gunter MJ, Ezzati M. Worldwide burden of cancer attributable to diabetes and high body-mass index: a comparative risk assessment. Lancet Diabetes Endocrinol. 2018;**6**(6):e6-e15.
3. Saltiel AR, Olefsky JM. Inflammatory mechanisms linking obesity and metabolic disease. *J Clin Invest.* 2017;**127**(1):1-4.
4. Hales CM, Carroll MD, Fryar CD, Ogden CL. Prevalence of Obesity Among Adults and Youth: United States, 2015-2016. *NCHS Data Brief.* 2017(288):1-8.

5. Arnold AP, Cassis LA, Eghbali M, Reue K, Sandberg K. Sex Hormones and Sex Chromosomes Cause Sex Differences in the Development of Cardiovascular Diseases. *Arterioscler Thromb Vasc Biol.* 2017;**37**(5):746-756.
6. Perez-Lopez FR, Larrad-Mur L, Kallen A, Chedraui P, Taylor HS. Gender differences in cardiovascular disease: hormonal and biochemical influences. *Reprod Sci.* 2010;**17**(6):511-531.
7. Yang Y, Kozloski M. Sex differences in age trajectories of physiological dysregulation: inflammation, metabolic syndrome, and allostatic load. *J Gerontol A Biol Sci Med Sci.* 2011;**66**(5):493-500.
8. Palmer BF, Clegg DJ. The sexual dimorphism of obesity. *Mol Cell Endocrinol.* 2015;**402**:113-119.
9. Karastergiou K, Smith SR, Greenberg AS, Fried SK. Sex differences in human adipose tissues - the biology of pear shape. *Biol Sex Differ.* 2012;**3**(1):13.
10. Pasquali R. The hypothalamic-pituitary-adrenal axis and sex hormones in chronic stress and obesity: pathophysiological and clinical aspects. *Ann N Y Acad Sci.* 2012;**1264**:20-35.
11. Bourke CH, Harrell CS, Neigh GN. Stress-induced sex differences: adaptations mediated by the glucocorticoid receptor. *Horm Behav.* 2012;**62**(3):210-218.
12. Pihlajoki M, Heikinheimo M, Wilson DB. Regulation of Adrenal Steroidogenesis. In: Levine AC, ed. *Adrenal Disorders*. Cham: Humana Press; 2018:15-66.
13. Jin JM, Yang WX. Molecular regulation of hypothalamus-pituitary-gonads axis in males. *Gene.* 2014;**551**(1):15-25.
14. Christensen A, Bentley GE, Cabrera R, Ortega HH, Perfito N, Wu TJ, Micevych P. Hormonal regulation of female reproduction. *Horm Metab Res.* 2012;**44**(8):587-591.
15. Luo E, Stephens SB, Chaing S, Munaganuru N, Kauffman AS, Breen KM. Corticosterone Blocks Ovarian Cyclicity and the LH Surge via Decreased Kisspeptin Neuron Activation in Female Mice. *Endocrinology.* 2016;**157**(3):1187-1199.
16. Juarez-Rojas L, Vigueras-Villasenor RM, Casillas F, Retana-Marquez S. Gradual decrease in spermatogenesis caused by chronic stress. *Acta Histochem.* 2017;**119**(3):284-291.
17. Mauvais-Jarvis F. Sex differences in metabolic homeostasis, diabetes, and obesity. *Biol Sex Differ.* 2015;**6**:14.
18. Kasinska MA, Drzewoski J, Sliwinska A. Epigenetic modifications in adipose tissue - relation to obesity and diabetes. *Arch Med Sci.* 2016;**12**(6):1293-1301.
19. Mauvais-Jarvis F, Clegg DJ, Hevener AL. The role of estrogens in control of energy balance and glucose homeostasis. *Endocr Rev.* 2013;**34**(3):309-338.
20. Navarro G, Allard C, Xu W, Mauvais-Jarvis F. The role of androgens in metabolism, obesity, and diabetes in males and females. *Obesity (Silver Spring).* 2015;**23**(4):713-719.
21. Kloting N, Bluher M. Adipocyte dysfunction, inflammation and metabolic syndrome. *Rev Endocr Metab Disord.* 2014;**15**(4):277-287.
22. Speakman JR. Chapter 26 - Obesity and thermoregulation. In: Romanovsky AA, ed. *Handbook of Clinical Neurology.* Vol 156: Elsevier; 2018:431-443.
23. Young P, Arch JR, Ashwell M. Brown adipose tissue in the parametrial fat pad of the mouse. *FEBS Lett.* 1984;**167**(1):10-14.
24. Frontini A, Vitali A, Perugini J, Murano I, Romiti C, Ricquier D, Guerrieri M, Cinti S. White-to-brown transdifferentiation of omental adipocytes in patients affected by pheochromocytoma. *Biochim Biophys Acta.* 2013;**1831**(5):950-959.
25. Kotzbeck P, Giordano A, Mondini E, Murano I, Severi I, Venema W, Cecchini MP, et al. Brown adipose tissue whitening leads to brown adipocyte death and adipose tissue inflammation. *J Lipid Res.* 2018;**59**(5):784-794.
26. Halberg N, Khan T, Trujillo ME, Wernstedt-Asterholm I, Attie AD, Sherwani S, Wang ZV, et al. Hypoxia-inducible factor 1alpha induces fibrosis and insulin resistance in white adipose tissue. *Mol Cell Biol.* 2009;**29**(16):4467-4483.
27. Kloting N, Fasshauer M, Dietrich A, Kovacs P, Schon MR, Kern M, Stumvoll M, Bluher M. Insulin-sensitive obesity. *Am J Physiol Endocrinol Metab.* 2010;**299**(3):E506-515.
28. Lundgren M, Svensson M, Lindmark S, Renstrom F, Ruge T, Eriksson JW. Fat cell enlargement is an independent marker of insulin resistance and 'hyperleptinaemia'. *Diabetologia.* 2007;**50**(3):625-633.
29. Salans LB, Knittle JL, Hirsch J. The role of adipose cell size and adipose tissue insulin sensitivity in the carbohydrate intolerance of human obesity. *J Clin Invest.* 1968;**47**(1):153-165.
30. Ghaben AL, Scherer PE. Adipogenesis and metabolic health. *Nat Rev Mol Cell Biol.* 2019;**20**(4):242-258.

31. Fukumura D, Ushiyama A, Duda DG, Xu L, Tam J, Krishna V, Chatterjee K, Garkavtsev I, Jain RK. Paracrine regulation of angiogenesis and adipocyte differentiation during in vivo adipogenesis. *Circ Res.* 2003;**93**(9):e88-97.
32. Chusyd DE, Wang D, Huffman DM, Nagy TR. Relationships between Rodent White Adipose Fat Pads and Human White Adipose Fat Depots. *Front Nutr.* 2016;**3**:10.
33. Guglielmi V, Sbraccia P. Obesity phenotypes: depot-differences in adipose tissue and their clinical implications. *Eat Weight Disord.* 2018;**23**(1):3-14.
34. Joe AW, Yi L, Even Y, Vogl AW, Rossi FM. Depot-specific differences in adipogenic progenitor abundance and proliferative response to high-fat diet. *Stem Cells.* 2009;**27**(10):2563-2570.
35. Macotela Y, Emanuelli B, Mori MA, Gesta S, Schulz TJ, Tseng YH, Kahn CR. Intrinsic differences in adipocyte precursor cells from different white fat depots. *Diabetes.* 2012;**61**(7):1691-1699.
36. Tchkonia T, Tchoukalova YD, Giorgadze N, Pirtskhalava T, Karagiannides I, Forse RA, Koo A, et al. Abundance of two human preadipocyte subtypes with distinct capacities for replication, adipogenesis, and apoptosis varies among fat depots. *Am J Physiol Endocrinol Metab.* 2005;**288**(1):E267-277.
37. Tchkonia T, Giorgadze N, Pirtskhalava T, Tchoukalova Y, Karagiannides I, Forse RA, DePonte M, et al. Fat depot origin affects adipogenesis in primary cultured and cloned human preadipocytes. *Am J Physiol Regul Integr Comp Physiol.* 2002;**282**(5):R1286-1296.
38. Durrer Schutz D, Busetto L, Dicker D, Farpour-Lambert N, Pryke R, Toplak H, Widmer D, Yumuk V, Schutz Y. European Practical and Patient-Centred Guidelines for Adult Obesity Management in Primary Care. *Obes Facts.* 2019;**12**(1):40-66.
39. Garvey WT, Mechanick JI, Brett EM, Garber AJ, Hurley DL, Jastreboff AM, Nadolsky K, Pessah-Pollack R, Plodkowski R, Reviewers of the AACEOCPG. American Association of Clinical Endocrinologists and American College of Endocrinology Comprehensive Clinical Practice Guidelines for Medical Care of Patients with Obesity. *Endocr Pract.* 2016;**22 Suppl 3**:1-203.
40. Arnett DK, Blumenthal RS, Albert MA, Buroker AB, Goldberger ZD, Hahn EJ, Himmelfarb CD, et al. 2019 ACC/AHA Guideline on the Primary Prevention of Cardiovascular Disease. *Circulation.* 2019:CIR0000000000000678.
41. Rosenzweig JL, Bakris GL, Berglund LF, Hivert MF, Horton ES, Kalyani RR, Murad MH, Verges BL. Primary Prevention of ASCVD and T2DM in Patients at Metabolic Risk: An Endocrine Society* Clinical Practice Guideline. *J Clin Endocrinol Metab.* 2019.
42. Gallagher D, Visser M, Sepulveda D, Pierson RN, Harris T, Heymsfield SB. How useful is body mass index for comparison of body fatness across age, sex, and ethnic groups? *Am J Epidemiol.* 1996;**143**(3):228-239.
43. Kuk JL, Lee S, Heymsfield SB, Ross R. Waist circumference and abdominal adipose tissue distribution: influence of age and sex. *Am J Clin Nutr.* 2005;**81**(6):1330-1334.
44. Wells JC. Sexual dimorphism of body composition. *Best Pract Res Clin Endocrinol Metab.* 2007;**21**(3):415-430.
45. Kautzky-Willer A, Harreiter J, Pacini G. Sex and Gender Differences in Risk, Pathophysiology and Complications of Type 2 Diabetes Mellitus. *Endocr Rev.* 2016;**37**(3):278-316.
46. Demerath EW, Sun SS, Rogers N, Lee M, Reed D, Choh AC, Couch W, et al. Anatomical patterning of visceral adipose tissue: race, sex, and age variation. *Obesity (Silver Spring).* 2007;**15**(12):2984-2993.
47. Macotela Y, Boucher J, Tran TT, Kahn CR. Sex and depot differences in adipocyte insulin sensitivity and glucose metabolism. *Diabetes.* 2009;**58**(4):803-812.
48. Pedersen SB, Jonler M, Richelsen B. Characterization of regional and gender differences in glucocorticoid receptors and lipoprotein lipase activity in human adipose tissue. *J Clin Endocrinol Metab.* 1994;**78**(6):1354-1359.
49. Taylor RW, Grant AM, Williams SM, Goulding A. Sex differences in regional body fat distribution from pre- to postpuberty. *Obesity (Silver Spring).* 2010;**18**(7):1410-1416.
50. Camhi SM, Bray GA, Bouchard C, Greenway FL, Johnson WD, Newton RL, Ravussin E, Ryan DH, Smith SR, Katzmarzyk PT. The relationship of waist circumference and BMI to visceral, subcutaneous, and total body fat: sex and race differences. *Obesity (Silver Spring).* 2011;**19**(2):402-408.
51. Svendsen OL, Hassager C, Christiansen C. Age- and menopause-associated variations in body composition and fat distribution in healthy women as measured by dual-energy X-ray absorptiometry. *Metabolism.* 1995;**44**(3):369-373.
52. Chang E, Varghese M, Singer K. Gender and Sex Differences in Adipose Tissue. *Curr Diab Rep.* 2018;**18**(9):69.

53. Wu Y, Lee MJ, Ido Y, Fried SK. High-fat diet-induced obesity regulates MMP3 to modulate depot- and sex-dependent adipose expansion in C57BL/6J mice. *Am J Physiol Endocrinol Metab*. 2017;**312**(1):E58-E71.
54. Stubbins RE, Holcomb VB, Hong J, Nunez NP. Estrogen modulates abdominal adiposity and protects female mice from obesity and impaired glucose tolerance. *Eur J Nutr*. 2012;**51**(7):861-870.
55. Litwak SA, Wilson JL, Chen W, Garcia-Rudaz C, Khaksari M, Cowley MA, Enriori PJ. Estradiol prevents fat accumulation and overcomes leptin resistance in female high-fat diet mice. *Endocrinology*. 2014;**155**(11):4447-4460.
56. Dakin RS, Walker BR, Seckl JR, Hadoke PW, Drake AJ. Estrogens protect male mice from obesity complications and influence glucocorticoid metabolism. *Int J Obes (Lond)*. 2015;**39**(10):1539-1547.
57. Seale P, Bjork B, Yang W, Kajimura S, Chin S, Kuang S, Scime A, et al. PRDM16 controls a brown fat/skeletal muscle switch. *Nature*. 2008;**454**(7207):961-967.
58. An Y, Wang G, Diao Y, Long Y, Fu X, Weng M, Zhou L, et al. A Molecular Switch Regulating Cell Fate Choice between Muscle Progenitor Cells and Brown Adipocytes. *Dev Cell*. 2017;**41**(4):382-391 e385.
59. Wang W, Kissig M, Rajakumari S, Huang L, Lim HW, Won KJ, Seale P. Ebf2 is a selective marker of brown and beige adipogenic precursor cells. *Proc Natl Acad Sci U S A*. 2014;**111**(40):14466-14471.
60. Shao M, Ishibashi J, Kusminski CM, Wang QA, Hepler C, Vishvanath L, MacPherson KA, et al. Zfp423 Maintains White Adipocyte Identity through Suppression of the Beige Cell Thermogenic Gene Program. *Cell Metab*. 2016;**23**(6):1167-1184.
61. Cristancho AG, Lazar MA. Forming functional fat: a growing understanding of adipocyte differentiation. *Nat Rev Mol Cell Biol*. 2011;**12**(11):722-734.
62. Villanueva CJ, Vergnes L, Wang J, Drew BG, Hong C, Tu Y, Hu Y, et al. Adipose subtype-selective recruitment of TLE3 or Prdm16 by PPARgamma specifies lipid storage versus thermogenic gene programs. *Cell Metab*. 2013;**17**(3):423-435.
63. Rosen ED, Spiegelman BM. What we talk about when we talk about fat. *Cell*. 2014;**156**(1-2):20-44.
64. Seale P. Transcriptional Regulatory Circuits Controlling Brown Fat Development and Activation. *Diabetes*. 2015;**64**(7):2369-2375.
65. Tontonoz P, Spiegelman BM. Fat and beyond: the diverse biology of PPARgamma. *Annu Rev Biochem*. 2008;**77**:289-312.
66. Barbatelli G, Murano I, Madsen L, Hao Q, Jimenez M, Kristiansen K, Giacobino JP, De Matteis R, Cinti S. The emergence of cold-induced brown adipocytes in mouse white fat depots is determined predominantly by white to brown adipocyte transdifferentiation. *Am J Physiol Endocrinol Metab*. 2010;**298**(6):E1244-1253.
67. Lafontan M. Advances in adipose tissue metabolism. *Int J Obes (Lond)*. 2008;**32 Suppl 7**:S39-51.
68. Luo L, Liu M. Adipose tissue in control of metabolism. *J Endocrinol*. 2016;**231**(3):R77-R99.
69. Marcelin G, Chua S, Jr. Contributions of adipocyte lipid metabolism to body fat content and implications for the treatment of obesity. *Curr Opin Pharmacol*. 2010;**10**(5):588-593.
70. Letexier D, Pinteur C, Large V, Frering V, Beylot M. Comparison of the expression and activity of the lipogenic pathway in human and rat adipose tissue. *J Lipid Res*. 2003;**44**(11):2127-2134.
71. Herman MA, Peroni OD, Villoria J, Schon MR, Abumrad NA, Bluher M, Klein S, Kahn BB. A novel ChREBP isoform in adipose tissue regulates systemic glucose metabolism. *Nature*. 2012;**484**(7394):333-338.
72. Ferre P, Foufelle F. SREBP-1c transcription factor and lipid homeostasis: clinical perspective. *Horm Res*. 2007;**68**(2):72-82.
73. Crewe C, Zhu Y, Paschoal VA, Joffin N, Ghaben AL, Gordillo R, Oh DY, Liang G, Horton JD, Scherer PE. SREBP-regulated adipocyte lipogenesis is dependent on substrate availability and redox modulation of mTORC1. *JCI Insight*. 2019;**5**.
74. Peckett AJ, Wright DC, Riddell MC. The effects of glucocorticoids on adipose tissue lipid metabolism. *Metabolism*. 2011;**60**(11):1500-1510.
75. Lebeck J. Metabolic impact of the glycerol channels AQP7 and AQP9 in adipose tissue and liver. *J Mol Endocrinol*. 2014;**52**(2):R165-178.

76. Furuhashi M, Saitoh S, Shimamoto K, Miura T. Fatty Acid-Binding Protein 4 (FABP4): Pathophysiological Insights and Potent Clinical Biomarker of Metabolic and Cardiovascular Diseases. *Clin Med Insights Cardiol.* 2014;**8**(Suppl 3):23-33.
77. Sztalryd C, Brasaemle DL. The perilipin family of lipid droplet proteins: Gatekeepers of intracellular lipolysis. *Biochim Biophys Acta Mol Cell Biol Lipids.* 2017;**1862**(10 Pt B):1221-1232.
78. Lee RA, Harris CA, Wang JC. Glucocorticoid Receptor and Adipocyte Biology. *Nucl Receptor Res.* 2018;**5**.
79. Bluher M. Adipose tissue dysfunction contributes to obesity related metabolic diseases. *Best Pract Res Clin Endocrinol Metab.* 2013;**27**(2):163-177.
80. Bluher M, Mantzoros CS. From leptin to other adipokines in health and disease: facts and expectations at the beginning of the 21st century. *Metabolism.* 2015;**64**(1):131-145.
81. Fasshauer M, Bluher M. Adipokines in health and disease. *Trends Pharmacol Sci.* 2015;**36**(7):461-470.
82. Zhang Y, Proenca R, Maffei M, Barone M, Leopold L, Friedman JM. Positional cloning of the mouse obese gene and its human homologue. *Nature.* 1994;**372**(6505):425-432.
83. Ingalls AM, Dickie MM, Snell GD. Obese, a new mutation in the house mouse. *J Hered.* 1950;**41**(12):317-318.
84. Hummel KP, Dickie MM, Coleman DL. Diabetes, a new mutation in the mouse. *Science.* 1966;**153**(3740):1127-1128.
85. Kennedy AJ, Ellacott KL, King VL, Hasty AH. Mouse models of the metabolic syndrome. *Dis Model Mech.* 2010;**3**(3-4):156-166.
86. Margetic S, Gazzola C, Pegg GG, Hill RA. Leptin: a review of its peripheral actions and interactions. *Int J Obes Relat Metab Disord.* 2002;**26**(11):1407-1433.
87. Park HK, Ahima RS. Physiology of leptin: energy homeostasis, neuroendocrine function and metabolism. *Metabolism.* 2015;**64**(1):24-34.
88. Kelesidis T, Kelesidis I, Chou S, Mantzoros CS. Narrative review: the role of leptin in human physiology: emerging clinical applications. *Ann Intern Med.* 2010;**152**(2):93-100.
89. Stern JH, Rutkowski JM, Scherer PE. Adiponectin, Leptin, and Fatty Acids in the Maintenance of Metabolic Homeostasis through Adipose Tissue Crosstalk. *Cell Metab.* 2016;**23**(5):770-784.
90. Pan WW, Myers MG, Jr. Leptin and the maintenance of elevated body weight. *Nat Rev Neurosci.* 2018;**19**(2):95-105.
91. Scherer PE, Williams S, Fogliano M, Baldini G, Lodish HF. A novel serum protein similar to C1q, produced exclusively in adipocytes. *J Biol Chem.* 1995;**270**(45):26746-26749.
92. Hu E, Liang P, Spiegelman BM. AdipoQ is a novel adipose-specific gene dysregulated in obesity. *J Biol Chem.* 1996;**271**(18):10697-10703.
93. Maeda K, Okubo K, Shimomura I, Funahashi T, Matsuzawa Y, Matsubara K. cDNA cloning and expression of a novel adipose specific collagen-like factor, apM1 (AdiPose Most abundant Gene transcript 1). *Biochem Biophys Res Commun.* 1996;**221**(2):286-289.
94. Nakano Y, Tobe T, Choi-Miura NH, Mazda T, Tomita M. Isolation and characterization of GBP28, a novel gelatin-binding protein purified from human plasma. *J Biochem.* 1996;**120**(4):803-812.
95. Zhang Y, Matheny M, Zolotukhin S, Tumer N, Scarpace PJ. Regulation of adiponectin and leptin gene expression in white and brown adipose tissues: influence of beta3-adrenergic agonists, retinoic acid, leptin and fasting. *Biochim Biophys Acta.* 2002;**1584**(2-3):115-122.
96. Fujimoto N, Matsuo N, Sumiyoshi H, Yamaguchi K, Saikawa T, Yoshimatsu H, Yoshioka H. Adiponectin is expressed in the brown adipose tissue and surrounding immature tissues in mouse embryos. *Biochim Biophys Acta.* 2005;**1731**(1):1-12.
97. Delaigle AM, Jonas JC, Bauche IB, Cornu O, Brichard SM. Induction of adiponectin in skeletal muscle by inflammatory cytokines: in vivo and in vitro studies. *Endocrinology.* 2004;**145**(12):5589-5597.
98. Jasinski-Bergner S, Buttner M, Quandt D, Seliger B, Kielstein H. Adiponectin and Its Receptors Are Differentially Expressed in Human Tissues and Cell Lines of Distinct Origin. *Obes Facts.* 2017;**10**(6):569-583.
99. Choe SS, Huh JY, Hwang IJ, Kim JI, Kim JB. Adipose Tissue Remodeling: Its Role in Energy Metabolism and Metabolic Disorders. *Front Endocrinol (Lausanne).* 2016;**7**:30.
100. Ryo M, Nakamura T, Kihara S, Kumada M, Shibazaki S, Takahashi M, Nagai M, Matsuzawa Y, Funahashi T. Adiponectin as a biomarker of the metabolic syndrome. *Circ J.* 2004;**68**(11):975-981.

101. Lopez-Jaramillo P, Gomez-Arbelaez D, Lopez-Lopez J, Lopez-Lopez C, Martinez-Ortega J, Gomez-Rodriguez A, Triana-Cubillos S. The role of leptin/adiponectin ratio in metabolic syndrome and diabetes. *Horm Mol Biol Clin Investig*. 2014;**18**(1):37-45.
102. Fruhbeck G, Catalan V, Rodriguez A, Gomez-Ambrosi J. Adiponectin-leptin ratio: A promising index to estimate adipose tissue dysfunction. Relation with obesity-associated cardiometabolic risk. *Adipocyte*. 2018;**7**(1):57-62.
103. Schraw T, Wang ZV, Halberg N, Hawkins M, Scherer PE. Plasma adiponectin complexes have distinct biochemical characteristics. *Endocrinology*. 2008;**149**(5):2270-2282.
104. Pajvani UB, Hawkins M, Combs TP, Rajala MW, Doebber T, Berger JP, Wagner JA, et al. Complex distribution, not absolute amount of adiponectin, correlates with thiazolidinedione-mediated improvement in insulin sensitivity. *J Biol Chem*. 2004;**279**(13):12152-12162.
105. Bernasochi GB, Bell JR, Simpson ER, Delbridge LMD, Boon WC. Impact of Estrogens on the Regulation of White, Beige, and Brown Adipose Tissue Depots. *Compr Physiol*. 2019;**9**(2):457-475.
106. Belanger C, Hould FS, Lebel S, Biron S, Brochu G, Tchernof A. Omental and subcutaneous adipose tissue steroid levels in obese men. *Steroids*. 2006;**71**(8):674-682.
107. Kinoshita T, Honma S, Shibata Y, Yamashita K, Watanabe Y, Maekubo H, Okuyama M, Takashima A, Takeshita N. An innovative LC-MS/MS-based method for determining CYP 17 and CYP 19 activity in the adipose tissue of pre- and postmenopausal and ovariectomized women using 13C-labeled steroid substrates. *J Clin Endocrinol Metab*. 2014;**99**(4):1339-1347.
108. Morton NM. Obesity and corticosteroids: 11beta-hydroxysteroid type 1 as a cause and therapeutic target in metabolic disease. *Mol Cell Endocrinol*. 2010;**316**(2):154-164.
109. De Sousa Peixoto RA, Turban S, Battle JH, Chapman KE, Seckl JR, Morton NM. Preadipocyte 11beta-hydroxysteroid dehydrogenase type 1 is a keto-reductase and contributes to diet-induced visceral obesity in vivo. *Endocrinology*. 2008;**149**(4):1861-1868.
110. Nedergaard J, Bengtsson T, Cannon B. Unexpected evidence for active brown adipose tissue in adult humans. *Am J Physiol Endocrinol Metab*. 2007;**293**(2):E444-452.
111. Hany TF, Gharehpapagh E, Kamel EM, Buck A, Himms-Hagen J, von Schulthess GK. Brown adipose tissue: a factor to consider in symmetrical tracer uptake in the neck and upper chest region. *Eur J Nucl Med Mol Imaging*. 2002;**29**(10):1393-1398.
112. van Marken Lichtenbelt WD, Vanhommerig JW, Smulders NM, Drossaerts JM, Kemerink GJ, Bouvy ND, Schrauwen P, Teule GJ. Cold-activated brown adipose tissue in healthy men. *N Engl J Med*. 2009;**360**(15):1500-1508.
113. Virtanen KA, Lidell ME, Orava J, Heglind M, Westergren R, Niemi T, Taittonen M, et al. Functional brown adipose tissue in healthy adults. *N Engl J Med*. 2009;**360**(15):1518-1525.
114. Cypess AM, Lehman S, Williams G, Tal I, Rodman D, Goldfine AB, Kuo FC, et al. Identification and importance of brown adipose tissue in adult humans. *N Engl J Med*. 2009;**360**(15):1509-1517.
115. Zingaretti MC, Crosta F, Vitali A, Guerrieri M, Frontini A, Cannon B, Nedergaard J, Cinti S. The presence of UCP1 demonstrates that metabolically active adipose tissue in the neck of adult humans truly represents brown adipose tissue. *FASEB J*. 2009;**23**(9):3113-3120.
116. Morrison SF. Central neural control of thermoregulation and brown adipose tissue. *Auton Neurosci*. 2016;**196**:14-24.
117. Blondin DP, Labbe SM, Tingelstad HC, Noll C, Kunach M, Phoenix S, Guerin B, et al. Increased brown adipose tissue oxidative capacity in cold-acclimated humans. *J Clin Endocrinol Metab*. 2014;**99**(3):E438-446.
118. Ouellet V, Routhier-Labadie A, Bellemare W, Lakhal-Chaieb L, Turcotte E, Carpentier AC, Richard D. Outdoor temperature, age, sex, body mass index, and diabetic status determine the prevalence, mass, and glucose-uptake activity of 18F-FDG-detected BAT in humans. *J Clin Endocrinol Metab*. 2011;**96**(1):192-199.
119. Cannon B, Nedergaard J. Metabolic consequences of the presence or absence of the thermogenic capacity of brown adipose tissue in mice (and probably in humans). *Int J Obes (Lond)*. 2010;**34 Suppl 1**:S7-16.
120. Cypess AM, Weiner LS, Roberts-Toler C, Franquet Elia E, Kessler SH, Kahn PA, English J, et al. Activation of human brown adipose tissue by a beta3-adrenergic receptor agonist. *Cell Metab*. 2015;**21**(1):33-38.
121. Vosselman MJ, van der Lans AA, Brans B, Wierts R, van Baak MA, Schrauwen P, van Marken Lichtenbelt WD. Systemic beta-adrenergic stimulation of thermogenesis is not accompanied by brown adipose tissue activity in humans. *Diabetes*. 2012;**61**(12):3106-3113.

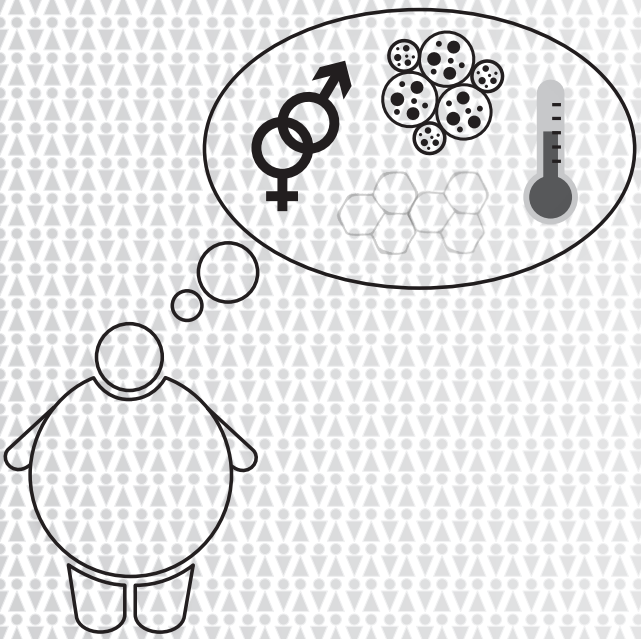
122. Agrawal A, Nair N, Baghel NS. A novel approach for reduction of brown fat uptake on FDG PET. *Br J Radiol.* 2009;**82**(980):626-631.
123. Soderlund V, Larsson SA, Jacobsson H. Reduction of FDG uptake in brown adipose tissue in clinical patients by a single dose of propranolol. *Eur J Nucl Med Mol Imaging.* 2007;**34**(7):1018-1022.
124. Louis SN, Nero TL, Iakovidis D, Jackman GP, Louis WJ. LK 204-545, a highly selective beta1-adrenoceptor antagonist at human beta-adrenoceptors. *Eur J Pharmacol.* 1999;**367**(2-3):431-435.
125. Li Y, Fromme T, Schweizer S, Schottl T, Klingenspor M. Taking control over intracellular fatty acid levels is essential for the analysis of thermogenic function in cultured primary brown and brite/beige adipocytes. *EMBO Rep.* 2014;**15**(10):1069-1076.
126. Labbe SM, Caron A, Bakan I, Laplante M, Carpentier AC, Lecomte R, Richard D. In vivo measurement of energy substrate contribution to cold-induced brown adipose tissue thermogenesis. *FASEB J.* 2015;**29**(5):2046-2058.
127. Haemmerle G, Lass A, Zimmermann R, Gorkiewicz G, Meyer C, Rozman J, Heldmaier G, et al. Defective lipolysis and altered energy metabolism in mice lacking adipose triglyceride lipase. *Science.* 2006;**312**(5774):734-737.
128. Kalinovich AV, de Jong JM, Cannon B, Nedergaard J. UCP1 in adipose tissues: two steps to full browning. *Biochimie.* 2017;**134**:127-137.
129. Evans BA, Merlin J, Bengtsson T, Hutchinson DS. Adrenoceptors in white, brown, and brite adipocytes. *Br J Pharmacol.* 2019;**176**(14):2416-2432.
130. Hao Q, Yadav R, Basse AL, Petersen S, Sonne SB, Rasmussen S, Zhu Q, et al. Transcriptome profiling of brown adipose tissue during cold exposure reveals extensive regulation of glucose metabolism. *Am J Physiol Endocrinol Metab.* 2015;**308**(5):E380-392.
131. de Jong JM, Larsson O, Cannon B, Nedergaard J. A stringent validation of mouse adipose tissue identity markers. *Am J Physiol Endocrinol Metab.* 2015;**308**(12):E1085-1105.
132. Law J, Bloor I, Budge H, Symonds ME. The influence of sex steroids on adipose tissue growth and function. *Horm Mol Biol Clin Investig.* 2014;**19**(1):13-24.
133. Kim SN, Jung YS, Kwon HJ, Seong JK, Granneman JG, Lee YH. Sex differences in sympathetic innervation and browning of white adipose tissue of mice. *Biol Sex Differ.* 2016;**7**:67.
134. Rodriguez AM, Quevedo-Coli S, Roca P, Palou A. Sex-dependent dietary obesity, induction of UCPs, and leptin expression in rat adipose tissues. *Obes Res.* 2001;**9**(9):579-588.
135. Rodriguez-Cuenca S, Pujol E, Justo R, Frontera M, Oliver J, Gianotti M, Roca P. Sex-dependent thermogenesis, differences in mitochondrial morphology and function, and adrenergic response in brown adipose tissue. *J Biol Chem.* 2002;**277**(45):42958-42963.
136. Quarta C, Mazza R, Pasquali R, Pagotto U. Role of sex hormones in modulation of brown adipose tissue activity. *J Mol Endocrinol.* 2012;**49**(1):R1-7.
137. Rodriguez AM, Monjo M, Roca P, Palou A. Opposite actions of testosterone and progesterone on UCP1 mRNA expression in cultured brown adipocytes. *Cell Mol Life Sci.* 2002;**59**(10):1714-1723.
138. Rodriguez-Cuenca S, Monjo M, Gianotti M, Proenza AM, Roca P. Expression of mitochondrial biogenesis-signaling factors in brown adipocytes is influenced specifically by 17beta-estradiol, testosterone, and progesterone. *Am J Physiol Endocrinol Metab.* 2007;**292**(1):E340-346.
139. Valle A, Catala-Niell A, Colom B, Garcia-Palmer FJ, Oliver J, Roca P. Sex-related differences in energy balance in response to caloric restriction. *Am J Physiol Endocrinol Metab.* 2005;**289**(1):E15-22.
140. Choi DK, Oh TS, Choi JW, Mukherjee R, Wang X, Liu H, Yun JW. Gender difference in proteome of brown adipose tissues between male and female rats exposed to a high fat diet. *Cell Physiol Biochem.* 2011;**28**(5):933-948.
141. Zimmerman MA, Budish RA, Kashyap S, Lindsey SH. GPER-novel membrane oestrogen receptor. *Clin Sci (Lond).* 2016;**130**(12):1005-1016.
142. Oliver-Williams C, Glisic M, Shahzad S, Brown E, Pellegrino Baena C, Chadni M, Chowdhury R, Franco OH, Muka T. The route of administration, timing, duration and dose of postmenopausal hormone therapy and cardiovascular outcomes in women: a systematic review. *Hum Reprod Update.* 2019;**25**(2):257-271.
143. Bae JM, Yoon BK. The Role of Menopausal Hormone Therapy in Reducing All-cause Mortality in Postmenopausal Women Younger than 60 Years: An Adaptive Meta-analysis. *J Menopausal Med.* 2018;**24**(3):139-142.

144. Xu Y, Lopez M. Central regulation of energy metabolism by estrogens. *Mol Metab.* 2018;**15**:104-115.
145. Gambacciani M, Ciaponi M, Cappagli B, De Simone L, Orlandi R, Genazzani AR. Prospective evaluation of body weight and body fat distribution in early postmenopausal women with and without hormonal replacement therapy. *Maturitas.* 2001;**39**(2):125-132.
146. Haarbo J, Marslew U, Gotfredsen A, Christiansen C. Postmenopausal hormone replacement therapy prevents central distribution of body fat after menopause. *Metabolism.* 1991;**40**(12):1323-1326.
147. Elbers JM, Asscheman H, Seidell JC, Gooren LJ. Effects of sex steroid hormones on regional fat depots as assessed by magnetic resonance imaging in transsexuals. *Am J Physiol.* 1999;**276**(2):E317-325.
148. Clegg DJ, Brown LM, Woods SC, Benoit SC. Gonadal hormones determine sensitivity to central leptin and insulin. *Diabetes.* 2006;**55**(4):978-987.
149. Jones ME, Thorburn AW, Britt KL, Hewitt KN, Wreford NG, Proietto J, Oz OK, et al. Aromatase-deficient (ArKO) mice have a phenotype of increased adiposity. *Proc Natl Acad Sci U S A.* 2000;**97**(23):12735-12740.
150. Heine PA, Taylor JA, Iwamoto GA, Lubahn DB, Cooke PS. Increased adipose tissue in male and female estrogen receptor-alpha knockout mice. *Proc Natl Acad Sci U S A.* 2000;**97**(23):12729-12734.
151. Lindberg MK, Alatalo SL, Halleen JM, Mohan S, Gustafsson JA, Ohlsson C. Estrogen receptor specificity in the regulation of the skeleton in female mice. *J Endocrinol.* 2001;**171**(2):229-236.
152. Lindberg MK, Weihua Z, Andersson N, Moverare S, Gao H, Vidal O, Erlandsson M, et al. Estrogen receptor specificity for the effects of estrogen in ovariectomized mice. *J Endocrinol.* 2002;**174**(2):167-178.
153. Davis KE, M DN, Sun K, W MS, J DB, J AZ, Zeve D, et al. The sexually dimorphic role of adipose and adipocyte estrogen receptors in modulating adipose tissue expansion, inflammation, and fibrosis. *Mol Metab.* 2013;**2**(3):227-242.
154. Nilsson M, Dahlman I, Ryden M, Nordstrom EA, Gustafsson JA, Arner P, Dahlman-Wright K. Oestrogen receptor alpha gene expression levels are reduced in obese compared to normal weight females. *Int J Obes (Lond).* 2007;**31**(6):900-907.
155. Wang A, Luo J, Moore W, Alkhalidy H, Wu L, Zhang J, Zhen W, et al. GPR30 regulates diet-induced adiposity in female mice and adipogenesis in vitro. *Sci Rep.* 2016;**6**:34302.
156. Sharma G, Hu C, Brigman JL, Zhu G, Hathaway HJ, Prossnitz ER. GPER deficiency in male mice results in insulin resistance, dyslipidemia, and a proinflammatory state. *Endocrinology.* 2013;**154**(11):4136-4145.
157. Arita Y, Kihara S, Ouchi N, Takahashi M, Maeda K, Miyagawa J, Hotta K, et al. Paradoxical decrease of an adipose-specific protein, adiponectin, in obesity. *Biochem Biophys Res Commun.* 1999;**257**(1):79-83.
158. Leung KC, Xu A, Craig ME, Martin A, Lam KS, O'Sullivan AJ. Adiponectin isoform distribution in women--relationship to female sex steroids and insulin sensitivity. *Metabolism.* 2009;**58**(2):239-245.
159. Tamakoshi K, Yatsuya H, Wada K, Matsushita K, Otsuka R, Yang PO, Sugiura K, et al. The transition to menopause reinforces adiponectin production and its contribution to improvement of insulin-resistant state. *Clin Endocrinol (Oxf).* 2007;**66**(1):65-71.
160. Auer MK, Ebert T, Pietzner M, Defreyne J, Fuss J, Stalla GK, T'Sjoen G. Effects of Sex Hormone Treatment on the Metabolic Syndrome in Transgender Individuals: Focus on Metabolic Cytokines. *J Clin Endocrinol Metab.* 2018;**103**(2):790-802.
161. Elbers JM, Asscheman H, Seidell JC, Frolich M, Meinders AE, Gooren LJ. Reversal of the sex difference in serum leptin levels upon cross-sex hormone administration in transsexuals. *J Clin Endocrinol Metab.* 1997;**82**(10):3267-3270.
162. Machinal-Quelin F, Dieudonne MN, Pecquery R, Leneveu MC, Giudicelli Y. Direct in vitro effects of androgens and estrogens on ob gene expression and leptin secretion in human adipose tissue. *Endocrine.* 2002;**18**(2):179-184.
163. Combs TP, Berg AH, Rajala MW, Klebanov S, Iyengar P, Jimenez-Chillaron JC, Patti ME, Klein SL, Weinstein RS, Scherer PE. Sexual differentiation, pregnancy, calorie restriction, and aging affect the adipocyte-specific secretory protein adiponectin. *Diabetes.* 2003;**52**(2):268-276.
164. Bryzgalova G, Lundholm L, Portwood N, Gustafsson JA, Khan A, Efendic S, Dahlman-Wright K. Mechanisms of antidiabetogenic and body weight-lowering effects of estrogen in high-fat diet-fed mice. *Am J Physiol Endocrinol Metab.* 2008;**295**(4):E904-912.

165. Kim JH, Meyers MS, Khuder SS, Abdallah SL, Muturi HT, Russo L, Tate CR, et al. Tissue-selective estrogen complexes with bazedoxifene prevent metabolic dysfunction in female mice. *Mol Metab.* 2014;**3**(2):177-190.
166. Garg D, Ng SSM, Baig KM, Driggers P, Segars J. Progesterone-Mediated Non-Classical Signaling. *Trends Endocrinol Metab.* 2017;**28**(9):656-668.
167. Lof M, Hilakivi-Clarke L, Sandin SS, de Assis S, Yu W, Weiderpass E. Dietary fat intake and gestational weight gain in relation to estradiol and progesterone plasma levels during pregnancy: a longitudinal study in Swedish women. *BMC Womens Health.* 2009;**9**:10.
168. Clark MK, Dillon JS, Sowers M, Nichols S. Weight, fat mass, and central distribution of fat increase when women use depot-medroxyprogesterone acetate for contraception. *Int J Obes (Lond).* 2005;**29**(10):1252-1258.
169. Africander D, Verhoog N, Hapgood JP. Molecular mechanisms of steroid receptor-mediated actions by synthetic progestins used in HRT and contraception. *Steroids.* 2011;**76**(7):636-652.
170. Kahn HS, Curtis KM, Marchbanks PA. Effects of injectable or implantable progestin-only contraceptives on insulin-glucose metabolism and diabetes risk. *Diabetes Care.* 2003;**26**(1):216-225.
171. Stelmanska E, Kmiec Z, Swierczynski J. The gender- and fat depot-specific regulation of leptin, resistin and adiponectin genes expression by progesterone in rat. *J Steroid Biochem Mol Biol.* 2012;**132**(1-2):160-167.
172. Stelmanska E, Swierczynski J. Up-regulation of lipogenic enzyme genes expression in inguinal white adipose tissue of female rats by progesterone. *J Steroid Biochem Mol Biol.* 2013;**134**:37-44.
173. Lacasa D, Le Liepvre X, Ferre P, Dugail I. Progesterone stimulates adipocyte determination and differentiation 1/sterol regulatory element-binding protein 1c gene expression. potential mechanism for the lipogenic effect of progesterone in adipose tissue. *J Biol Chem.* 2001;**276**(15):11512-11516.
174. Zhang Y, Nadeau M, Faucher F, Lescelleur O, Biron S, Daris M, Rheaume C, Luu-The V, Tchernof A. Progesterone metabolism in adipose cells. *Mol Cell Endocrinol.* 2009;**298**(1-2):76-83.
175. Pektas M, Kurt AH, Un I, Tiftik RN, Buyukafsar K. Effects of 17beta-estradiol and progesterone on the production of adipokines in differentiating 3T3-L1 adipocytes: Role of Rho-kinase. *Cytokine.* 2015;**72**(2):130-134.
176. Miller WL, Auchus RJ. The molecular biology, biochemistry, and physiology of human steroidogenesis and its disorders. *Endocr Rev.* 2011;**32**(1):81-151.
177. Corona G, Monami M, Rastrelli G, Aversa A, Sforza A, Lenzi A, Forti G, Mannucci E, Maggi M. Type 2 diabetes mellitus and testosterone: a meta-analysis study. *Int J Androl.* 2011;**34**(6 Pt 1):528-540.
178. Wang N, Zhai H, Han B, Li Q, Chen Y, Chen Y, Xia F, Lin D, Lu Y. Visceral fat dysfunction is positively associated with hypogonadism in Chinese men. *Sci Rep.* 2016;**6**:19844.
179. Smith MR, Finkelstein JS, McGovern FJ, Zietman AL, Fallon MA, Schoenfeld DA, Kantoff PW. Changes in body composition during androgen deprivation therapy for prostate cancer. *J Clin Endocrinol Metab.* 2002;**87**(2):599-603.
180. Smith MR, Lee H, Nathan DM. Insulin sensitivity during combined androgen blockade for prostate cancer. *J Clin Endocrinol Metab.* 2006;**91**(4):1305-1308.
181. Grossmann M. Hypogonadism and male obesity: Focus on unresolved questions. *Clin Endocrinol (Oxf).* 2018;**89**(1):11-21.
182. Corona G, Rastrelli G, Monami M, Saad F, Luconi M, Lucchese M, Facchiano E, et al. Body weight loss reverts obesity-associated hypogonadotropic hypogonadism: a systematic review and meta-analysis. *Eur J Endocrinol.* 2013;**168**(6):829-843.
183. Woodhouse LJ, Gupta N, Bhasin M, Singh AB, Ross R, Phillips J, Bhasin S. Dose-dependent effects of testosterone on regional adipose tissue distribution in healthy young men. *J Clin Endocrinol Metab.* 2004;**89**(2):718-726.
184. Kalinchenko SY, Tishova YA, Mskhalaya GJ, Gooren LJ, Giltay EJ, Saad F. Effects of testosterone supplementation on markers of the metabolic syndrome and inflammation in hypogonadal men with the metabolic syndrome: the double-blinded placebo-controlled Moscow study. *Clin Endocrinol (Oxf).* 2010;**73**(5):602-612.
185. Page ST, Amory JK, Bowman FD, Anawalt BD, Matsumoto AM, Bremner WJ, Tenover JL. Exogenous testosterone (T) alone or with finasteride increases physical performance, grip strength, and lean body mass in older men with low serum T. *J Clin Endocrinol Metab.* 2005;**90**(3):1502-1510.

186. Marin P, Holmang S, Gustafsson C, Jonsson L, Kvist H, Elander A, Eldh J, Sjostrom L, Holm G, Bjorntorp P. Androgen treatment of abdominally obese men. *Obes Res.* 1993;**1**(4):245-251.
187. Ghanim H, Dhindsa S, Abuaysheh S, Batra M, Kuhadiya ND, Makdissi A, Chaudhuri A, Dandona P. Diminished androgen and estrogen receptors and aromatase levels in hypogonadal diabetic men: reversal with testosterone. *Eur J Endocrinol.* 2018;**178**(3):277-283.
188. Finkelstein JS, Lee H, Burnett-Bowie SA, Pallais JC, Yu EW, Borges LF, Jones BF, et al. Gonadal steroids and body composition, strength, and sexual function in men. *N Engl J Med.* 2013;**369**(11):1011-1022.
189. Aoki A, Fujitani K, Takagi K, Kimura T, Nagase H, Nakanishi T. Male Hypogonadism Causes Obesity Associated with Impairment of Hepatic Gluconeogenesis in Mice. *Biol Pharm Bull.* 2016;**39**(4):587-592.
190. Moverare-Skrtic S, Venken K, Andersson N, Lindberg MK, Svensson J, Swanson C, Vanderschueren D, Oscarsson J, Gustafsson JA, Ohlsson C. Dihydrotestosterone treatment results in obesity and altered lipid metabolism in orchidectomized mice. *Obesity (Silver Spring).* 2006;**14**(4):662-672.
191. Rana K, Fam BC, Clarke MV, Pang TP, Zajac JD, MacLean HE. Increased adiposity in DNA binding-dependent androgen receptor knockout male mice associated with decreased voluntary activity and not insulin resistance. *Am J Physiol Endocrinol Metab.* 2011;**301**(5):E767-778.
192. Fan W, Yanase T, Nomura M, Okabe T, Goto K, Sato T, Kawano H, Kato S, Nawata H. Androgen receptor null male mice develop late-onset obesity caused by decreased energy expenditure and lipolytic activity but show normal insulin sensitivity with high adiponectin secretion. *Diabetes.* 2005;**54**(4):1000-1008.
193. Lin HY, Xu Q, Yeh S, Wang RS, Sparks JD, Chang C. Insulin and leptin resistance with hyperleptinemia in mice lacking androgen receptor. *Diabetes.* 2005;**54**(6):1717-1725.
194. Yu IC, Lin HY, Liu NC, Wang RS, Sparks JD, Yeh S, Chang C. Hyperleptinemia without obesity in male mice lacking androgen receptor in adipose tissue. *Endocrinology.* 2008;**149**(5):2361-2368.
195. Page ST, Herbst KL, Amory JK, Coviello AD, Anawalt BD, Matsumoto AM, Bremner WJ. Testosterone administration suppresses adiponectin levels in men. *J Androl.* 2005;**26**(1):85-92.
196. Xu A, Chan KW, Hoo RL, Wang Y, Tan KC, Zhang J, Chen B, et al. Testosterone selectively reduces the high molecular weight form of adiponectin by inhibiting its secretion from adipocytes. *J Biol Chem.* 2005;**280**(18):18073-18080.
197. Nishizawa H, Shimomura I, Kishida K, Maeda N, Kuriyama H, Nagaretani H, Matsuda M, et al. Androgens decrease plasma adiponectin, an insulin-sensitizing adipocyte-derived protein. *Diabetes.* 2002;**51**(9):2734-2741.
198. Barber TM, Golding SJ, Alvey C, Wass JA, Karpe F, Franks S, McCarthy MI. Global adiposity rather than abnormal regional fat distribution characterizes women with polycystic ovary syndrome. *J Clin Endocrinol Metab.* 2008;**93**(3):999-1004.
199. Carmina E, Bucchieri S, Esposito A, Del Puente A, Mansueto P, Orio F, Di Fede G, Rini G. Abdominal fat quantity and distribution in women with polycystic ovary syndrome and extent of its relation to insulin resistance. *J Clin Endocrinol Metab.* 2007;**92**(7):2500-2505.
200. Toulis KA, Goulis DG, Farmakiotis D, Georgopoulos NA, Katsikis I, Tarlatzis BC, Papadimas I, Panidis D. Adiponectin levels in women with polycystic ovary syndrome: a systematic review and a meta-analysis. *Hum Reprod Update.* 2009;**15**(3):297-307.
201. Manneras-Holm L, Leonhardt H, Kullberg J, Jennische E, Oden A, Holm G, Hellstrom M, et al. Adipose tissue has aberrant morphology and function in PCOS: enlarged adipocytes and low serum adiponectin, but not circulating sex steroids, are strongly associated with insulin resistance. *J Clin Endocrinol Metab.* 2011;**96**(2):E304-311.
202. van Houten EL, Kramer P, McLuskey A, Karels B, Themmen AP, Visser JA. Reproductive and metabolic phenotype of a mouse model of PCOS. *Endocrinology.* 2012;**153**(6):2861-2869.
203. Caldwell ASL, Edwards MC, Desai R, Jimenez M, Gilchrist RB, Handelsman DJ, Walters KA. Neuroendocrine androgen action is a key extraovarian mediator in the development of polycystic ovary syndrome. *Proc Natl Acad Sci U S A.* 2017;**114**(16):E3334-E3343.
204. Granner DK, Wang JC, Yamamoto KR. Regulatory Actions of Glucocorticoid Hormones: From Organisms to Mechanisms. *Adv Exp Med Biol.* 2015;**872**:3-31.
205. Pihlajoki M, Dorner J, Cochran RS, Heikinheimo M, Wilson DB. Adrenocortical zonation, renewal, and remodeling. *Front Endocrinol (Lausanne).* 2015;**6**:27.

206. Curtis JR, Westfall AO, Allison J, Bijlsma JW, Freeman A, George V, Kovac SH, Spettell CM, Saag KG. Population-based assessment of adverse events associated with long-term glucocorticoid use. *Arthritis Rheum*. 2006;**55**(3):420-426.
207. Huscher D, Thiele K, Gromnica-Ihle E, Hein G, Demary W, Dreher R, Zink A, Buttgereit F. Dose-related patterns of glucocorticoid-induced side effects. *Ann Rheum Dis*. 2009;**68**(7):1119-1124.
208. Pivonello R, Isidori AM, De Martino MC, Newell-Price J, Biller BM, Colao A. Complications of Cushing's syndrome: state of the art. *Lancet Diabetes Endocrinol*. 2016;**4**(7):611-629.
209. Veilleux A, Laberge PY, Morency J, Noel S, Luu-The V, Tchernof A. Expression of genes related to glucocorticoid action in human subcutaneous and omental adipose tissue. *J Steroid Biochem Mol Biol*. 2010;**122**(1-3):28-34.
210. Lee MJ, Fried SK, Mundt SS, Wang Y, Sullivan S, Stefanni A, Daugherty BL, Hermanowski-Vosatka A. Depot-specific regulation of the conversion of cortisone to cortisol in human adipose tissue. *Obesity (Silver Spring)*. 2008;**16**(6):1178-1185.
211. Rebuffe-Scrive M, Krotkiewski M, Elfverson J, Bjorntorp P. Muscle and adipose tissue morphology and metabolism in Cushing's syndrome. *J Clin Endocrinol Metab*. 1988;**67**(6):1122-1128.
212. Xu C, He J, Jiang H, Zu L, Zhai W, Pu S, Xu G. Direct effect of glucocorticoids on lipolysis in adipocytes. *Mol Endocrinol*. 2009;**23**(8):1161-1170.
213. Campbell JE, Peckett AJ, D'Souza A M, Hawke TJ, Riddell MC. Adipogenic and lipolytic effects of chronic glucocorticoid exposure. *Am J Physiol Cell Physiol*. 2011;**300**(1):C198-209.
214. Pantoja C, Huff JT, Yamamoto KR. Glucocorticoid signaling defines a novel commitment state during adipogenesis in vitro. *Mol Biol Cell*. 2008;**19**(10):4032-4041.
215. Lee MJ, Fried SK. The glucocorticoid receptor, not the mineralocorticoid receptor, plays the dominant role in adipogenesis and adipokine production in human adipocytes. *Int J Obes (Lond)*. 2014;**38**(9):1228-1233.
216. Masuzaki H, Ogawa Y, Hosoda K, Miyawaki T, Hanaoka I, Hiraoka J, Yasuno A, et al. Glucocorticoid regulation of leptin synthesis and secretion in humans: elevated plasma leptin levels in Cushing's syndrome. *J Clin Endocrinol Metab*. 1997;**82**(8):2542-2547.
217. De Vos P, Lefebvre AM, Shrivo I, Fruchart JC, Auwerx J. Glucocorticoids induce the expression of the leptin gene through a non-classical mechanism of transcriptional activation. *Eur J Biochem*. 1998;**253**(3):619-626.
218. Fallo F, Scarda A, Sonino N, Paoletta A, Boscaro M, Pagano C, Federspil G, Vettor R. Effect of glucocorticoids on adiponectin: a study in healthy subjects and in Cushing's syndrome. *Eur J Endocrinol*. 2004;**150**(3):339-344.
219. Degawa-Yamauchi M, Moss KA, Bovenkerk JE, Shankar SS, Morrison CL, Lelliott CJ, Vidal-Puig A, Jones R, Considine RV. Regulation of adiponectin expression in human adipocytes: effects of adiposity, glucocorticoids, and tumor necrosis factor alpha. *Obes Res*. 2005;**13**(4):662-669.
220. Bangasser DA, Valentino RJ. Sex differences in stress-related psychiatric disorders: neurobiological perspectives. *Front Neuroendocrinol*. 2014;**35**(3):303-319.
221. Kitay JI. Pituitary-Adrenal Function in the Rat after Gonadectomy and Gonadal Hormone Replacement. *Endocrinology*. 1963;**73**:253-260.
222. Seale JV, Wood SA, Atkinson HC, Bate E, Lightman SL, Ingram CD, Jessop DS, Harbuz MS. Gonadectomy reverses the sexually diergic patterns of circadian and stress-induced hypothalamic-pituitary-adrenal axis activity in male and female rats. *J Neuroendocrinol*. 2004;**16**(6):516-524.
223. Spaanderman DCE, Nixon M, Buurstede JC, Sips HC, Schilperoort M, Kuipers EN, Backer EA, et al. Androgens modulate glucocorticoid receptor activity in adipose tissue and liver. *J Endocrinol*. 2019;**240**(1):51-63.
224. de Souza CF, Stopa LRS, Santos GF, Takasumi LCN, Martins AB, Garnica-Siqueira MC, Ferreira RN, et al. Estradiol protects against ovariectomy-induced susceptibility to the anabolic effects of glucocorticoids in rats. *Life Sci*. 2019;**218**:185-196.



Chapter 1B

General Introduction

Sex Difference in Thermoregulation, a Mechanism beyond Brown Adipose Tissue Activation

1

1. Introduction

Adipose tissue can be generally categorized into two main types with opposite function: energy-storing white adipose tissue (WAT) and energy-burning brown adipose tissue (BAT), reviewed in chapter 1A. Upon cold stimulation, BAT utilizes energy-rich substrates, mainly fatty acids, to fuel thermogenesis, a mitochondrial process in which the activity of uncoupling protein 1 (UCP1) is pivotal. Upon confirmation of the presence of active BAT in adult humans in 2009 (1-3), BAT has become a "hot organ" for metabolic research. Induced utilization of excess calories through BAT activation is considered a promising strategy for obesity treatment (4). As discussed in chapter 1A, studies in humans and rodents have shown that females generally have a higher BAT prevalence and activity than males (5-7). The thermogenic function of BAT is part of the whole-body thermal homeostasis. This chapter will therefore provide an overview of body temperature regulation and highlight the role of BAT herein. In addition, this chapter will discuss whether thermoregulation is sex-dependently modulated.

2. Body temperature regulation

Humans and rodents are endotherms whose core body temperature (T_c) is tightly controlled at ~37°C in a narrow range, e.g. 35.4–37.8°C for humans (8). This is because cellular proteins are irreversibly denatured at a T_c above 42°C and cellular reactions slow down at a T_c below the normal range, resulting in, for instance, fatal cardiac failure at T_c ~27°C (9,10). In general, core temperatures, e.g. in the brain and the thoracoabdominal compartment, are usually higher than peripheral temperatures, e.g. skin temperatures (T_{sk}) measured at the limbs. Heat is transferred between the core and the periphery via the circulation, which is an important regulator of body thermal homeostasis (10). To achieve optimal thermoregulation, the body integrates three main components for autonomic responses: afferent (input) sensing, central integration, and efferent (output) responses (9,11), see **Figure 1** for a schematic overview.

The skin serves as the principal sensory organ since it contains sensory nerve endings of afferent sensory neurons located in the dorsal root ganglia (DRG) (11). The neuronal receptive endings contain temperature sensors (or thermoreceptors) that belong to the transient receptor potential (TRP) superfamily of cation channels of which the voltage-dependent receptors are affected by different temperatures. Examples of these sensors include the heat-sensitive channel TPRV1, the warm-sensitive channel TRPM2, and the cool-sensitive channel TRPM8 (12,13). Via these sensors, information about the T_{sk} is directed to the thermosensory neurons: first-order neurons in the DRG, second-order neurons in the dorsal horn (DH) of the spinal cord, and third-order neurons in

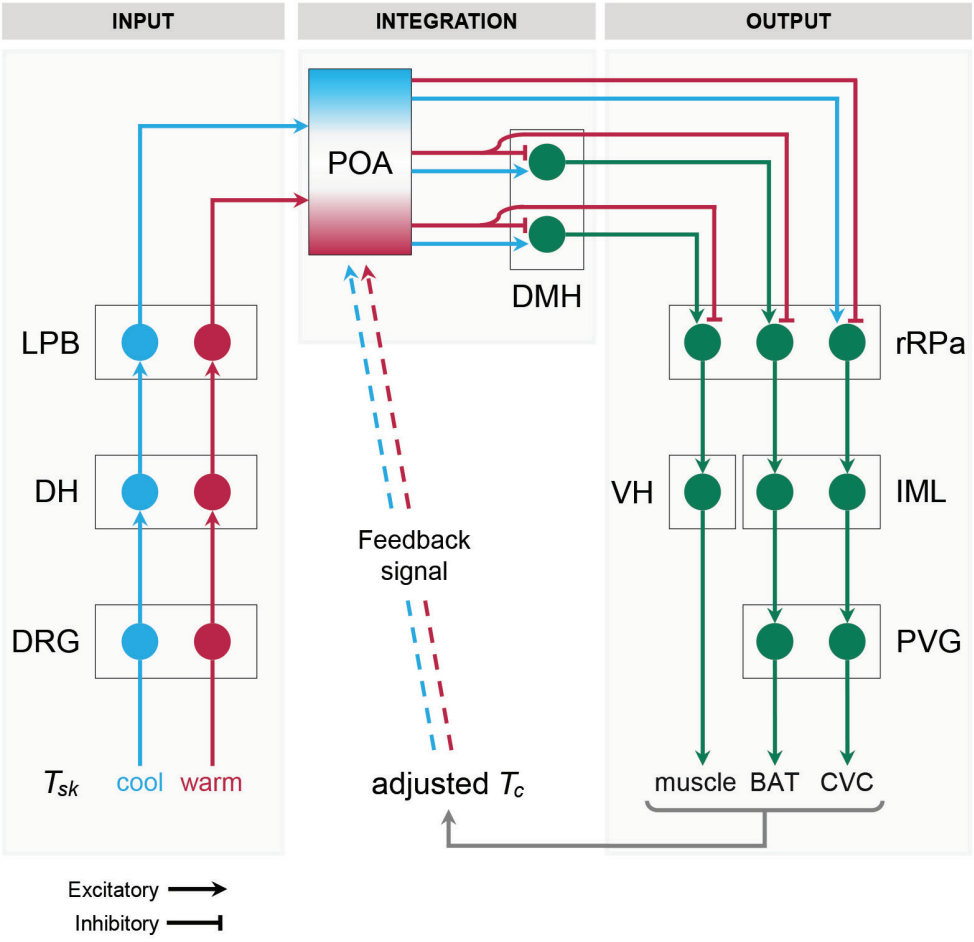


Figure 1 Overview of autonomic thermoregulation

Abbreviations (from input to output): T_{sk}, skin temperature; DRG, dorsal root ganglia; DH, dorsal horn of the spinal cord; LPB, lateral parabrachial nucleus of the pons; POA, preoptic area of the anterior hypothalamus; DMH, dorsomedial hypothalamic nucleus; rRPa, rostral raphe pallidus nucleus in the medulla oblongata; VH, ventral horn; IML, intermediolateral cell column of the spinal cord; PVG, paravertebral ganglia; BAT, brown adipose tissue; CVC, cutaneous vasoconstriction; and T_c, core body temperature.

the lateral parabrachial nucleus (LPB) of the pons. The LPB, in turn, transmits the signal to the preoptic area (POA) of the anterior hypothalamus, the master thermoregulatory sensorimotor integration area (9,11). Of note, warm and cool signals are transmitted through separate populations of afferent neurons that project to distinct populations of neurons in POA to distinguish between warm and cool sensory inputs (14).

The warm-activated neurons and cold-activated neurons in the POA are interconnected to integrate the signals from the LPB, and hence transmit inhibitory or excitatory signals to the dorsomedial hypothalamic nucleus [DMH, the key regulator for body temperature, body weight, and metabolic function (15)] in the posterior hypothalamus and/or to the rostral raphe pallidus nucleus (rRPa) in the medulla oblongata. The DMH contains BAT sympathoexcitatory neurons and shivering promoting neurons, while the rRPa contains cutaneous vasoconstriction (CVC) premotor neurons, BAT sympathetic premotor neurons, and skeletal muscle shivering premotor neurons. In other words, cold exposure leads to excitatory commands to the DMH and the rRPa that stimulate the cold-defensive responses such as a reduction in heat loss by peripheral vasoconstriction and inductions in heat production by skeletal muscle shivering and BAT activation. By contrast, warm exposure inhibits the excitatory commands to the DMH and the rRPa (9,11).

For vasoconstriction and BAT thermogenesis during cold exposure, signals from the premotor neurons in the rRPa are transmitted through the preganglionic neurons in the intermediolateral cell column (IML) of the spinal cord, which synapse onto the postganglionic neurons in the paravertebral ganglia (PVG), and in turn stimulate the effector organs, cutaneous blood vessels or BAT depots. For shivering, the involuntary somatic premotor neurons in the rRPa drive the α- and γ-motoneurons in the ventral horn (VH) of the spinal cord to initiate repeated skeletal muscle contractions (11). Although shivering leads to rapid, maximal heat production during cold stress, it is a more energy-consuming process than BAT activation, even when the cold is rather mild. Hence, shivering thermogenesis is initiated only when the energy-inexpensive vasoconstriction is at its maximum. Non-shivering BAT thermogenesis is enhanced and preferred during habitual/prolonged cold conditions (9,16,17). For example, mice exposed to 4°C for 3 weeks had ~4-fold induction in UCP1 mRNA and protein expression in their BAT, compared to mice housed at 21°C (18). Rats housed at 10°C for 3 weeks also had higher oxidative activity in BAT, as well as a larger BAT depot, indicating a more metabolically active BAT compared to BAT of rats kept at 27°C (19). These rodent findings underscore the importance of non-shivering thermogenesis in BAT for chronic cold-defensive responses.

In contrast, warm exposure leads to peripheral vasodilation for heat dissipation, through inhibition of the sympathetic CVC tone. In hot conditions, warm-activated neurons also activate the sympathetic outflow through sympathetic cholinergic nerves that induce active vasodilation of peripheral vessels and perspiration of eccrine sweat glands, resulting in evaporative heat loss. When the ambient temperature (T_a) exceeds T_c, evaporative cooling is the only possible mechanism for heat dissipation (9,11,20).

After thermal effectors have responded to external cues, e.g. changes in T_a or T_{sk}, temperature sensors in internal organs recheck the adjusted T_c, and send a feedback signal to the POA in order to optimize the thermoregulatory circuit (11,21). In fact, only changes in T_c initiate the autonomic thermoregulatory responses and sensory information from T_c contributes more than T_{sk} to the autonomic responses (22). Furthermore, physiological thermoregulatory mechanismsalso involve behavioral adaptations, e.g. changing body posture or clothing [reviewed in (23)], which are beyond the scope of this chapter.

3. Sex difference in thermoregulation

Studies in humans and rodents have revealed sex differences in several of the thermoregulatory control mechanisms. A remarkable sex difference is the sex hormone-driven alterations in T_c. Women during the luteal phase of the menstrual cycle and women taking hormonal contraceptives have a 0.3–0.5°C higher T_c than men and women during the follicular phase (24,25). Of note, the T_c fluctuation during reproductive cycles is also observed in mice (26). This fluctuation in T_c in females is mediated by progesterone, resulting in decreased firing rates of warm-sensitive neurons and increased firing rates of cold-sensitive neurons in the POA (27). In addition, a study comparing the thermoeffector responses between the early follicular phase and the midluteal phase found that T_c at which women started shivering, sweating, and displaying cutaneous vasodilation were all increased in the luteal phase, confirming the set point altering effects of progesterone (28). Interestingly, estrogen seems to counteract this effect of progesterone on the temperature set point since the T_c of women taking combined estrogen-progestin (a synthetic progesterone) contraceptives was ~0.5°C lower than T_c of women taking progestin-only contraceptives (29).

An important factor for thermoregulation is body composition, an anthropometric feature that is profoundly different between men and women. A higher body surface area (BSA) directly results in a higher rate of heat loss, whereas body mass positively correlates with the rate of heat production and storage. The BSA-to-mass ratio thus dictates the net heat transfer from the body to its surroundings (30,31). With similar adiposity, women generally have a larger

BSA but a smaller body mass than men, indicating a higher heat loss and a lower heat production capacity compared to men. Therefore, the sex difference in body composition, with women having in general a higher BSA-to-mass ratio than men, suggests that women have a higher basal rate of heat loss than men (30-32). Intriguingly, a recent study found that the BSA-to-mass ratio was almost the sole determinant for heat-dissipating responses during an exercise with matched heat-loss requirements and that women favored dry heat loss (vasodilation) whereas men depended more on evaporative heat loss (sweating) (33).

The thermoeffector responses, e.g., adaptations of cutaneous vessels, sweating, and BAT thermogenesis, have been shown to display a certain degree of sex-dependent regulation. A human study in which the researchers infused warm or cold saline to manipulate T_c while keeping T_{sk} constant found that the average T_c at which women started vasoconstriction, sweating, and shivering were all ~0.3°C higher than the T_c in men (34). However, the inter-threshold range of shifting from vasoconstriction to sweating was ~0.2°C, which was equal in both sexes (34).

Concerning direct effects of sex hormones on vascular tones, 17β-estradiol (E2) directly causes rapid vasodilation by estrogen receptor (ER)-stimulated production of nitric oxide, an endothelium-derived relaxing factor, in vascular walls (35). The effects of progesterone and testosterone on blood vessels have also been studied, but the findings are contradictory. For example, while acute testosterone treatment increased vasodilation, chronic testosterone treatment led to impaired vascular relaxation and augmented vasoconstriction (36,37).

Studies have shown sex differences in sweating. Men had a greater maximal sweat rate than women when they were locally infused with acetylcholine, without a difference in cutaneous blood flow or the number of active sweat glands (38,39). Furthermore, although the acetylcholine-induced sweat rate was increased in physically trained subjects, the sweat rate remained higher in the physically trained men than in trained women (38). The same sex difference was also found when volunteers were exposed to a controlled whole-body heating protocol. Although men had a higher maximal sweat rate than women, the change in T_c and the T_{sk} at which sweating started, as well as cutaneous vasodilation measured by cutaneous vascular conductance, did not differ between the sexes (39).

As reviewed in chapter 1A, BAT is more prevalent and active in females than in males (5-7,40) and studies have demonstrated that the female sex hormones E2 and progesterone stimulate while the male sex hormone testosterone inhibits BAT activities (41,42). In addition, E2 can directly influence the central nervous system through ERα in the ventromedial hypothalamus (VMH),

which subsequently activates the rRPA, resulting in sympathetic nervous system activation, and hence BAT activation (43,44). Female mice lacking ERα in the VMH-specific steroidogenic factor-1 neurons had a reduction in sympathetic outflow activity and impaired BAT thermogenesis, confirming the direct E2-activated BAT thermogenesis in the VMH (45).

4. Thermal perception: a matter of sex?

In terms of thermal perception, thermal comfort is not only the sensation of T_a, but also a subjective interpretation of an individual how satisfied he/she is with T_a. Of interest, research has shown that women are more sensitive to and more often dissatisfied to fluctuations in T_a than men (46-50). In other words, women have a smaller comfort range of T_a than do men, though the findings have not always been statistically significant (50,51). This sex-dependent thermal preference has been nicely demonstrated in mouse studies. For instance, if mice were allowed to choose to reside at either 20, 25, or 30°C, females preferred to spend more time in the 30°C cage and less time in the 20°C cage, compared to males (52,53). One of the possible mechanisms for this sex difference in thermal sensation is the sensitivity of the cool-sensitive TRPM8. Females had higher sensitivity (lower threshold) and greater signal transmission of TRPM8 in the DRG neurons than males. The sex-dependent effects in TRPM8 were lost upon removal of E2 and testosterone from the culture media (54), implicating a role for sex steroids in thermal sensation.

5. Conclusion

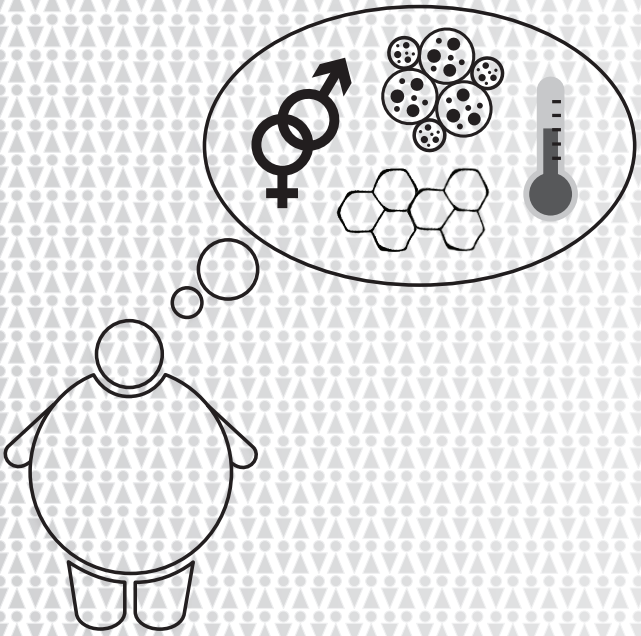
Optimal control of T_c is achieved by coordination of thermal somatosensory organs, central integrating centers in the hypothalamus, and thermoeffector organs. Sex differences in thermoregulation are evident in multiple aspects, such as anthropometric characteristics and effects of sex hormones on BAT. However, it is still largely unknown whether sex hormones are involved in the differences in thermal perception. Also, whether the different sensitivities to T_a in males and females are causative for the sex differences in thermoeffector responses requires more dedicated investigations.

References

1. van Marken Lichtenbelt WD, Vanhommerig JW, Smulders NM, Drossaerts JM, Kemerink GJ, Bouvy ND, Schrauwen P, Teule GJ. Cold-activated brown adipose tissue in healthy men. *N Engl J Med.* 2009;**360**(15):1500-1508.
2. Cypess AM, Lehman S, Williams G, Tal I, Rodman D, Goldfine AB, Kuo FC, et al. Identification and importance of brown adipose tissue in adult humans. *N Engl J Med.* 2009;**360**(15):1509-1517.
3. Virtanen KA, Lidell ME, Orava J, Heglind M, Westergren R, Niemi T, Taittonen M, et al. Functional brown adipose tissue in healthy adults. *N Engl J Med.* 2009;**360**(15):1518-1525.

4. Virtanen KA. The rediscovery of BAT in adult humans using imaging. *Best Pract Res Clin Endocrinol Metab*. 2016;**30**(4):471-477.
5. Ouellet V, Routhier-Labadie A, Bellemare W, Lakhal-Chaieb L, Turcotte E, Carpentier AC, Richard D. Outdoor temperature, age, sex, body mass index, and diabetic status determine the prevalence, mass, and glucose-uptake activity of 18F-FDG-detected BAT in humans. *J Clin Endocrinol Metab*. 2011;**96**(1):192-199.
6. Rodriguez AM, Quevedo-Coli S, Roca P, Palou A. Sex-dependent dietary obesity, induction of UCPs, and leptin expression in rat adipose tissues. *Obes Res*. 2001;**9**(9):579-588.
7. Law J, Bloor I, Budge H, Symonds ME. The influence of sex steroids on adipose tissue growth and function. *Horm Mol Biol Clin Investig*. 2014;**19**(1):13-24.
8. Sund-Levander M, Forsberg C, Wahren LK. Normal oral, rectal, tympanic and axillary body temperature in adult men and women: a systematic literature review. *Scand J Caring Sci*. 2002;**16**(2):122-128.
9. Tansey EA, Johnson CD. Recent advances in thermoregulation. *Adv Physiol Educ*. 2015;**39**(3):139-148.
10. Kuht J, Farmery AD. Body temperature and its regulation. *Anaesthesia & Intensive Care Medicine*. 2014;**15**(6):273-278.
11. Morrison SF, Nakamura K. Central Mechanisms for Thermoregulation. *Annu Rev Physiol*. 2019;**81**:285-308.
12. Voets T, Droogmans G, Wissenbach U, Janssens A, Flockerzi V, Nilius B. The principle of temperature-dependent gating in cold- and heat-sensitive TRP channels. *Nature*. 2004;**430**(7001):748-754.
13. Tan CH, McNaughton PA. The TRPM2 ion channel is required for sensitivity to warmth. *Nature*. 2016;**536**(7617):460-463.
14. Nakamura K, Morrison SF. A thermosensory pathway mediating heat-defense responses. *Proc Natl Acad Sci U S A*. 2010;**107**(19):8848-8853.
15. Cardinali DP. Third Level: The Hypothalamus. *Autonomic Nervous System*. Cham: Springer; 2018:175-244.
16. Blondin DP, Labbe SM, Phoenix S, Guerin B, Turcotte EE, Richard D, Carpentier AC, Haman F. Contributions of white and brown adipose tissues and skeletal muscles to acute cold-induced metabolic responses in healthy men. *J Physiol*. 2015;**593**(3):701-714.
17. Blondin DP, Labbe SM, Tingelstad HC, Noll C, Kunach M, Phoenix S, Guerin B, et al. Increased brown adipose tissue oxidative capacity in cold-acclimated humans. *J Clin Endocrinol Metab*. 2014;**99**(3):E438-446.
18. Kalinovich AV, de Jong JM, Cannon B, Nedergaard J. UCP1 in adipose tissues: two steps to full browning. *Biochimie*. 2017;**134**:127-137.
19. Labbe SM, Caron A, Bakan I, Laplante M, Carpentier AC, Lecomte R, Richard D. In vivo measurement of energy substrate contribution to cold-induced brown adipose tissue thermogenesis. *FASEB J*. 2015;**29**(5):2046-2058.
20. Johnson JM, Minson CT, Kellogg DL, Jr. Cutaneous vasodilator and vasoconstrictor mechanisms in temperature regulation. *Compr Physiol*. 2014;**4**(1):33-89.
21. Werner J. System properties, feedback control and effector coordination of human temperature regulation. *Eur J Appl Physiol*. 2010;**109**(1):13-25.
22. Frank SM, Raja SN, Bulcao CF, Goldstein DS. Relative contribution of core and cutaneous temperatures to thermal comfort and autonomic responses in humans. *J Appl Physiol (1985)*. 1999;**86**(5):1588-1593.
23. Terrien J, Perret M, Aujard F. Behavioral thermoregulation in mammals: a review. *Front Biosci (Landmark Ed)*. 2011;**16**:1428-1444.
24. Kattapong KR, Fogg LF, Eastman CI. Effect of Sex, Menstrual Cycle Phase, and Oral Contraceptive Use on Circadian Temperature Rhythms. *Chronobiol Int*. 1995;**12**(4):257-266.
25. Baker FC, Waner JI, Vieira EF, Taylor SR, Driver HS, Mitchell D. Sleep and 24 hour body temperatures: a comparison in young men, naturally cycling women and women taking hormonal contraceptives. *J Physiol*. 2001;**530**(Pt 3):565-574.
26. Sanchez-Alavez M, Alboni S, Conti B. Sex- and age-specific differences in core body temperature of C57Bl/6 mice. *Age (Dordr)*. 2011;**33**(1):89-99.
27. Nakayama T, Suzuki M, Ishizuka N. Action of progesterone on preoptic thermosensitive neurones. *Nature*. 1975;**258**(5530):80.
28. Hessemer V, Bruck K. Influence of menstrual cycle on shivering, skin blood flow, and sweating responses measured at night. *J Appl Physiol (1985)*. 1985;**59**(6):1902-1910.
29. Stachenfeld NS, Silva C, Keefe DL. Estrogen modifies the temperature effects of progesterone. *J Appl Physiol (1985)*. 2000;**88**(5):1643-1649.

30. Anderson GS. Human morphology and temperature regulation. *Int J Biometeorol.* 1999;**43**(3):99-109.
31. Castellani JW, Young AJ. Human physiological responses to cold exposure: Acute responses and acclimatization to prolonged exposure. *Auton Neurosci.* 2016;**196**:63-74.
32. MacAuley D. Exercise physiology: Gender and performance. *Oxford Handbook of Sport and Exercise Medicine*. 2nd ed. Oxford: Oxford University Press; 2012:164-165.
33. Notley SR, Park J, Tagami K, Ohnishi N, Taylor NAS. Variations in body morphology explain sex differences in thermoeffector function during compensable heat stress. *Exp Physiol.* 2017;**102**(5):545-562.
34. Lopez M, Sessler DI, Walter K, Emerick T, Ozaki M. Rate and gender dependence of the sweating, vasoconstriction, and shivering thresholds in humans. *Anesthesiology.* 1994;**80**(4):780-788.
35. Guo X, Razandi M, Pedram A, Kassab G, Levin ER. Estrogen induces vascular wall dilation: mediation through kinase signaling to nitric oxide and estrogen receptors alpha and beta. *J Biol Chem.* 2005;**280**(20):19704-19710.
36. Salerni S, Di Francescomarino S, Cadeddu C, Acquistapace F, Maffei S, Gallina S. The different role of sex hormones on female cardiovascular physiology and function: not only oestrogens. *Eur J Clin Invest.* 2015;**45**(6):634-645.
37. Iliescu R, Reckelhoff JF. Testosterone and vascular reactivity. *Clin Sci (Lond).* 2006;**111**(4):251-252.
38. Inoue Y, Ichinose-Kuwahara T, Funaki C, Ueda H, Tochihara Y, Kondo N. Sex differences in acetylcholine-induced sweating responses due to physical training. *J Physiol Anthropol.* 2014;**33**:13.
39. Gagnon D, Crandall CG, Kenny GP. Sex differences in postsynaptic sweating and cutaneous vasodilation. *J Appl Physiol (1985).* 2013;**114**(3):394-401.
40. Rodriguez-Cuenca S, Pujol E, Justo R, Frontera M, Oliver J, Gianotti M, Roca P. Sex-dependent thermogenesis, differences in mitochondrial morphology and function, and adrenergic response in brown adipose tissue. *J Biol Chem.* 2002;**277**(45):42958-42963.
41. Quarta C, Mazza R, Pasquali R, Pagotto U. Role of sex hormones in modulation of brown adipose tissue activity. *J Mol Endocrinol.* 2012;**49**(1):R1-7.
42. Monjo M, Rodriguez AM, Palou A, Roca P. Direct effects of testosterone, 17 beta-estradiol, and progesterone on adrenergic regulation in cultured brown adipocytes: potential mechanism for gender-dependent thermogenesis. *Endocrinology.* 2003;**144**(11):4923-4930.
43. Martinez de Morentin PB, Gonzalez-Garcia I, Martins L, Lage R, Fernandez-Mallo D, Martinez-Sanchez N, Ruiz-Pino F, et al. Estradiol regulates brown adipose tissue thermogenesis via hypothalamic AMPK. *Cell Metab.* 2014;**20**(1):41-53.
44. Contreras C, Gonzalez F, Ferno J, Dieguez C, Rahmouni K, Nogueiras R, Lopez M. The brain and brown fat. *Ann Med.* 2015;**47**(2):150-168.
45. Xu Y, Nedungadi TP, Zhu L, Sobhani N, Irani BG, Davis KE, Zhang X, et al. Distinct hypothalamic neurons mediate estrogenic effects on energy homeostasis and reproduction. *Cell Metab.* 2011;**14**(4):453-465.
46. Karjalainen S. Gender differences in thermal comfort and use of thermostats in everyday thermal environments. *Build Environ.* 2007;**42**(4):1594-1603.
47. Kim J, de Dear R, Candido C, Zhang H, Arens E. Gender differences in office occupant perception of indoor environmental quality (IEQ). *Build Environ.* 2013;**70**:245-256.
48. Maykot JK, Rupp RF, Ghisi E. Assessment of gender on requirements for thermal comfort in office buildings located in the Brazilian humid subtropical climate. *Energy Build.* 2018;**158**:1170-1183.
49. Schweiker M, Huebner GM, Kingma BRM, Kramer R, Pallubinsky H. Drivers of diversity in human thermal perception - A review for holistic comfort models. *Temperature (Austin).* 2018;**5**(4):308-342.
50. Wang Z, de Dear R, Luo M, Lin B, He Y, Ghahramani A, Zhu Y. Individual difference in thermal comfort: A literature review. *Build Environ.* 2018;**138**:181-193.
51. Fato I, Martellotta F, Chiancarella C. Thermal comfort in the climatic conditions of Southern Italy. *ASHRAE Transactions.* 2004;**110**(2):578-593.
52. Gaskill BN, Rohr SA, Pajor EA, Lucas JR, Garner JP. Some like it hot: Mouse temperature preferences in laboratory housing. *Appl Anim Behav Sci.* 2009;**116**(2-4):279-285.
53. Gaskill BN, Rohr SA, Pajor EA, Lucas JR, Garner JP. Working with what you've got: Changes in thermal preference and behavior in mice with or without nesting material. *J Therm Biol.* 2011;**36**(3):193-199.
54. Kondrats'kyi AP, Kondrats'ka KO, Skryma R, Prevars'ka N, Shuba Ia M. [Gender differences in cold sensitivity: role of hormonal regulation of TRPM8 channel]. *Fiziol Zh.* 2009;**55**(4):91-99.



Chapter 1C

General Introduction

Aims and Outline of this Thesis

1

Males and females display differences in adipose tissue distribution and function, as well as in thermoregulation, as reviewed in **Chapters 1A** and **1B**. Yet, the mechanisms that underlie this sex dimorphism are not completely understood. So far, most human and rodent studies have studied the role of the sex steroids estrogens and androgens in adipocyte metabolism, proliferation, and function. However, little is known whether other factors, such as glucocorticoids and sensory input, contribute to sex differences in white adipose tissue (WAT) and brown adipose tissue (BAT) function and whether these factors modulate the actions of sex steroids on adipose tissue.

This thesis therefore aims to investigate intrinsic and glucocorticoid-induced sex differences in adipose tissue function. In addition, this thesis aims to unravel the role of sex hormones in thermal perception, as this might be an important factor in controlling sex differences in BAT activity.

Chapter 2 presents a study on the effects of treatment with high-dose corticosterone (the endogenous rodent glucocorticoid) on whole-body glucose metabolism and adipose tissue adaptation in male and female mice. WAT and BAT are studied by morphological, molecular, and functional approaches. One of the aims of this study was to investigate whether biological sex modulates the adverse metabolic consequences caused by the intensive glucocorticoid treatment.

Chapter 3 focuses on sex differences in mouse BAT transcriptome with the aim to identify molecular mechanisms that contribute to sex differences in BAT morphology and activity. In addition, the effects of the less studied female sex hormone progesterone on cultured adipocytes were analyzed to gain insight into its regulatory role in BAT activity.

Chapter 4 demonstrates another aspect of BAT regulation, namely a sensory input for BAT activation. Because exposure to cold (an ambient temperature lower than the thermoneutral zone) is a major driver of BAT thermogenesis for maintaining an optimal body temperature, this study explored whether the thermal preference between young adult male and female mice differs. Since sex hormones are important physiological regulators of the temperature set point in the central nervous system, gonadectomy was performed to study the role of sex steroids herein.

Chapter 5 is a translational and confirmatory study on the thermal preference in young adult men and women. In this study, the water-filled cooling blanket was used as a body-cooling instrument since this is a common method for studying BAT activity. The temperature at which participants started shivering,

was used as the primary outcome since this is a sensitive and quantitative assessment for evaluating cold perception threshold in humans.

Chapter 6 provides a general discussion about the findings of the studies presented in this thesis and incorporates some recent updates on interactions between sex and stress hormones influencing adipose tissue function. Finally, conclusions and some future perspectives are provided.