Chapter 6

General Discussion

6

Overview

Adipose tissue is a multifunctional organ essential for maintaining whole-body energy homeostasis. The first known function of adipose tissue is an energy reservoir for the body by storing excess energy as triglycerides in intracellular lipid droplets which can be supplied in energy-deprived conditions. Second, adipose tissue acts as an endocrine organ since it secretes a variety of adipokines that systematically reflect the metabolic status. Apart from the classical type of adipose tissue [white adipose tissue (WAT)] which serves the above-mentioned functions, brown adipose tissue (BAT) is another specialized adipose tissue that can utilize energy substrates for non-shivering thermogenesis. In this latter process, the uncoupling protein 1 (UCP1) plays a crucial role (1). Currently, increasing energy expenditure by BAT activation or by "browning" of WAT is considered a promising tool for the treatment of obesity and metabolic diseases (2).

Despite being relatively overlooked, studies in rodents and humans have shown that males and females display many sex-dependent characteristics in WAT and BAT (3). Although the precise mechanisms underlying these sex differences are still not completely understood, studies have revealed crucial roles for sex hormones, particularly estrogens and androgens herein. This thesis broadens the understanding of sex differences in adipose tissue function upon modulation by corticosterone [an endogenous rodent glucocorticoid (GC)] or progesterone [a circulating female sex steroid that has been limitedly investigated]. Furthermore, this thesis also demonstrates sex differences in thermal perception, the sensory signal for whole-body thermoregulatory system, in which activation of BAT thermogenesis is its major autonomic response.

Factors affecting sex differences in WAT

The most prominent characteristic of sex differences in WAT is sexual dimorphism in fat distribution. Men and male rodents are prone to accumulate fat in visceral depots, known as the apple-shaped or android fat distribution which correlates to worsened metabolic health, whereas women and female rodents store fat in subcutaneous depots, known as the pear-shaped or gynoid fat distribution which is relatively protective against metabolic diseases (4). The sex-differential fat distribution is principally driven by sex steroids because it becomes apparent during puberty and reverses at menopause when women gain more visceral fat (4-6).

WAT expansion is a coordinated response by multiple cells in the adipose tissue, namely adipocytes, endothelial cells, fibroblasts, and immune cells (7). A healthy adipose tissue expansion is accomplished by four reciprocal steps:

1) transient hypoxic stress caused by a limitation in oxygen diffusion of enlarged adipocytes, 2) sufficient angiogenesis induced by local hypoxia and upregulations of angiogenic factors, such as vascular endothelial growth factors, 3) formation of new adipocytes through recruitment and differentiation of the perivascular adipose progenitors, and 4) remodeling of the extracellular matrix to promote further expansion (7,8). Studies in rodents have shown that female WAT depots exhibit a higher angiogenic induction, a greater differentiation capacity of adipose progenitor cells, and more optimal remodeling in extracellular matrix proteins than male WAT depots (9,10).

Endogenous GCs are adrenal hormones crucial for diverse physiological processes, including regulation of glucose homeostasis. Exogenous GCs are widely used due to their anti-inflammatory properties. Elevated endogenous or chronic exposure to exogenous GCs can cause many adverse effects in a variety of organ systems leading to Cushing syndrome that includes weight gain, fat redistribution towards truncal obesity (Cushingoid appearance), increased insulin resistance, and worsening of glycemic control or new-onset diabetes mellitus (11,12). GC-induced diabetes or insulin resistance predominantly presents with postprandial hyperglycemia through increased hepatic gluconeogenesis and reduced glucose uptake in skeletal muscle and adipose tissue (13). Although male sex has been suggested to be a risk factor to develop GC-induced diabetes (14), other studies have reported that the GC-induced insulin resistance was not sexually dimorphic (12,15,16). The study in **Chapter 2**, therefore, investigated the glucometabolic effects of a high dose of corticosterone in male and female mice. The results of this study demonstrate that corticosterone induces nonfasting hyperglycemia only in male mice but the compensatory hyperinsulinemia is profound in both sexes. Upon corticosterone treatment, serum total adiponectin and high-molecular-weight adiponectin levels, which are tightly correlated with improved insulin sensitivity (17), were higher in female mice than in male mice. In addition, the hyperplastic expansion of adipose tissue, which is associated with better insulin sensitivity compared with the hypertrophic expansion (8), was more evident in female adipocytes than in male adipocytes. Altogether, **Chapter 2** suggests a more beneficial adaptation in WAT depots of female mice upon GC administration than that of male mice.

Factors affecting sex differences in BAT and browning of WAT

GCs are known to suppress BAT thermogenesis, likely through the glucocorticoid receptor (GR) (18,19), but interestingly most studies have only been performed in males. Whether GCs affect BAT in a sex-dependent manner has not been extensively studied. The study in **Chapter 2** also demonstrates that treatment with a supraphysiological dose of corticosterone reduced *Ucp1*

mRNA expression and induced lipid accumulation in BAT in a sex-independent manner. In contrast, Gasparini *et al.* (20) found that corticosterone treatment induced lipid accumulation (whitening) in BAT only in male mice, not in female mice, but corticosterone treatment marginally reduced the amount of UCP1 in BAT. The discrepancy between findings in this thesis and the study of Gasparini *et al.* (20) is likely due to a higher dose of administered corticosterone and higher circulating corticosterone levels after treatment in our study, suggesting a sex difference in dose-sensitivity. Nevertheless, a recent study in mice with BAT-specific GR knockdown presented contradictory findings, i.e., GR signaling for BAT thermogenesis and metabolic activity was negligible upon cold exposure or high-fat diet–induced obesity (21). This observation could suggest that the deleterious effect of systemic GC administration on BAT *in vivo* is an indirect consequence of systemic metabolic disturbances via modulation of efferent signals from the central nervous system to BAT, or that the direct effect of GCs on BAT is signaled through other receptors or modulated by the interaction of GR and those receptors. Hence, the GC-GR-BAT axis still requires further validation.

A major plausible explanation for sexual dimorphism in BAT activity is likely a difference in circulating sex hormones. Detection of active BAT in adults by the positron emission tomography/computed tomography (PET/CT) imaging suggests that BAT is more prevalent in women than in men and that its presence is inversely correlated with outdoor temperatures, underscoring its thermogenic function (22). Another PET/CT study under thermoneutral conditions confirmed that women have higher BAT activity and BAT mass than men (23). This study also reported that BAT activity declines with age, which might be due to a decline in sex hormones (23). A similar sex difference is found in rodents: female rats have a larger BAT depot, a higher *Ucp1* mRNA expression in BAT, as well as greater lipolytic and thermogenic activation by β_3-adrenergic receptor (β_3-ADR) agonists than male rats (24). An *in vitro* study in primary brown adipocytes isolated from adult mice also supported direct effects of sex steroids on brown adipocytes. Testosterone dose-dependently inhibited norepinephrine-induced *Ucp1* mRNA expression, while 17β-estradiol (E2) and progesterone had no significant effects or slightly upregulated *Ucp1* mRNA expression (25). Moreover, E2 and progesterone were shown to reduce the α_{2A}/β_3-ADR protein ratio in primary brown adipocytes, reflecting a greater thermogenic and lipolytic capacity, whereas testosterone did not alter the α_{2A}/β_3-ADR ratio (26). Concerning the browning of WAT to reach a maximal thermogenic capacity, female sex hormones are important factors to induce WAT browning since CL316,243 (a β_3-ADR agonist) stimulation was able to induce browning of gonadal WAT only in female but not male C57BL/6J mice (27). This is remarkable since this depot is considered the most refractory WAT depot for browning in this mouse strain (28,29). Moreover,

the CL316,243-induced gonadal WAT browning was abolished when female mice had chemically induced ovarian failure (27), suggesting an interaction with female gonadal factors.

To uncover more sex-specific features in BAT, gene expression profiling was performed on BAT of male and female mice of which the data are described in **Chapter 3**. This study reveals that BAT gene expression profiles of male and female mice were indeed different, especially for genes encoding proteins involved in cellular structure, cell-cell contact, and cell adhesion. As expected, E2, progesterone, and dihydrotestosterone (DHT; a potent and active form of testosterone) were identified as possible upstream regulators for the sex-differential expression profile. Although expression of thermogenic markers *Ucp1* and *Ppargc1a* (the transcription factor of *Ucp1*) in BAT and primary brown adipocytes were not significantly sex-dependent, they show a tendency to be higher in female brown adipocytes.

In literature, the effects of progesterone on BAT activities are conflicting. Some studies have shown a stimulatory effect of progesterone on brown adipocytes (25,26), whereas *in vivo* observations show that BAT becomes inactive and atrophied during pregnancy (when progesterone concentrations are high), likely to conserve maternal energy for fetal growth (1). BAT of pregnant mice showed whitening morphological changes (enlarging intracellular lipid droplets) with decreased expression of thermogenic genes and increased expression of WAT markers (30). In **Chapter 3**, high concentrations of progesterone inhibited basal and norepinephrine-stimulated *Ucp1* and *Ppargc1a* mRNA expression in T37i cells, a female brown adipocyte cell line, in accordance with the findings of McIlvride *et al.* (30). The studies described in **Chapter 3** also test the effects of progesterone on primary brown adipocytes differentiated from stromal vascular fraction cells in BAT of male and female mice. In contrast to data in T37i cells, progesterone stimulation did not significantly affect *Ucp1* and *Ppargc1a* mRNA expression in primary brown adipocytes, but it dose-dependently reduced mRNA expression of *Adipoq*, the gene encoding the metabolically favorable adipokine adiponectin. Interestingly, the inhibitory effect of progesterone was likely due to the enhanced GR signaling. This suggests an interesting interaction between sex steroid and glucocorticoid signaling that requires further research to unravel the full physiological consequences. In addition, female primary brown adipocytes tended to express thermogenic genes and some cell-cell contact or structural genes at higher levels than male adipocytes although the cells were maintained in similar culture conditions, including sex steroid concentrations. This suggests intrinsic roles for the sex origin of the cells, driven by epigenetic programming or sex chromosomes. This finding stresses the need for stable cell lines generated from BAT of both male and female mice, which are currently lacking, in order

to understand these additional mechanisms beyond or in interaction with sex steroids.

At 22 °C (a usual temperature for housing conditions of laboratory animals), female rats have been reported to have higher *Ucp1* mRNA expression levels and higher mitochondrial activity in BAT than male rats (31). Surprisingly, this sex difference disappeared when the thermogenic capacity of BAT was maximally stimulated by exposing animals to cold (4 °C) since *Ucp1* mRNA expression was upregulated to a similar level in both sexes (31). These data suggest that a sex difference in thermal perception at 22 °C might result in sexually dimorphic BAT activity, a plausible explanation that will be discussed in the following section.

Factors affecting sex differences in thermal perception

Males and females pose many differences in the thermoregulatory system to achieve optimal thermal homeostasis. The major contributing factors are sexual dimorphisms in body composition and anthropometric characteristics implicating that women (or female rodents) generally have larger body surface area (BSA)-to-mass ratios, which result in greater net heat loss to the surroundings, than men (or male rodents) (32,33). Since mice are >3,000-fold smaller in body mass and have a larger BSA-to-mass ratio than humans, mice present some unique characteristics in thermoregulation. Compared to humans, mice prefer higher ambient temperatures (T_a) with a thermoneutral zone (TNZ) around 30–32 °C and mice have a lower vasomotor dependency, higher metabolic rates, larger BAT relative to body mass, and more diurnal variations in the core body temperature (T_c) (34,35).

Thermal perception is a broader term than thermal sensation since it incorporates thermal sensation (neural thermal reception), individual interpretation of thermal sensation (e.g. cold, cool, neutral, warm, or hot), and thermal comfort or satisfaction (comfortable vs. uncomfortable or satisfied vs. dissatisfied) (36). Many experimental and field studies indicate that women are more sensitive to deviations from a preferred T_a and women experience more dissatisfaction in a similar thermal environment than men (37,38). Some studies demonstrate that women prefer a higher T_a than men to achieve their thermal comfort (39); however, this observation is not always consistent since some studies show no sex-dependency in thermal preference (37,38).

To study thermal perception in animals, temperature preference tests, i.e. behavioral observations to study where animals prefer to reside among a range of T_a's, are usually performed. That is because behavioral thermoregulatory responses, i.e. movement to a preferred T_a, are energy-inexpensive mechanisms that animals use to optimize thermal needs (34). The temperature preference test

in adult mice presented in **Chapter 4** shows that female mice spend more time in cages with a higher T_a than male mice, especially during the light (inactive) phase, which is in line with a previous study (40). More specifically, the study in **Chapter 4** is the first to demonstrate that females prefer a T_a close to an upper limit of TNZ (32 °C) whereas male mice show no preference to either an upper limit (32 °C) or lower limit (29 °C) of TNZ.

The cool-sensitive thermoreceptor TRPM8 of females has been reported to be more sensitive than that of males and the *ex vivo* study in isolated dorsal root ganglion neurons showed that this difference was depended on the presence of E2 and testosterone (41). Surprisingly, the study in **Chapter 4** reveals that gonadectomy in adult mice does not affect the sex-specific thermal preference. This might suggest that an effect of gonadal hormones on thermal sensory inputs alone cannot explain the sex difference in thermal preference of adult mice and/or that sex hormones may have influenced the thermal neural circuits during earlier stages of life, such as the two most critical periods of brain development: pubertal and neonatal stages (42). In literature, it has been demonstrated that the preoptic area (POA) of the anterior hypothalamus (the thermoregulatory integration area) is responsive to gonadal hormones during development and shows striking sexual dimorphism in both molecular and morphological components, and hence causes several sex-specific behaviors in adults (42). Hence, it would be of interest to repeat this study using mice at prepubertal age.

The study in male and female volunteers in **Chapter 5** supports the findings in mice. Experiencing a gradually cooling protocol using a temperature-controlled water-perfused blanket, women start shivering at a higher temperature (a quantitative outcome for cold sensation) and sense/perceive a similar temperature colder and less comfortable than men. A subgroup analysis that matches the BSA-to-mass ratio between men and women does not change the sex-dependent outcomes, suggesting that body composition is not the only factor for the sex difference in thermal demand and that thermal preference has been determined in a sex-specific fashion. Hence, further (animal) studies investigating the roles of sex hormones before puberty are needed to obtain further insight into the underlying mechanism of the sex-dimorphic thermal preference.

Future perspectives for studies of sex difference in adipose tissue function

Apart from sex hormones, sex chromosomes are likely independent factors regulating adiposity (43). The four core genotypes (FCG) mouse model in which the testis-determining gene *Sry* is relocated from the Y chromosome to an autosome, has been used to investigate the segregation of the effects of the

gonadal sex and gonad-secreting factors from the sex chromosome complement (44). Comparing mice with a similar gonadal type in early adulthood, mice with XX chromosomes were slightly but significantly heavier than those with XY chromosomes (45). When gonadectomy was performed to remove acute effects of sex hormones, the XX mice had a substantial increase in total fat mass and plasma leptin levels, compared to XY mice, regardless of their original gonads (45). This suggests that the sex chromosomes themselves have effects on adipose tissue function. Indeed, an increase in adiposity by the number of X chromosomes was also observed in the XY* mouse model in which the paternal unusual Y* chromosome produces offspring equivalent to XO, XX, XY, and XXY genotypes (44,45). Metabolic challenges with a high-fat diet revealed sex-chromosomal effects on fat distribution: XX mice had a larger inguinal WAT depot whereas XY mice had a larger gonadal WAT depot, independent of gonads (45). All these studies were performed with mice of the C57BL/6J strain, but similar genetic models in mice of the MF1 strain confirmed a role of the total number of sex chromosomes on adiposity and metabolism (46), but showed no differential effects whether these were X or Y chromosomes. Thus, animal studies indicate that the number of the sex chromosome is an independent factor for body fat accumulation. In humans, men with Klinefelter syndrome (47,XXY) and women with Turner syndrome (e.g. 45,X) have increased risks of abdominal obesity and metabolic diseases relative to their normal karyotype controls (47,48). Because patients with Klinefelter and Turner syndromes also suffer from hypogonadism, effects of sex chromosomes and sex hormones on adiposity in humans cannot be individually evaluated.

The effect of sex chromosomes on BAT function has not been studied in detail. Thus far, there has been only one study investigating BAT of the FCG mice, in which no effect of sex chromosomes on *Ucp1* and *Ppargc1a* mRNA expression in BAT at 4 weeks or 10 months after gonadectomy was described (45). Interestingly, in a similar sex-chromosomal status at 4 weeks after gonadectomy, orchiectomized gonadal male mice had a slightly higher *Ucp1* mRNA expression level in BAT than ovariectomized gonadal female mice (45), stating a role of gonadal hormones rather than sex chromosome complements. Nevertheless, since the study in **Chapter 3** reveals sex-dependent gene expression patterns in primary brown adipocytes independent of sex steroids, further analysis of the role of sex chromosomes and their interaction with sex steroids, particularly in pathological conditions in which cells are exposed to sex steroids opposite to their sex-chromosomal status, is warranted.

Genome-wide association studies (GWAS) of genetic variants, such as single-nucleotide polymorphisms (SNPs) and short deletions or insertions, revealed many genetic loci associated with body mass index (BMI; a surrogate

for obesity) and waist-to-hip ratio adjusted for BMI (WHR_{adjBMI}; an index for abdominal obesity) (49). A study in 339,224 individuals identified 97 autosomal genetic loci associated with BMI of which only 2 loci were marginally sex-dependent (50). Another study in 224,459 individuals identified 49 loci associated with WHR_{adjBMI} of which 20 loci were sex-dependent (51). Interestingly, there was no overlap in the identified loci for general and abdominal obesity [see a comprehensive list in (49)]. A subsequent study in 320,485 individuals revealed that 15 BMI-associated loci showed age-specific effects and 44 WHR_{adjBMI}-associated loci showed sex-specific effects, but interestingly there was neither a sex-specific effect on BMI-associated loci nor an age-specific effect on WHR_{adjBMI}-associated loci (52). Therefore, these GWAS data suggest that genetic variants in autosomal DNA might contribute to sex dimorphisms in the fat distribution pattern, but not for general obesity. Of note, variants in sex chromosomes were excluded from these GWAS due to technical reasons.

Epigenetic modifications, such as DNA methylation, histone modifications, and environmental factors, have been shown to play an important role in WAT programming (53), which includes some sex-differential features. For example, a single injection of testosterone in female mice and rats during the early postnatal period resulted in obesity, increased visceral fat accumulation, and insulin resistance at adult age (54,55). These phenotypes likely resulted from imprinting or epigenetic programming due to neonatal testosterone exposure. The epigenetic effect of androgens is supported by a study in women with polycystic ovary syndrome (PCOS) whose classical manifestations include hyperandrogenism (56). Several DNA methyltransferases (*DNMT*s; major enzymes that maintain methylation patterns upon DNA replication) were differentially methylated and *DMAP1* (DNMT1 associated protein 1; a protein involved in DNA methylation and obesity-related inflammation) was differentially expressed in abdominal subcutaneous WAT of women with PCOS compared to those of healthy controls. In addition, 30 differentially expressed genes were identified with changes in DNA methylation sites (56), suggesting the potential influence of DNA methylation on corresponding gene expression.

Epigenetic regulation is also important for BAT function at many levels (57). For example, a retrospective study found that individuals with active BAT in PET/CT imaging were more likely to have been conceived in the colder period of the year (October–February) than in the warmer period (April–September) (58). Studies in mice showed that paternal cold exposure before mating led to upregulated basal and cold-stimulated UCP1 protein levels in BAT and inguinal WAT in the offspring of both sexes, which is likely due to a differential methylation status in the sperm of the cold-exposed males compared to control fathers (58). Some histone deacetylases (HDACs), which remove acetyl groups from histones,

compact the chromatin structure, and generally suppress gene transcription, are recognized as negative regulators for BAT thermogenesis (57). For instance, acute cold exposure or an injection of CL316,243 in mice downregulated *Hdac1* mRNA expression in BAT and inhibitions of HDAC1 upregulated *Ucp1* mRNA and UCP1 protein levels in cultured brown adipocytes (59). Another relevant observation is that a single injection of testosterone during the neonatal period in female mice increased lipid accumulation and reduced mRNA expression of *Ucp1* and other BAT-specific genes in BAT in later life (55), suggesting lifelong epigenetic modulation by postnatal sex steroid exposure. However, our preliminary unpublished study observed no apparent sex difference in the global methylation pattern in BAT of adult male and female mice housed at normal housing conditions. Hence, whether sex-dependent epigenetic programming in BAT at physiological conditions exists and whether it affects sex differences in BAT function warrants further studies.

Concerning the secretory function of adipose tissue apart from conventional adipokines, WAT and BAT produce and secrete exosomes containing microRNAs (miRNAs) which are 19–22-nucleotide long non-coding RNAs that regulate gene expression and translation in other tissues, such as liver (60). A study in the FCG mice revealed that sex differences in miRNA expression levels in gonadal WAT were mainly driven by the gonadal type, thus likely by sex hormones (61). When gonadectomy was performed to eliminate the acute effects of sex hormones, sex chromosome complements were also found to play a role in miRNA profiles, but to a lower extent than sex hormones (61). To the best of my knowledge, sex differences in miRNA expression levels in BAT have not been investigated. It is worth mentioning that circulating levels of exosomal miRNAs, such as miR-34c, miR-92a, and miR-122, are possibly a non-invasive biomarker to reflect *in vivo* BAT activity in mice or humans (62-64). However, our preliminary unpublished data and a subsequent study by Okamatsu-Ogura *et al.* (64) could not confirm a correlation of miR-92a levels and BAT activity. It would, therefore, be of interest to study the circulating concentrations of miRNAs in males and females with an emphasis on the role of sex steroids in the circulating miRNA levels.

As mentioned earlier, effects of sex steroids or other factors could differ depending on the sex origin of the cells. The human-induced pluripotent stem cell (hiPSC) is a promising tool to produce an *in vitro* functional white or brown adipocyte model that is most analogous to *in vivo* adipocytes to study sex-dimorphic features. Somatic cells, such as skin fibroblasts, can be used to generate iPSCs (65). Since iPSCs are capable of long-term self-renewal and can be expanded into large numbers, many biological or translational tests can be performed. In addition, obtaining iPSCs is more feasible than obtaining other

primary human cells, namely human embryonic stem cells (which raises ethical issues) or adipose-derived stromal vascular cells (which have limited proliferative potential and may quickly lose differentiation capacities) (66,67). However, one might argue that the traditional cell culture in which cells are maintained in monolayer cultures is not an ideal model for adipose tissue structure. To mimic the natural structure, three-dimensional cultures may be preferred and this would also allow studying interactions with additional cell types such as endothelial cells and immune cells. Protein scaffolds of biomaterials, e.g. silk or elastin-like polypeptides conjugated to polyelectrolytes, have been shown to enhance long-term tissue sustainability, structural organization, and functional activities (68,69). Three-dimensional cultures may facilitate the understanding if sex difference in adipose tissue structure persists *ex vivo* and whether this leads to the functional discrepancy.

WAT shows many sex- and depot-specific morphology and composition in its microenvironment, an aspect that has been limitedly studied in BAT. Our transcriptional data in **Chapter 3** suggest that the microenvironment also plays an important role in regulation of sex-specific BAT function. Our data furthermore implicates that interactions of brown adipocytes with various cells present in the BAT depot contribute to sex-specific BAT function. Compared to WAT, BAT has a higher number of endothelial cells and denser vasculature in the tissue (70,71), as well as a higher norepinephrine turnover rate per depot, which indicates a dense innervation of sympathetic nerves (72,73). These features of BAT facilitate its thermogenic function in both mice and humans, as blood flow to BAT, uptake of glucose or fatty acids in BAT, and skin temperature overlaying BAT depots are markedly increased by norepinephrine injection or cold exposure (71,74,75). Female BAT is more responsive to adrenergic stimulation than male BAT (24), but whether the adrenergic innervation or the blood supply to BAT is sexually dimorphic has not been studied. Our data showing sex-dimorphic expression of structural genes in BAT, not being expressed in primary adipocytes, suggest that these additional features may be interesting targets to activate BAT.

Steroid hormone receptors such as the GR, mineralocorticoid receptor (MR), estrogen receptor (ER), progesterone receptor (PR), and androgen receptor (AR) are members of the nuclear receptor superfamily 3 and they therefore share several molecular and functional characteristics. Upon ligand binding, the ligand-receptor complex is translocated from the cytoplasm to the nucleus, binds to the response elements and/or interacts with coregulatory proteins, and hence activates or inhibits transcription of target genes (76,77). The coregulatory proteins, e.g. the steroid receptor coactivator (SRC) family, also known as the nuclear receptor coactivator (NCoA), have been shown to regulate adipocyte differentiation and energy expenditure (78). However, whether coregulatory

proteins influence sex-dependent transcriptional profile in adipose tissue has not been systematically evaluated. Since studies in **Chapters 2–3** suggest interactions of sex hormones and GR signaling on adipose tissue functional activities, nuclear receptor coregulatory complexes, as well as crosstalk among sex hormones and nuclear receptors, should be investigated. The most relevant study of hormonal crosstalk described so far in literature was performed only in male mice. Upon corticosterone treatment, DHT cotreatment potentiated transcription of GR-target genes in WAT and BAT, and cotreatment with the AR antagonist enzalutamide attenuated the GR signaling in WAT (79). The GR-inhibiting effect of enzalutamide in WAT is likely driven by reduced activity of the enzyme 11β-hydroxysteroid dehydrogenase type 1 (11β-HSD1; an intra-tissue GC reactivating enzyme) (79).

Summary

Altogether, this thesis has broadened our understanding of sex differences in control of WAT and BAT function in various aspects, namely sex-dependent adaptation of WAT upon corticosterone exposure, sex-specific transcriptional profile in BAT at basal conditions, and sex-biased thermal perception (the afferent signals essential for BAT activation and WAT browning). These findings generally support that inclusion of sex should be considered as an independent risk factor when considering individualized treatment options for patients with obesity. However, more studies are warranted to fully understand this complex picture of sexual dimorphisms in adipose tissue biology.

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