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Sex Differences in Brown Adipose Tissue Function: Sex Hormones, Glucocorticoids, and Their Crosstalk

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Excessive fat accumulation in the body causes overweight and obesity. To date, research has confirmed that there are two types of adipose tissue with opposing functions: lipid-storing white adipose tissue (WAT) and lipid-burning brown adipose tissue (BAT). After the rediscovery of the presence of metabolically active BAT in adults, BAT has received increasing attention especially since activation of BAT is considered a promising way to combat obesity and associated comorbidities. It has become clear that energy homeostasis differs between the sexes, which has a significant impact on the development of pathological conditions such as type 2 diabetes. Sex differences in BAT activity may contribute to this and, therefore, it is important to address the underlying mechanisms that contribute to sex differences in BAT activity. In this review, we discuss the role of sex hormones in the regulation of BAT activity under physiological and some pathological conditions. Given the increasing number of studies suggesting a crosstalk between sex hormones and the hypothalamic-pituitary-adrenal axis in metabolism, we also discuss this crosstalk in relation to sex differences in BAT activity.

Keywords: androgens, estrogens, glucocorticoids, progesterone, sex characteristics, sex chromosomes, steroid receptors, brown adipocytes

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INTRODUCTION

Excessive or abnormal fat accumulation in the body causes overweight and obesity. The recent report of the World Health Organization (WHO) showed that 39.1% of adults worldwide in 2016 were overweight and 13.2% (or over 650 million) were obese, which is approximately three times as high as in 1975 (1). Obesity is a major risk for a variety of chronic diseases, including diabetes mellitus and cardiovascular diseases (CVD), mainly heart disease and stroke (1). Although the prevalence of obesity among adults is only marginally higher in women than in men (2, 3), the incidence and severity of CVD are lower in premenopausal women than in men and rise after menopause (4). Multiple determinants, such as sex hormones, sex chromosomes, physical exercise, smoking, and environmental factors, have been described to account for this sex difference in CVD and metabolic risks (5).

The major contributors to the global trend of obesity are the increased energy-rich food consumption and a physically inactive lifestyle (1), resulting in a net excess caloric intake. These

excess calories are stored as triglycerides (TG) in adipose tissue which was previously considered a passive organ that stores excess energy and provides metabolic substrates for the body when needed. Nowadays, the adipose tissue is also considered an endocrine organ as it secretes various hormones called adipokines that play a role in the adaptation to different physiological and pathological conditions and that contribute to the regulation of whole-body energy homeostasis/metabolism (6).

Adipose tissue is generally categorized into two types with opposing functions: lipid-storing white adipose tissue (WAT) and lipid-burning brown adipose tissue (BAT). The latter tissue can dissipate energy (i.e., lipids) as heat instead of adenosine triphosphate (ATP), a process that is mediated by the mitochondrial uncoupling protein 1 (UCP1). Previously, it was thought that BAT was only present and active in hibernating animals, small mammals, and human infants. The traditional concept was that BAT regressed in the early years of life, leading to an absence of BAT in adults (7). Studies using positron emission tomography/computed tomography (PET/CT) in which the uptake of energy substrates indicates the metabolically active tissues, as well as UCP1 immunohistochemistry have revealed the existence of active BAT in healthy adults since 2009 (8–11). BAT is located in the cervico-supraclavicular region (between the shoulder blades), but depots are also found in the axillary, mediastinal, paravertebral, perirenal, and peri-aortic regions (12). After the rediscovery of functioning BAT in human adults, BAT has received renewed interest since its lipid oxidizing properties are considered ideal to battle the obesity pandemic.

Multiple studies have demonstrated sex differences in BAT activity. Female rodents have higher prevalence of active BAT and greater BAT mass than males (13–15). Under normal animal housing conditions at 22°C, female rats have larger mitochondria with more cristae and a higher amount of mitochondrial proteins including UCP1 in BAT than male rats (15, 16). Female BAT also displays higher protective metabolic adaptations than male BAT under energy-excess conditions. Several studies showed that when fed a high-fat diet (HFD) or a high-fat high-sugar diet, female rats increased their energy expenditure by maintaining higher amounts of thermogenic proteins such as UCP1 and PGC1α (the transcriptional coactivator of UCP1 and mitochondrial biogenesis), as well as those involved in lipid oxidation in their BAT than male rats (15, 17). Moreover, when rats were energy-deprived (i.e., received 60% of the calories of *ad libitum* fed animals for 100 days), females had a larger decline in BAT thermogenesis than males resulting in a reduced energy expenditure and preserved vital organs (18, 19).

Studies in humans also suggest that women have a higher BAT activity than men. Metabolically active BAT was detected in nearly 6% of participants in retrospective PET/CT studies (20, 21). In these studies, sex was an independent determinant of BAT activity, with women having more often detectable BAT on a fluorodeoxyglucose-PET/CT scan (20, 21). Others, however, report conflicting results, which may be because human BAT is more dispersed than the classical BAT in rodents (22). This is clear from a recent PET/CT study in non-obese adults in which it was found that women have a lower supraclavicular BAT volume but a comparable activity as men while BAT activity in the superficial dorsocervical region was more prevalent in women (23). This finding of sex differences in the distribution of active BAT adds to the complexity to study BAT function in humans.

BAT activity changes with age. Unlike the old belief, BAT mass does not decrease but even increases with age in children, a process associated with the degree of sexual maturation (24). Baseline and cold-induced BAT activity appeared greater in prepubertal girls than in boys (25). Also in young adults, cold-induced BAT activity was higher in women than in men, although the tissue density was less (26). In contrast, BAT activity in adults declines with increasing age, reflected by lipid accumulation and a decline in UCP1 expression (21, 27, 28). Interestingly, the sex dimorphism in BAT activity disappears when women become postmenopausal. This suggests that the age-related decline in circulating levels of sex hormones may contribute to this loss of BAT activity (29–31). In addition, it has been proposed that a decrease in sex hormone levels leads to a relative increase in inhibitory actions of glucocorticoids (GC) on BAT, thereby contributing to the loss of BAT activity (32), although this intriguing concept still needs to be confirmed experimentally. Species differences in BAT characteristics are summarized in **Table 1** and sex differences in BAT activity are summarized in **Table 2**.

This review will address the mechanisms that contribute to sex differences in the regulation of BAT activity. We will focus on the roles of sex hormones in the regulation of BAT activity, i.e. thermogenesis, under physiological and pathological conditions. In addition, we will discuss the crosstalk between sex hormones and GCs in the regulation of BAT activity. Understanding the mechanisms that underlie the sex dimorphism in BAT activity will not only improve our understanding of BAT biology but will also help to better understand sex differences in metabolic diseases.

TABLE 1 | Comparison of BAT characteristics between rodents and humans.

Characteristics	Rodents	Humans
BAT distribution	Well-defined fat pads, i.e., interscapular and dorsocervical BAT	Found dispersed in many regions of the body, e.g., supraclavicular, interscapular, paravertebral/dorsal, axillary, perirenal areas
Cellular composition of BAT	Mostly homogeneous brown adipocytes	Mixture of brown, beige, and white adipocytes
ADR subtype involving BAT thermogenesis	β_3-ADR	Likely β_2-ADR
Effect of aging on BAT activity	Minimal decline (BAT remains active in old rodents.)	Gradual decline with age

ADR, adrenergic receptor; BAT, brown adipose tissue.

TABLE 2 | Comparison of BAT features between males and females.

BAT features	Species	Findings (Females vs Males)	References
BAT mass or BAT volume (relative to body mass)	Rodents	Females > Males	(15, 16, 33–35)
		Females < Males	(31)
		No sex difference	(36)
	Humans	Females > Males	(10, 20, 28, 37)
		No sex difference	(23, 38)
BAT activity detected by PET/CT imaging	Humans	Females > Males	(10, 20, 21, 28, 37–39)
		No sex difference	(23, 40)
UCP1 protein levels	Rodents	Females > Males	(16, 17, 19, 31, 34, 35, 41, 42)
		Trend of Females > Males	(15)
Ucp1 mRNA expression	Rodents	Females > Males	(17, 35)
		Trend of Females > Males	(15, 36, 43)
		No sex difference	(34, 41)
BAT thermogenesis or response upon adrenergic stimulation	Rodents	Females > Males	(16, 35)
		No sex difference	(15, 19)

BAT, brown adipose tissue; PET/CT, positron emission tomography/computed tomography; UCP1, uncoupling protein 1.

ADIPOSE TISSUE CHARACTERISTICS AND FUNCTION

Adipose Tissue Plasticity

As mentioned above, adipose tissue is principally classified into WAT and BAT. Studies have revealed the high capacity of cellular plasticity in adipose tissue. WAT can transdifferentiate into brown-like tissue by, for instance, prolonged adrenergic stimulation and acute or sustained exposure to low-temperature conditions, which are all factors that also activate BAT thermogenesis by means of stimulated lipid oxidation (44, 45). This activation process is called 'browning' and the resulting adipose tissue is called brite (brown-in-white) or beige adipose tissue. Of interest, the potential of cold to induce browning of WAT declines with age in mice and humans (45). In contrast, BAT can undergo 'whitening' as is seen, for instance, with β-adrenergic signaling impairment, chronic inflammation, high-temperature acclimation, and aging (46).

WAT Storage Function and Distribution

WAT is not a static tissue: white adipocytes can expand through hypertrophy (increase in cell size of existing adipocytes) and/or hyperplasia (increase in number by forming new adipocytes from preadipocytes or progenitor cells) to store excess energy. Hypertrophic expansion is associated with adverse metabolic consequences because the enlarged cells exceed a maximum limit of oxygen diffusion which might lead to hypoxia and even fibrosis and inflammation, and hence insulin resistance (47–50). In contrast, hyperplastic expansion is linked with favorable metabolic outcomes and it occurs simultaneously with angiogenesis, allowing the supply of nutrients and oxygen to growing adipocytes through the newly formed blood vessels (51, 52). Of interest, angiogenesis and adipogenesis are reciprocally regulated by vascular endothelial growth factor (VEGF) and peroxisome proliferator-activated receptor-γ (PPARγ), as VEGF inhibition and loss of PPARγ activity reduce vascular formation and preadipocyte differentiation (53).

Regarding fat distribution in the body, WAT can generally be categorized into two groups by anatomical deposition: subcutaneous depots, such as anterior (axillary) and posterior (inguinal) subcutaneous depots for rodents or abdominal subcutaneous and gluteofemoral depots for humans; and visceral depots, e.g. mesenteric, gonadal, omental, and retroperitoneal depots (54, 55). Differences in WAT expansion between anatomical depots are evident in both rodent and human studies. In general, visceral obesity is associated with increased risks of metabolic complications and, therefore, measurement of waist circumference has been suggested as a reasonable proxy and indicator for the risk to develop CVDs and metabolic diseases (56–59).

Of interest, there is sexual dimorphism in fat accumulation. At an equivalent body mass index (BMI), women generally have a higher percentage of body fat than men (60–62). Moreover, women typically accumulate body fat around hips and thighs, resembling a pear-shaped body, whereas men accumulate fat around the abdomen, resembling an apple-shaped body (62–64). In other words, women, as well as female rodents, have relatively less visceral fat and more subcutaneous fat than age-matched males (65–69). This sex-dependent fat distribution becomes apparent after puberty, implying the role of sex hormones herein (70). In line with the suspected role of sex hormones, this sex difference is diminished in postmenopausal women since they gain visceral fat and their body shape converts into the male-like fat distribution (71, 72). A discussion on the effects of sex hormones on WAT function and distribution is beyond the scope of this review and has been comprehensively reviewed elsewhere (63, 73).

BAT Thermogenesis and Metabolic Function

Under physiological circumstances, a low ambient temperature stimulates thermoreceptors in the skin and cutaneous layer that transmit sensory inputs to the preoptic area of the hypothalamus. The hypothalamic neuronal circuit then stimulates the sympathetic neurons that activate vasoconstriction in the skin and cutaneous layer in order to reduce heat loss. If rapid heat generation is required, the sympathetic neurons also induce skeletal muscle shivering and promote non-shivering thermogenesis in BAT (74).

Using fluorodeoxyglucose-PET/CT scans, it has been shown that BAT activity negatively correlates with outdoor temperatures and is more prevalent during winter than other seasons (20). In humans, chronic or repeated exposure to cold, e.g. 2 hours per day for 4 weeks, increases the volume and oxidative metabolic rate of BAT (75).

Upon cold exposure, sympathetic nerves in BAT secrete norepinephrine to induce BAT thermogenesis *via* β-adrenergic receptors (β-ADR). In mice, β_3-ADR has been shown to be the major β-ADR controlling BAT thermogenesis (76). However, it is still under debate if the β-ADR subtype involved in BAT activation is the same in mice and humans. A recent study suggested that β_2-ADR is likely responsible for BAT activity in humans (77), but some studies have also shown that a β_3-ADR agonist can increase BAT activity (78, 79). The current hypothesis on how lipid metabolism links to thermogenesis in brown adipocytes is depicted in **Figure 1**. Sympathetic activation of BAT results in intracellular lipolysis of TGs stored in lipid droplets catalyzed by adipose triglyceride lipase (ATGL), hormone-sensitive lipase (HSL), and monoacylglycerol lipase (MGL), resulting in the release of fatty acids which are the main substrate for mitochondrial respiration (80). In brown adipocytes, this respiration does not generate ATP molecules but instead will generate heat *via* the actions of UCP1 (80). Additionally, cold exposure also stimulates the uptake of fatty acids and glucose from the circulation into BAT, leading to a decline in plasma levels of free fatty acids, TG, and glucose (81). Once the fatty acids enter brown adipocytes, they will be esterified into TG and integrated into lipid droplets before they are hydrolyzed to yield the substrates for uncoupling thermogenesis (81). The importance of intracellular TG was confirmed by the impaired BAT thermogenesis in male and female ATGL-deficient mice during acute cold exposure (82). Moreover, cold exposure induces *UCP1* mRNA transcription and UCP1 protein abundance together with higher transcription and activity of crucial proteins in substrate turnover (81, 83–85). Chronic cold exposure also induces browning of WAT, as well as mRNA and protein expression of UCP1 in WAT of rodents (83, 86, 87) and humans (88). Of note, this browning is more pronounced in subcutaneous WAT than in visceral WAT (89).

Research has shown the significance of BAT in the regulation of body homeostasis through various mechanisms that include crosstalk with multiple organs [see for a comprehensive review (90)]. For instance, BAT contributes to controlling glucose homeostasis and energy balance since BAT transplantation improves insulin sensitivity, reduces body weight and WAT

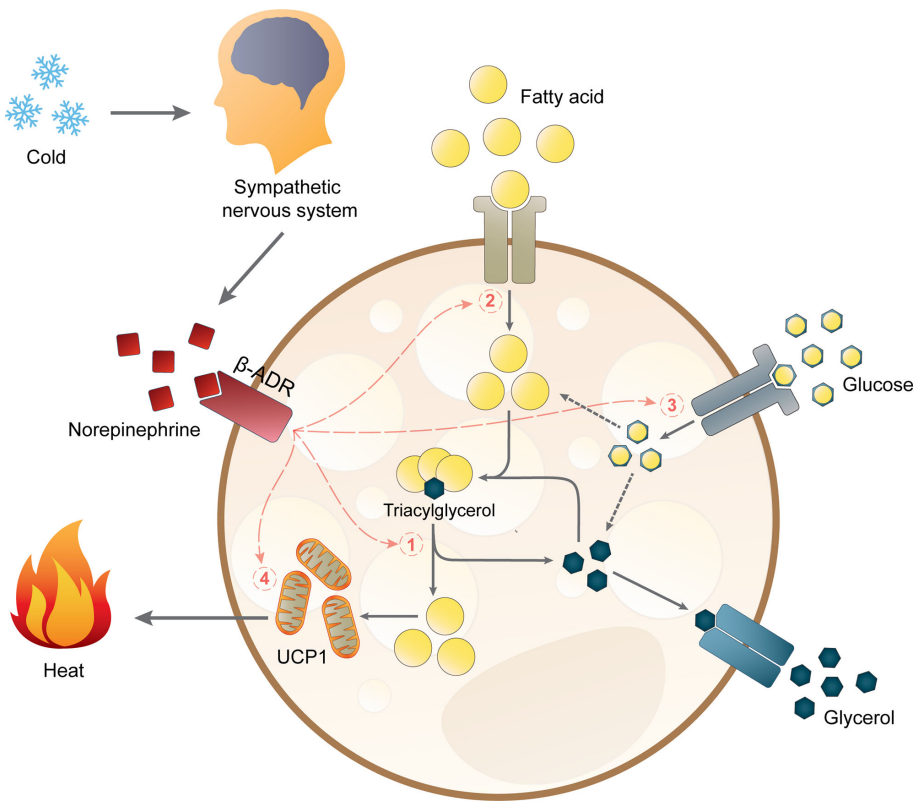

FIGURE 1 | Non-shivering thermogenesis and lipid metabolism in brown adipocytes. Acute thermogenic responses by activating β-adrenergic receptors (β-ADR) which hence stimulate (1) intracellular lipolysis, (2) fatty acid uptake, and (3) glucose uptake. Altogether, these processes increase the availability of intracellular free fatty acids for thermogenesis by mitochondrial uncoupling protein 1 (UCP1). Prolonged cold exposure also induces adaptive thermogenesis by (4) upregulating *UCP1* mRNA expression.

mass, and attenuates HFD-induced glucose intolerance (91, 92). The metabolic function of BAT thermogenesis is also supported by studies in the male UCP1-deficient mice, which show impaired glucose tolerance upon HFD feeding (93). Moreover, BAT is also an endocrine organ since it secretes various adipokines, such as the well-known adipokine adiponectin, recognized for its anti-diabetic and anti-inflammatory properties, and the more likely BAT-specific adipokines, e.g., bone morphogenetic protein 8B (BMP8B) and neuregulin-4 (NRG4) (94).

EFFECTS OF SEX HORMONES AND GLUCOCORTICOIDS ON BAT

Physiological Principles

The production of sex hormones is under tight regulation of the hypothalamic-pituitary-gonadal (HPG) axis (95). In brief, the gonadotropin-releasing hormone (GnRH) neurons in the hypothalamus secrete GnRH in a pulsatile fashion to induce the production and secretion of gonadotropins [follicle-stimulating hormone (FSH) and luteinizing hormone (LH)] from the anterior pituitary. In turn, gonadotropins stimulate the gonads to produce sex hormones.

In fertile women, the ovaries secrete estrogens during the follicular phase of the menstrual cycle, and estrogens and progesterone during the luteal phase. The main circulating estrogen is 17β-estradiol (E2). At target organs, estrogens typically bind to the nuclear estrogen receptors (ER), i.e., ERα and ERβ. Nuclear translocation of the estrogen-ER complex is followed by DNA binding to an estrogen response element or to other transcription factors ultimately leading to transcriptional activation or inhibition of target genes (65). In addition, estrogens can bind to the G protein-coupled estrogen receptor (GPER, previously known as GPR30) or other membrane-associated receptors to rapidly initiate non-genomic responses (96). In addition to the ovaries, progesterone is produced by the placenta during pregnancy and to some extent by the adrenal glands. The classical progesterone signaling pathway involves the nuclear progesterone receptor (PR). However, progesterone can also mediate its effects *via* non-classical pathways through receptors such as the PR membrane components (PGRMC) 1 and 2 and the membrane-associated progesterone receptors (mPR), which are members of the progestin and adipoQ receptor (PAQR) family (97).

In men, the principal circulating androgen is testosterone, produced by the testes but also in small amounts by the adrenal glands. The adrenal glands also synthesize androgenic precursors (so-called adrenal androgens), such as androstenedione, dehydroepiandrosterone (DHEA), and DHEA sulfate (98, 99). In various tissues, e.g. the prostate, the liver, and many brain regions, testosterone is converted to the very potent androgen dihydrotestosterone (DHT) by the enzyme 5α-reductase. The physiological actions of testosterone and DHT are mediated by the nuclear androgen receptor (AR). Furthermore, circulating testosterone can locally be aromatized to E2 by the enzyme aromatase (CYP19A1), thereby contributing to increased local estrogen levels. This holds true for various WAT depots, although sex differences in *CYP19A1* expression may exist (100–102). However, studies in male mice suggest that BAT does not express *Cyp19a1* and, in line, has undetectable E2 levels (102–104). Our RNA-sequencing data suggest that also in female BAT *Cyp19a1* expression is absent (43). Whether this is also true for human BAT remains to be determined, as human BAT is more heterogeneous, showing a mix of brown and white-like adipocytes (22, 105).

Studies have shown that BAT expresses the major sex hormone receptors (106), supporting the hypothesis that differences in sex hormone levels may directly contribute to the sexual dimorphism in BAT function. Given that sex hormones also regulate energy metabolism through central mechanisms, these hormones could also mediate indirect effects on BAT activity.

However, when analyzing sex differences in energy metabolism and the role of sex hormones therein, potential crosstalk with other pathways also has to be taken into account. An important crosstalk to highlight is the sex hormone-GC crosstalk as an increasing number of papers suggest that this bidirectional crosstalk may contribute to sex differences in metabolism, and potentially also in BAT activity (32, 107). GCs (cortisol in humans and corticosterone in rodents) are secreted by the adrenal cortex under the control of the hypothalamic-pituitary-adrenal (HPA) axis. GCs are involved in a broad range of physiological processes, including glucose and lipid metabolism. The effects of GCs are mediated by the nuclear glucocorticoid receptor (GR), expressed throughout the body. In addition, GCs can also signal through the mineralocorticoid receptor (MR) in certain cell types (108). Chronic exposure to elevated GC levels, as observed under stress or in Cushing's syndrome, induces obesity not only by direct effects on adipose tissue but also at the neuroendocrine level (109). Of interest, GC synthesis and GC sensitivity show sex-specific differences (107, 110).

The graphical overview for the effects of sex hormones, GCs, and its crosstalk on BAT activity is illustrated in **Figure 2** and the supporting evidence will be discussed in the following sections.

Effects of Estrogens on BAT

Circulating sex hormones, particularly estrogens, are likely among the most significant regulators of BAT activity and differentiation (111). The thermogenic activity and *Ucp1* mRNA expression in BAT are reduced by ovariectomy (surgical removal of ovaries) (112, 113), while systemic administration of E2 to ovariectomized mice induces protein and mRNA expression of UCP1 in BAT (114). Moreover, estrogens, as well as cold exposure, induce whereas ovariectomy reduces transcription of BMP8B, a BAT adipokine involved in tissue remodeling for adaptive thermogenesis, in BAT of female mice (41). Using cell cultures, it has also been confirmed that E2 has a direct activating effect on brown adipocytes, for instance, by inducing the norepinephrine-induced lipolysis (a preliminary step in BAT thermogenesis) and mitochondrial biogenesis factors (115, 116). The mechanism by which estrogens promote brown adipocyte proliferation and

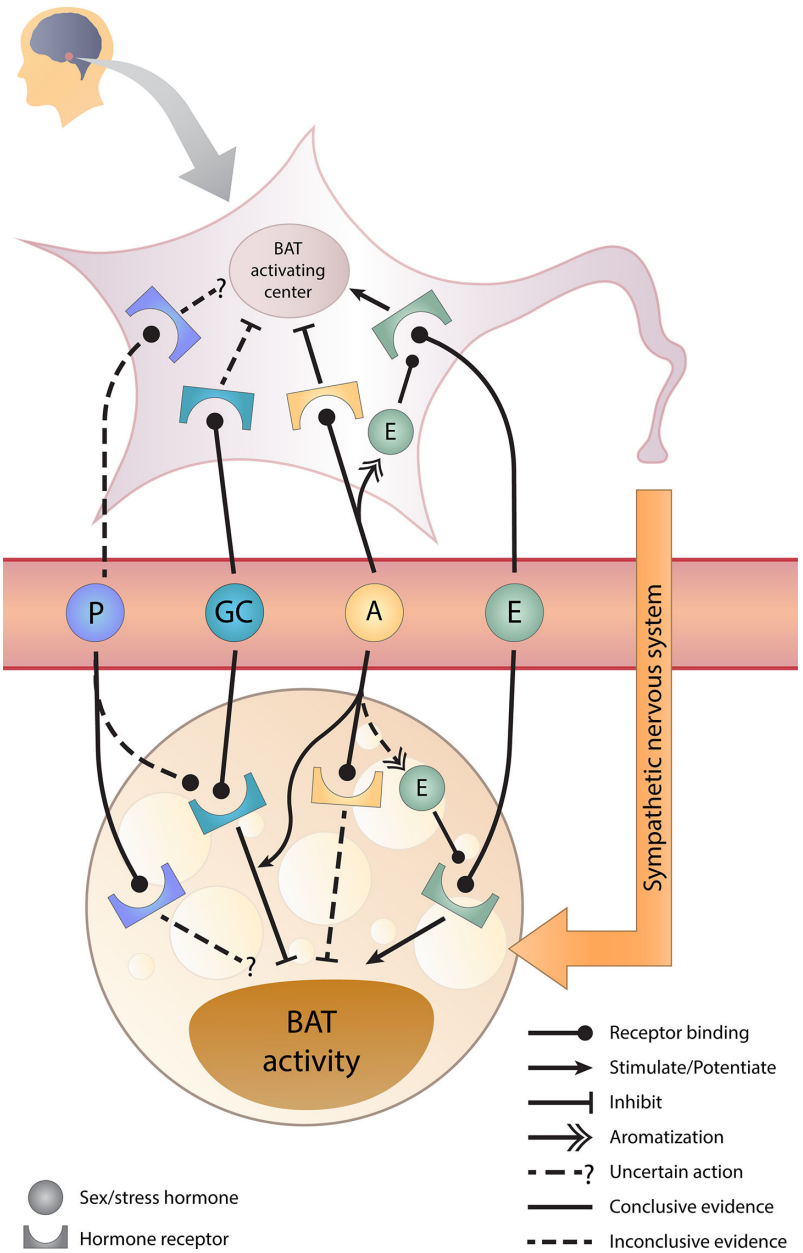

FIGURE 2 | Sex hormones, glucocorticoids, and their crosstalk in BAT regulation. Estrogens stimulate whereas androgens inhibit brown adipose tissue (BAT) activity directly and indirectly *via* the brain. Glucocorticoids directly inhibit BAT activity and androgens potentiate this inhibition. However, the effect of progesterone requires further studies. A, androgens; E, estrogens; GC, glucocorticoids; P, progesterone.

differentiation, including *Ucp1* mRNA expression, is likely driven by ERα (117). BAT activity in whole-body ERα knockout mice has not been fully analyzed, although BAT weights were similar in both male and female ERα-knockout mice compared to wild-type mice, despite increased obesity of the knockout mice (118). Interestingly, in female mice ERα expression is two-fold higher compared to male mice and upregulated upon cold exposure (119). Furthermore, female mice with BAT-specific ERα deficiency had lower basal and cold-induced *Ucp1* expression in BAT, showed whitening of brown adipocytes, had a lower body temperature, and altered substrate metabolism in BAT during a cold challenge test compared to control mice (119). Thus, estrogens may have a direct role *via* ERα in BAT function in female mice. However, in male mice this may be less likely given that male mice have undetectable circulation estrogen levels and aromatization of testosterone in BAT of male mice is absent, as discussed above (102–104). Unfortunately, results in male BAT-specific ERα deficient mice were not reported (119). A role for ERβ in the regulation of energy metabolism is less clear. Energy metabolism seems unaltered in whole-body male ERβ-deficient

mice fed a chow diet (120). However, ERβ-selective ligands appear to have anti-obesity effects. Treatment of HFD-fed mice with ERβ-selective ligands prevented the HFD-induced lipid accumulation in BAT and induced expression of mitochondrial biogenesis markers in BAT and WAT in both males and females (121, 122). Finally, whole-body GPER-knockout mice display subtle sex differences in the regulation of energy homeostasis, with more pronounced adverse effects on energy expenditure and adiposity in males. BAT of both male and female GPER-knockout mice displayed lipid accumulation and reduced mRNA expression of β_3-ADR, while *Ucp1* gene expression was reduced in males only (123). These findings strongly indicate an important role for estrogens and their receptors in regulating thermogenic and metabolic activity of BAT. Of note, some studies demonstrated only a slight activation or no direct influence of E2 on isolated brown adipocytes (116, 124).

Apart from direct stimulating effects on brown adipocytes, estrogens also enhance BAT activity and thermogenesis *via* the brain, especially at the hypothalamic neuronal circuit, leading to an activated sympathetic nervous system (125). Intracerebroventricular administration of E2 in female rats resulted in elevated UCP1 protein content in BAT, together with a rise in the supraclavicular temperature and core body temperature (114), confirming central BAT-activating effects of estrogens. A study in ovariectomized mice illustrated the crucial potent central effects of estrogens in the regulation of BAT activity because only intracerebroventricular but not subcutaneous E2 treatment led to increased BAT *Ucp1* mRNA expression and core body temperature (126). Overall, it is evident that estrogens induce BAT activity and thermogenesis by both direct actions and indirect actions through activation of the sympathetic nervous system.

Effects of Progesterone on BAT

The effects of progesterone on BAT have limitedly been studied and are, therefore, poorly understood. Our recent study suggests that progesterone might be involved in sex differences in BAT function (43). Analysis of the murine interscapular BAT transcriptome by RNA-sequencing identified 295 genes showing ≥2-fold sex-differential expression pattern. *In silico* analysis identified progesterone, in addition to estrogens and androgens, as one of the upstream regulators of the identified genes (43). However, *in vitro* studies of the effects of progesterone on murine brown adipocytes have provided contradictory results. While our study and others showed that progesterone reduced basal *Ucp1* mRNA expression and inhibited norepinephrine-stimulated *Ucp1* mRNA expression and lipolysis in cultured male and female brown adipocytes (43, 127), other studies showed that progesterone had a stimulatory effect on these parameters (115, 124). In part, these conflicting results can be explained by the difference in progesterone concentrations used, with high concentrations having an inhibitory effect. Additionally, it was shown that during pregnancy, when progesterone levels are high, murine BAT was less active, reflected by a reduction in mitochondrial content, thermogenic activity, and mRNA expression of *Ucp1* and other thermogenic genes (127). The reduced BAT activity during pregnancy is likely to conserve maternal energy for fetal growth (128). This inhibitory effect of progesterone on BAT thermogenic markers was confirmed in ovariectomized mice treated with progesterone (127). Information on the effects of progesterone in humans is scarce and if present indirect. Although BAT activity was not measured directly, supraclavicular temperature as a proxy of BAT thermogenesis was higher during the luteal phase compared to the follicular phase, which correlated with progesterone levels (129).

Mechanistically, it remains to be determined how the effects of progesterone on BAT are mediated. Our results suggest that when progesterone concentrations are high, part of the effects may be driven by the GR (43). Progesterone may also signal through to the MR, which was shown to be expressed in BAT (130). Effects of MR signaling are briefly discussed in the section – effects of GC on BAT. Furthermore, effects *via* progesterone membrane receptors cannot be ruled out. PGRMC1 and PGRMC2 both play a role in adipose tissue metabolism but appear to have opposing roles. Male adipose-specific PGRMC1 knockout mice are less prone to HFD-induced lipid accumulation in BAT (131), while male and female adipose-specific PGRMC2 knockout mice are defective in cold-induced thermogenesis (132). Thus, while it is clear that E2 stimulates BAT activity, the role of the 'other' female sex hormone progesterone in BAT physiology requires more research.

Effects of Androgens on BAT

In contrast to estrogens, the effects of androgens on BAT activity are more difficult to unravel. *In vitro* studies suggest that androgens reduce BAT activity. In primary brown adipocytes of rodents, testosterone inhibited mitochondrial biogenesis, brown adipocyte differentiation, and norepinephrine-induced lipolysis (115, 116, 124). In immortalized mouse brown adipose cells, DHT (a nonaromatizable androgen) dose-dependently inhibited differentiation, isoproterenol-stimulated *Ucp1* mRNA expression, and mitochondrial respiration, and these findings were confirmed in explants of mouse BAT (133). However, direct *in vivo* effects of androgens appear controversial. Orchiectomy (surgical removal of testes) increased mRNA and protein expression of UCP1 in murine BAT, together with an elevated body temperature (134, 135). Yet, some studies have shown that prolonged exposure to DHT in orchiectomized male mice or in female mice did not reduce *Ucp1* mRNA expression in BAT (136–138). Furthermore, AR-knockout male mice developed late-onset obesity and displayed a reduction in the expression of thermogenic genes in BAT (139). This latter study also describes the presence of an androgen response element in the *Ucp1* promoter and showed a stimulation of an *Ucp1* promoter construct by DHT (139). Clinical studies suggest that changes in androgen levels may have an opposite effect in women and men. Hyperandrogenism in women, as in the prevalent disorder polycystic ovary syndrome (PCOS), and hypogonadism in men are both associated with abdominal obesity and obesity-related disorders (140). Compared to controls, women with PCOS have been suggested to have lower BAT activity, based on a lower supraclavicular skin temperature (141). In androgen-induced mouse models of PCOS, we and others observed that androgen excess reduced the mRNA expression of *Ucp1* and other genes involved in mitochondrial

function in BAT, likely contributing to a lower body temperature (41, 142, 143).

Similar to estrogens, in addition to direct effects, androgens can have indirect effects on BAT through central mechanisms. Chronic DHT exposure in female mice reduced hypothalamic leptin sensitivity, which blunted leptin-induced BAT thermogenesis through changes in the melanocortin system (137). Whole-body AR knockout male mice also develop leptin resistance (144).

Importantly, it should be noted that part of the androgen effects could be explained by locally increased estrogen levels due to intratissue aromatization of androgens, particularly in WAT and the brain (145). Thus, part of the observed effects of testosterone may in fact be ER-mediated. A recent study showed that the testosterone-induced reduction of white fat mass in obese hypogonadal male mice requires ERα expression in the brain (104). Hence, it seems likely that *in vivo* androgens affect BAT function through central mechanisms, likely through central estrogen signaling, although the latter requires more detailed studies.

Effects of Glucocorticoids on BAT

GCs are well known to have metabolic effects, and GC excess leads to obesity in both rodents and humans (109). Several studies in rodents have shown that upon chronic GC exposure BAT thermogenesis is inhibited. Studies from our laboratory showed that corticosterone treatment resulted in lipid accumulation and a decrease in basal and norepinephrine-induced UCP1 mRNA and protein content in BAT of both male and female mice (36, 146, 147). These effects were confirmed by others in a study in which male rats were treated with corticosterone for 21 days (148). In *in vitro* differentiated brown adipocytes, GCs reduced the norepinephrine-stimulated *Ucp1* mRNA expression (147, 149). GC inhibition of BAT activity is likely mediated through the GR since a GR-specific antagonist reversed the inhibitory effects of GC on *Ucp1* mRNA expression in mice (150–152). However, it has to be kept in mind that GC can also signal through the MR, which is also expressed in BAT (130). Activation of the MR in a mouse brown adipocyte cell line stimulates the differentiation of pre-adipocytes into mature adipocytes (153), and suppresses thermogenic activity by reducing isoproterenol-stimulated *Ucp1* transcription (154). In male mice, adipose tissue-specific MR deficiency prevents lipid accumulation in BAT upon HFD treatment (155). MR antagonist treatment also resulted in browning of WAT in diet-induced obese female mice (156). In agreement, in a small study of healthy male and female volunteers, treatment with a MR antagonist for two weeks increased BAT activity and volume (157). Whether these MR effects are mediated by GC may depend on the intratissue availability of GCs, discussed below.

Some recent studies also present contradictory data on GR signaling in BAT. GC treatment only reduced the total mRNA and protein content of UCP1 in BAT when male mice were housed at thermoneutrality (30°C) while total UCP1 protein content was not affected when mice were housed under standard housing conditions (21°C), but the mice developed obesity to a similar extent at both temperatures (158). This reported difference in GC effects on UCP1 expression can in part be explained by the way UCP1 levels were expressed, i.e. per μg protein or per total depot weight. Interestingly, in UCP1-deficient mice, GC-induced obesity was not worsened compared to wild-type mice (158), suggesting UCP1-independent effects of GCs on BAT function. Furthermore, based on a study analyzing BAT-specific GR knockout (GR^{BATKO}) male mice, the role of GR in BAT function is debatable, as GR^{BATKO} mice did not differ from wild-type mice in terms of BAT thermogenesis and HFD-induced metabolic consequences (159). It should be noted that in the GR^{BATKO} male mice the HPA axis was not affected and that these mice had normal corticosterone levels. Interestingly, adrenalectomy resulted in increased BAT activity in obese male mice (160). Adrenalectomy also resulted in a differential effect on substrate uptake by BAT, as adrenalectomy in male mice abolished the circadian rhythm of glucose uptake but had no effect on its rhythms in fatty acid uptake (161). Although these studies might underscore the effects of endogenous GCs on BAT, adrenalectomy has effects beyond a reduction in GC levels as it also leads to reduced catecholamines and elevated adrenocorticotropic hormone (ACTH) levels, which have been shown to induce BAT activity in male mice (147).

Although studies are limited, also in humans GCs may inhibit BAT activity. In Cushing's syndrome, characterized by hypercortisolism due to ACTH-secreting pituitary tumors or cortisol-secreting adrenal tumors, increased lipid accumulation occurs in the supraclavicular fat depot (162). Furthermore, prolonged GC treatment resulted in significantly fewer patients (men and women) with detectable BAT compared to controls (163), and lower cold-induced BAT activity (42). However, opposite effects were observed when comparing acute and chronic GC exposure, because acute GC treatment was shown to induce BAT activity as assessed by the supraclavicular temperature in male volunteers (163, 164). Furthermore, species difference in GC regulation of BAT has been demonstrated since GC was shown to inhibit UCP1 expression in cultured brown adipocytes of male mice whereas the opposite effect was observed in cultured human brown adipocytes (163).

The effects of GCs also depend on its intratissue concentration, which is regulated by the enzymes 11β-hydroxysteroid dehydrogenase type 1 (11β-HSD1) and 11β-HSD2. 11β-HSD1 converts the inactive GC isoform, cortisone in humans or 11-dehydrocorticosterone in rodents, into the bioactive GC isoform, cortisol in humans or corticosterone in rodents, whereas 11β-HSD2 inactivates the intratissue GCs (165). The GC-inactivating enzyme 11β-HSD1 is mostly expressed in the classical MR-target tissues, such as kidneys, while the GC-activating enzyme 11β-HSD1 is highly expressed in most metabolic tissues including BAT (166, 167). Thus, 11β-HSD1 contributes to the intratissue availability of GC and thereby is an important factor influencing GC effects on BAT activity. Lipid accumulation in BAT upon GC treatment and aging was strongly diminished in 11β-HSD1-deficient male mice (167). In HFD-fed male mice, treatment with an 11β-HSD1 inhibitor increased *Ucp1* mRNA expression and also reduced lipid accumulation in BAT (168). Since GCs can stimulate the expression of 11β-HSD1, thereby increasing GC

exposure to impair BAT function (158), pharmacological inhibition of 11β-HSD1 has been proposed as a target in the treatment of obesity.

Altogether, these studies suggest that the detrimental effects of GC on BAT activity become visible under conditions where GC levels are elevated, such as under chronic stress or prolonged systemic GC administration. Hypercortisolism may also affect other endocrine systems, such as the HPG axis, and thereby sex hormone levels.

Sex Hormone-Glucocorticoid Crosstalk in BAT

It is well-accepted that the HPA axis exhibits some sexually dimorphic features. For example, many stress-related disorders, such as anxiety and depressive disorders, are more prevalent and severe in women than in men (169). Female rats also show higher basal levels and stress-induced levels of neuroendocrine responses of the HPA axis, including corticosterone and ACTH, than male rats. Interestingly, baseline and stress-induced levels of corticosterone and ACTH were indistinguishable between the sexes after gonadectomy (170, 171), implicating a role for sex hormones in the regulation of the HPA axis. A sex-dependent bidirectional crosstalk between the HPA axis and sex hormones in metabolism has been demonstrated and proposed to play a role in the developmental misprogramming of metabolism (172). Whether such a crosstalk also plays a role in the regulation of BAT activity is less clear but we recently showed that treatment of mice with corticosterone elevated the expression of GR-target genes *Fkbp5* and *Tsc22d3* more profoundly in BAT of male than of female mice while it induced BAT whitening and reduced *Ucp1* mRNA expression in BAT to a similar extent in both sexes (36). Another study, using a lower concentration of corticosterone, also reported a negative effect of GCs on BAT, which was more pronounced in male than in female mice (135). Interestingly, the corticosterone-induced lipid accumulation in BAT of mice was absent after orchidectomy, suggesting an androgen-dependent effect of GC on BAT (135). Furthermore, ovariectomy did not sensitize BAT to glucocorticoids, but DHT treatment of ovariectomized mice did (135). Also in an *in vitro* study, DHT was shown to potentiate GC-induced GR signaling in brown adipocytes, as illustrated by an upregulation in transcriptional levels of the GR-responsive genes *Fkbp5*, *Tsc22d3*, and *Mt2a* (138). Sex hormones may also contribute to GC intratissue availability. It has been shown that androgens increase whereas estrogens decrease 11β-HSD1 expression and activity in WAT (107, 138). Interestingly, studies using human primary white adipocytes suggest that the effect of sex hormones on 11β-HSD1 expression may be sex-specific (173). Of interest, postmenopausal women have higher 11β-HSD1 activity in their adipose tissue (174), underscoring the physiological relevance of the role of estrogens on 11β-HSD1. Whether sex hormones also regulate 11β-HSD1 expression in BAT remains to be determined. These studies suggest that a crosstalk between androgens and GCs in the regulation of BAT activity may exist. It also suggests that changes in the balance of sex hormone levels and glucocorticoid levels may impact BAT activity. This could be relevant upon aging in women when sex hormone levels decline, thereby reinforcing the inhibitory effect of GC on BAT.

Sex Hormone-Independent Factors Contributing to Sex Differences in BAT Activity

Sex differences in metabolism can be explained by differences in the levels of sex hormones between the sexes. This difference is driven to a large extent by the difference in sex chromosomes. However, this genetic difference also results in sex-hormone independent effects. For instance, random X-inactivation in female cells and genomic imprinting of autosomes have been shown to contribute to sex-specific gene expression (175). Indeed, cultured primary brown adipocytes, isolated and differentiated from BAT of male and female mice, maintained a sex-differential expression profile even though they were cultured and differentiated under the same standard culture conditions (43). In addition, human adipocytes differentiated from pre-adipocytes isolated from female perirenal fat had a higher *UCP1* mRNA expression level than those isolated from male perirenal fat despite similar culture conditions (176).

The contribution of this intrinsic genetic difference in the regulation of BAT differentiation and activity has not been studied in great detail. In humans, dissecting the effects of genetic sex and sex hormones is difficult. In disorders of sex differentiation, an altered number of X or Y chromosomes is often associated with abnormal gonadal differentiation and function (177). So far, only the use of the four core genotypes (FCG) mice has provided a tool to study effects of genetic sex and gonadal function separately. The FCG is a mouse model in which the Y-chromosomal *Sry* gene that functions as a testis-determining factor is relocated to an autosome, allowing the generation of four types of mice: XX mice with ovaries, XX mice with testes, XY mice with testes, and XY mice with ovaries (175). In young gonadectomized FCG mice, BAT *Ucp1* expression tended to be suppressed in the presence of the Y chromosome. However, the existing gonads (testes or ovaries) were more influential than the sex chromosomes, as the orchiectomized XX or XY mice had a slightly but significantly higher BAT *Ucp1* mRNA expression than the ovariectomized XX or XY mice (178). Although further studies are needed, this study suggests that gonadal hormones have a more prominent role than sex chromosomes in the regulation of BAT activity.

Another factor that may contribute to sex differences in metabolism is epigenetic programming. Epigenetic programming has been shown to modulate BAT and WAT activity through several mechanisms, such as DNA methylation and histone modifications. Mouse models in which epigenetic mechanisms were inhibited or stimulated displayed changes in the transcriptional control of BAT and hence BAT thermogenesis (179). Furthermore, sex-specific epigenetic marks in adipose tissue were identified in the effects of early-life social disadvantage on adulthood BMI (180). However, a role in sex-differential control of BAT thermogenesis remains to be determined. Intriguingly, a single dose of testosterone administration in the neonatal period of female mice was sufficient to induce whitening of BAT and

downregulate *Ucp1* and other BAT-specific gene transcriptional levels at adulthood (181). Whether this involves epigenetic programming remains to be determined. However, it does show that early life events can have lifelong effects on BAT activity and contribute to sex differences in BAT activity.

CONCLUDING REMARKS

Studies in rodents show that sex hormones regulate BAT activity in a sex-specific manner through direct and indirect mechanisms. Estrogens induce a stimulatory effect on BAT activity, adding to the healthier metabolic actions of estrogens in females. Androgens appear to have an inhibitory effect, while the actions of progesterone on BAT function require further research. The crosstalk between sex hormones and GCs adds to the mechanisms that control sexually dimorphic BAT activity. This crosstalk also illustrates that tipping the balance in sex hormones and GC levels, but likely other factors as well, alters the effect of each of these hormones on BAT. In that respect, more knowledge about mechanisms that regulate intracellular availability and sensitivity of these hormones is warranted as these may contribute to the sex-specific sensitivity of BAT to sex hormones and GCs. Studies in humans suggest comparable effects but require further studies. Analysis of BAT activity under pathophysiological conditions may aid to gain a better understanding.

Given the proposed role of BAT as a target to battle obesity, changes in sex hormone levels may be one of the mechanisms contributing to sex differences in BAT physiology and thereby sex differences in the onset and development of obesity-related disorders.

AUTHOR CONTRIBUTIONS

KK and JV drafted the manuscript. All authors contributed to the article and approved the submitted version.

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