REVIEW

Role of sex hormones in modulation of brown adipose tissue activity

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Abstract

The recent demonstration that metabolically active brown adipose tissue (BAT) is present with a high prevalence in humans undoubtedly represents one of the major advancements in the field of metabolic research in the last few years. The increasing interest in BAT is justified by preclinical observations highlighting an important role of this tissue in energy dissipation and metabolic clearance of substrates from the blood. These findings imply that stimulation of BAT activity may represent a new therapeutic approach for obesity and associated comorbidities. However, before proposing BAT as a target organ for therapeutics in a clinical setting, many further notions about BAT function and modulation need to be explored. Keeping in mind the importance of sex dimorphism in energy metabolism control under physiological and pathological conditions, sex hormones may play a relevant role in the regulation of BAT activity in both males and females. Much of the evidence acquired in the past supports the concept of an important role for different sex hormones in BAT thermogenesis and indicates that this tissue mediates the ability of sex hormones to modulate energy balance. These findings make it plausible that a modified interaction between BAT and sex hormones may contribute to the development and the maintenance of obesity and associated metabolic complications.

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Brown adipose tissue and obesity

The key role played by brown adipose tissue (BAT) in energy balance regulation of mammals has been known for decades (Rothwell & Stock 1979, Cannon & Nedergaard 2004). Because of the high number of mitochondria and the presence of uncoupling protein 1 (UCP1), brown adipocytes are known to efficiently generate heat in order to maintain a constant body temperature. This process is mainly regulated by the sympathetic nervous system (SNS), which controls BAT activity through noradrenaline (NE)-mediated mechanisms involving β3 adrenergic receptors (Cannon & Nedergaard 2004). NE-mediated activation of BAT thermogenesis promotes dissipation into heat of the energy contained in substrates as triglycerides and free fatty acids. Therefore, besides thermoregulation, it was originally postulated that substrate oxidation is used by BAT for the combustion of excess energy intake, with the purpose of 'extracting' essential food nutrients (Rothwell & Stock 1979, Cannon & Nedergaard 2004). This property implies that a reduction in BAT thermogenesis favors fat mass and body weight accumulation, whereas stimulation of the number/activity of brown adipocytes increases energy dissipation, thereby promoting fat mass and body weight loss in overweight/obese subjects.

Indeed, an increased propensity for obesity has been found in both young (Feldmann *et al.* 2009) and old mice (Kontani *et al.* 2005) lacking UCP1 activity. Recent results from studies on genetically modified mice have consistently shown that an increased BAT amount protects from body weight gain and metabolic dysregulation induced by high fat feeding (Cederberg *et al.* 2001, Seale *et al.* 2011).

Notably, in addition to the positive properties regarding the control of fat stores and body weight, BAT might have an even more determinant role in controlling whole-body lipid content and glucose metabolism. Indeed, when active, this tissue is able to oxidize high amounts of lipids and glucose, inducing a substantial clearance of these substrates from the blood. When BAT thermogenesis is activated by cold exposure, brown adipocytes are able to massively take up plasma triglycerides, thereby restoring hyperlipidemia in mouse models of obesity associated with diabetes/dyslipidemia (Bartelt *et al.* 2011). In addition, the important role of BAT in glucoregulation is demonstrated by the

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notion that this tissue displays a high rate of glucose uptake in both basal and stimulated conditions and is one of the most insulin-sensitive tissues with respect to stimulation of glucose uptake (Ferrè *et al.* 1986). Moreover, recent evidence has shown that increasing the amount of BAT depots by genetic approaches ameliorates glucose homeostasis and insulin sensitivity in diet-induced obese mice (Cederberg *et al.* 2001, Seale *et al.* 2011).

Therefore, persuasive preclinical evidence indicates that modulation of BAT activity may represent a new promising strategy in promoting energy dissipation and restoring metabolic dysregulation, such as dyslipidemia and type 2 diabetes in obese subjects.

Although metabolically active BAT was considered, until recently, to be present exclusively in animals and newborns, the introduction of positron emmission tomography/computed tomography (PET/CT) imaging technology has made it possible to demonstrate that even healthy adult humans possess, with a high prevalence, metabolically active areas of BAT (Cypess *et al.* 2009, Saito *et al.* 2009, Van Marken Lichtenbelt *et al.* 2009, Virtanen *et al.* 2009, Nedergaard *et al.* 2010). Most importantly, activation of human BAT by cold exposure has been shown to be associated with an increase in total energy expenditure (Ouellet *et al.* 2012), providing evidence that human BAT can contribute to whole-body modifications in energy balance. These important notions highlight the possibility that modulation of BAT activity may represent an effective therapeutic strategy to tackle obesity and related health complications.

However, it is not yet completely understood how such BAT modulation might be achieved; in particular, the role of various endocrine factors in affecting BAT activity in physiological and pathological conditions still needs to be explored.

Understanding hormonal modulation of BAT is of interest for endocrinologists because BAT may contribute to the phenotypical presentation of states of pathological hormone excess or deficiency. In this context, several observations support an important influence of sex hormones on BAT metabolic activity.

Sex hormones, white adipose tissue, and energy balance

Sex hormones have long been proposed as leading actors in the determination of fat mass distribution, and function, in both females and males; however, most of the knowledge so far accumulated is related to the influence of sex hormones on white adipose tissue (WAT). A full discussion on the plethora of action of sex steroids in energy balance and WAT modulation is beyond the scope of the present work and has already been reviewed in detail (Haifei *et al.* 2009, Brown *et al.* 2010). We will summarize here some dominant concepts to better comprehend the physiological and pathophysiological roles of sex steroids in the regulation of energy homeostasis and adipose tissue function.

The important role played by sex hormones in energy balance regulation is supported by the notion that an appropriate androgen to estrogen ratio is a key regulator of adipose tissue homeostasis. When this ratio is altered, fat mass accumulation and body weight gain may occur. Indeed, original studies on mice knocked out for the aromatase gene (aromatase is the enzyme responsible for converting androgens to estrogens) revealed that a reduced conversion of androgens into estrogens leads to body weight gain and obesity-related metabolic complications in both genders (Jones *et al.* 2000).

Further evidence in humans confirms the important role played by the androgen to estrogen ratio in energy balance regulation. Aromatase deficiency, a rare disorder in which affected individuals cannot synthesize endogenous estrogen, is associated with metabolic abnormalities including obesity in humans (Jones *et al.* 2007). In women, it is well known that hyperandrogenism is often associated with increased intra-abdominal fat accumulation and the metabolic syndrome (as for the case of polycystic ovary syndrome; Gambineri *et al.* 2009), and the decline of estrogen after menopause is known to determine an increase in intra-abdominal fat mass (Lobo 2008). In men, testosterone deficiency in hypogonadic individuals is associated with a significant change in body composition (especially featured by an increase in fat mass) and with the frequent presence of dysmetabolisms (Barrett-Connor 1992, Barud *et al.* 2002, Grossmann *et al.* 2010, Traish *et al.* 2011). Testosterone replacement therapy in patients with reduced testosterone levels has been shown to produce a significant metabolic amelioration, not only related to body weight but also related to the glucose and lipid profile (Traish *et al.* 2011). These data suggest that appropriate levels of ovarian hormones in females and androgens in males are critical for the regulation of energy homeostasis.

The mechanisms by which sex hormones regulate energy balance and influence adiposity are not completely understood. A direct action, produced by these hormones by binding their own specific receptors located on white adipocytes, is one of the mechanisms proposed. It has been demonstrated that white adipocytes express the whole battery of sex steroid receptors as progesterone receptors (PRs), androgen receptors (ARs), and estrogen receptors (ERs; Crandall *et al.* 1998). Subcutaneous adipose tissue seems to be the site where higher concentrations of ERs and PRs are present, whereas, compared with subcutaneous adipose tissue, visceral adipose tissue apparently expresses higher concentrations of ARs (Sjogren *et al.* 1995,

Björntorp 1997). The AR is expressed in both male and female WAT; in females, testosterone action to AR seems to promote fat mass accumulation, a possibility supported by the clinical notion that hyperandrogenic women tend to accumulate excessive visceral fat mass. AR expressed on adipose tissue can be down-regulated by estrogen, suggesting that, in women, estrogen provides protection from androgenic effects (Björntorp 1997).

The ability of sex hormones to modulate leptin secretion from adipocytes is a clear indication of the involvement of these hormones in the regulation of energy balance at the WAT level (Haifei *et al.* 2009). After puberty, estrogen and testosterone modulate leptin synthesis and secretion from adipocytes, via sex steroid-dependent transcriptional mechanisms (Machinal *et al.* 1999, Haifei *et al.* 2009). Moreover, exposure of human adipose cells to testosterone or 5α-dihydrotestosterone (DHT), inhibits leptin expression (Wabitsch *et al.* 1997), whereas 17β-estradiol (E_2) treatment increases adipose tissue leptin mRNA levels in female rats (Kristensen *et al.* 1999).

Sex hormones and BAT

In analogy to what occurs on WAT, BAT may also represent a potential target of sex hormones, although the evidence is not as compelling as that found for WAT.

Some differences in BAT thermogenic capacity, detected between males and females in response to diet-induced obesity and cold exposure (Quevedo *et al.* 1998, Roca *et al.* 1999, Rodriguez *et al.* 2001, Rodriguez-Cuenca *et al.* 2002), seem to point to a role played by sex hormones in the control of BAT activity.

These gender differences are probably due to a dimorphism in mitochondrial capacity; indeed, female BAT shows larger mitochondria and higher cristae density compared with the BAT present in males (Rodriguez-Cuenca *et al.* 2002). An augmented thermogenic activity, driven by the increased mitochondrial function of female BAT, may underlie the different body weight gain that female rodents exhibit following a high-fat diet (HFD) in comparison with males. Indeed, it has been shown that when exposed to a HFD, male rats gain more body weight than females, and this phenotype is paralleled in BAT with a lower expression of numerous proteins involved in thermogenesis and fat oxidation, as well as a higher expression of proteins contributing to fat synthesis (Choi *et al.* 2011).

These findings are corroborated by the evidence that PET/CT imaging studies on humans revealed a higher incidence of metabolically active BAT in females than in males (Cypess *et al.* 2009, Pfannenberg *et al.* 2010). The determination of gender-specific values of BAT prevalence from the available retrospective PET/CT studies, however, has several limitations (Nedergaard *et al.* 2010), and the true sex-specific prevalence of metabolically active BAT thus still needs to be verified. The important role played by sex hormones in the control of BAT function is also suggested by the evidence that physiological states characterized by profound modifications in sex hormone levels/activity are usually associated with a modification in BAT presence and metabolic activity.

During pregnancy and lactation, for example, the modified hormonal milieu is accompanied by BAT atrophy, a phenomenon that is probably necessary to reduce thermogenesis in order to save energy for fetus and newborn growth (Cannon & Nedergaard 2004). Moreover, as recently discussed by Nedergaard *et al.* (2010), the age-related decline in sex hormone levels may be responsible for an impaired BAT functional activity. Indeed, a balance between a stimulatory effect of sex hormones and an inhibitory effect of glucocorticoids could influence BAT recruitment. With age, the sex hormone influence would diminish, and the inhibitory effect of glucocorticoids would take over, leading to tissue involution.

Several sex steroid receptors are expressed in BAT-cultured cells derived from both male and female animals, indicating a property for these hormones to directly control BAT machinery in a similar manner to what is observed in WAT. Cultured brown adipocytes have been shown to express ARs (higher levels in males than in females (Rodriguez-Cuenca *et al.* 2007*a*), ERs type α (higher levels in males than in females; Wade & Gray 1978, Rodriguez-Cuenca *et al.* 2007*a*), and PRs A and B (both in males and in females; Rodriguez-Cuenca *et al.* 2007*a*). However, in close analogy to what is observed in WAT and already reviewed (Haifei *et al.* 2009), sex hormones may also indirectly regulate BAT activity by their ability to modulate different metabolic pathways controlling energy homeostasis. Estrogen, for example, may control BAT activity that has the ability to modulate the leptinergic–melatonergic system, which is a major controller of brown adipocyte thermogenesis (Richard *et al.* 2010).

Estrogen and melanocortin/leptin-mediated thermogenesis

The importance of estrogen for body composition/energy expenditure is known from previous studies (Landau & Zucker 1976) and is related to mechanisms other than food intake and physical activity. Supporting this notion, different studies on animals have shown that E_2 administration induces an increase in oxygen consumption (indicating increased energy expenditure; Laudenslager *et al.* 1980) and that ER knockout mice have a higher fat mass and decreased energy expenditure than their wild-type littermates (Heine

et al. 2000, Ohlsson *et al.* 2000). Therefore, the ability of estrogen to influence energy balance seems to be related to an effect of this hormone on energy dissipation. The interaction between estrogen and both the melanocortin and the leptinergic system probably underlies this property of the hormone.

Specifically, estrogen can activate hypothalamic pro-opiomelanocortin (POMC) neurons acting on ERα expressed in these cells (Pelletier *et al.* 2007). Following activation, POMC neurons are known to produce the neuropeptide α-melanocyte-stimulating hormone, which is able to induce a series of catabolic effects such as a reduction in food intake and an increase in energy expenditure. It has been shown that ovariectomy decreases POMC mRNA levels in POMC neurons, whereas E_2 replacement reestablishes normal POMC mRNA levels (Pelletier *et al.* 2007). Interestingly, E_2 administration rapidly increases excitatory synapses onto POMC neurons (Gao *et al.* 2007), and this synaptic rearrangement strictly parallels the positive effects that the hormone induces on energy expenditure and body weight of obese animals (Gao *et al.* 2007). Thus, the melanocortin system seems to mainly mediate the effects of estrogen on energy balance.

The leptinergic system may also mediate the ability to influence energy dissipation. Indeed, estrogen action in the hypothalamus increases the local action of leptin (Brown *et al.* 2010), whose catabolic effects in the brain are known to inhibit food intake and increase energy expenditure (Gautron & Elmquist 2011). Moreover, estrogen may regulate energy balance by enhancing leptin-induced activation of the SNS, which innervates the WAT, thereby reducing fat mass through stimulation of SNS-dependent lipolysis (Haifei *et al.* 2009).

Importantly, there is evidence that the activity of the melanocortin system is directly regulated by leptin and that leptin receptors and melanocortin receptors are part of the same homoeostatic pathway controlling SNS activity in adipose tissues and BAT thermogenesis (Richard *et al.* 2010). Therefore, SNS-mediated thermogenesis in BAT, triggered by estrogen action on the leptinergic/melanocortin system, probably contributes to the effect of the hormone on energy dissipation. In this context, even if direct experimental evidence has never been reported, the important role played by BAT in estrogen-induced modifications in energy balance is supported by a series of observations reported here: i) ovariectomized animals, which become spontaneously obese, display atrophy of their BAT (Pedersen *et al.* 2001). ii) BAT thermogenesis is mainly mediated by the melanocortin system. More than 80% of the neurons projecting to BAT express melanocortin receptors (Song *et al.* 2008), and administration of melanocortin receptor agonists induces UCP1 activation in BAT (Brito *et al.* 2007, Glavas *et al.* 2007) and enhances SNS activity to both WAT and BAT (Brito *et al.* 2007). Therefore, the stimulatory effect of estrogen on the melanocortin system also probably affects BAT thermogenesis. iii) Taking into account that estrogen positively influences leptin action at various levels, and that BAT is essential for leptin-induced thermogenesis in rodents (Cannon & Nedergaard 2004), it is possible to speculate that the increased leptin sensitivity induced by estrogen also produces an increase in BAT thermogenesis. iv) SNS signaling activating BAT has been shown to be very important for estrogen-mediated changes in energy balance. Indeed, a procedure of SNS denervation of BAT markedly impairs estrogen-induced increases in whole-body oxygen consumption (Bartness & Wade 1984). In conclusion, estrogen appears to be a potent stimulator of BAT in animals, the final product being the promotion of whole-body energy dissipation. The evidence discussed earlier linking estrogen, BAT, and energy balance is represented in Fig. 1.

Androgens and BAT thermogenesis

As experimental evidence attributing a role for estrogen on thermogenesis modulation varies, the effect of androgen signaling to BAT activity is a matter of controversy. Recent studies have demonstrated the existence of an androgen response element in the

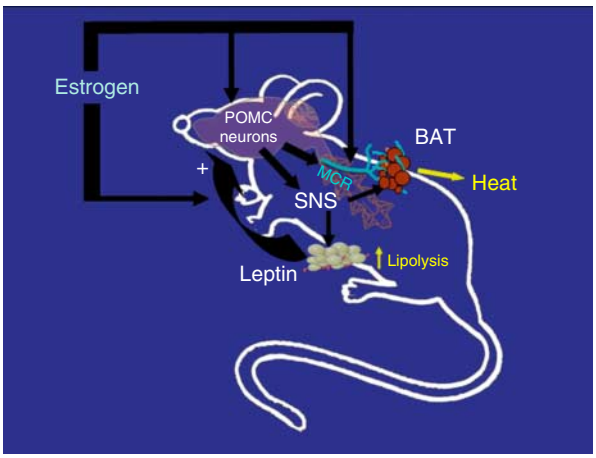

Figure 1 Regulation of BAT thermogenesis by estrogen. The ability of estrogen to promote energy dissipation may derive from its interaction with the leptinergic/melanocortin system. Estrogen can act either by activating POMC neurons in the brain or by sensitizing the hypothalamic action of leptin. Activation of both the leptinergic and melanocortin systems in the brain results in an increased peripheral SNS activity, which in turn promotes energy dissipation by stimulation of thermogenesis in the BAT and lipolysis in the WAT. In light of the evidence that the central action of leptin potentiates the melanocortin system, sensitization of leptin action in the brain by estrogen may amplify POMC-derived activation of BAT and WAT. Moreover, stimulation of BAT thermogenesis may also derive from estrogen-mediated activation of peripheral MCR (melanocortin receptor) expressing neurons (in blue) in the BAT.

UCP1 promoter that suggests a direct effect of androgens on UCP1 expression (Fan *et al.* 2005). Interestingly, *Ar* knockout male mice develop obesity as a consequence of decreased energy expenditure and display a substantially lower expression of *Ucp1* transcript in both WAT and BAT depots (Fan *et al.* 2005, Yanase *et al.* 2008). These findings indicate that androgens may directly contribute to determine appropriate UCP1 levels in adipocytes and that, in light of the important role played by UCP1 in the thermogenic process, this modulation may have an influence on energy homeostasis.

Nevertheless, the possibility of a stimulatory effect of androgens on BAT thermogenesis is in contrast with the observation that, following testosterone treatment, cultured brown adipocytes exhibit lower levels of *Ucp1* mRNA (Rodriguez *et al.* 2002) and a concomitant reduction in PGC1-α (PPARGC1A; Rodriguez-Cuenca *et al.* 2007*b*), a key master of *Ucp1* transcription. These discrepancies could be due to the experimental model used (*in vivo* vs *in vitro*), as indirect mechanisms dependent on hormonal action, such as adrenergic signaling, are obviously lost in cell culture paradigm.

The *in vivo* available evidence on the effect of androgen administration on BAT activity does not clarify the final effect that androgens have on BAT activity but still deserves attention. *In vivo* treatment with the androgen precursor dehydroepiandrosterone (DHEA) and with testosterone has been shown to reduce food intake, body weight, and fat mass in experimental male rats. The same treatment did not induce any apparent change in BAT thermogenic activity (Tagliaferro *et al.* 1986, Abelenda *et al.* 1992). These data would imply that BAT does not contribute to the positive effects on energy metabolism provided by androgen administration. However, as interestingly discussed by Cannon & Nedergaard (2004), because a decrease in food intake leads to BAT atrophy in animals, and because androgens have been shown to modify food intake in some reports (Cannon & Nedergaard 2004), a putative recruiting effect of androgens on BAT may be masked *in vivo* by secondary effects driven by the modifications in food intake. This possibility would explain why, in an experiment in which DHEA treatment did not result in food intake modifications (but results in modification in energy balance and reduction in body weight), BAT activity was increased by the hormone administration (LeBlanc *et al.* 1998).

In conclusion, whether androgens increase UCP1 and BAT thermogenic activity awaits a definitive answer. Direct experiments aimed at examining the *in vivo* effect of androgens on BAT functional activity by pair feeding have never been performed. These experiments would probably unveil whether androgens are able to modulate BAT activity *in vivo*.

Progesterone and BAT thermogenesis

Brown adipocytes have been shown to express, both in males and in females, the two types of PR (PRA and PRB; Rodriguez-Cuenca *et al.* 2007*a*).

Progesterone seems to exert an important role in BAT physiology by stimulating adrenergic signaling. Indeed, progesterone stimulates *Ucp1* mRNA expression (Rodriguez *et al.* 2002) and NE-mediated lipolysis (Monjo *et al.* 2003) in cultured brown adipocytes. Further indirect findings highlight the role of progesterone as an enhancer of BAT activity. During lactation in female rats, *Pra* and *Prb* mRNA levels are decreased in brown adipocytes (Rodriguez-Cuenca *et al.* 2007*a*). Bearing in mind that the downregulation of UCP1-dependent thermogenesis usually seen during lactation (Martin *et al.* 1995) has been mainly attributed to a decrease in the SNS activity (Trayhurn *et al.* 1982), one could speculate that an impaired responsiveness to progesterone during lactation could contribute to the impairment in BAT thermogenesis.

Mitochondrial biogenesis is another critical process involved in the ability of BAT to meet environmental and physiological stimuli, such as cold exposure, diet, and oxidative stress (Lee & Wei 2005, Nisoli *et al.* 2007). The recent *in vitro* observation that progesterone promotes mitochondrial biogenesis in BAT primary cultures of rodents (Rodriguez-Cuenca *et al.* 2007*b*) supports a possible role for this hormone as an enhancer of BAT thermogenic activity.

Conclusions

Clarifying hormonal factors regulating BAT thermogenesis is a crucial aspect in order to achieve effective therapeutic strategies to reduce fat mass and restore the altered metabolic profile of obese individuals.

Old and new preclinical evidence indicates that sex hormones display profound effects on BAT activity through both direct and indirect mechanisms. Even if the mechanisms underlying this interaction still need to be clarified, these data support the possibility that BAT may contribute to the effect of several sex hormones on whole-body energy balance.

In light of the emerging role of BAT as a new therapeutic target for obesity and related metabolic consequences, this evidence highlights the intriguing possibility that a modified interaction between BAT and sex hormones may contribute to the progression of diseases in which an altered estrogen to androgen ratio leads to the metabolic syndrome.

Declaration of interest

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

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