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 β_3 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

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 emission 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 5 α -dihydrotestosterone (DHT), inhibits leptin expression (Wabitsch *et al.* 1997), whereas 17 β -estradiol (E₂) 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, Pfannenberger *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.* 2007a), ERs type α (higher levels in males than in females; Wade & Gray 1978, Rodriguez-Cuenca *et al.* 2007a), and PRs A and B (both in males and in females; Rodriguez-Cuenca *et al.* 2007a). 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 E₂ 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 ER α 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 E₂ replacement reestablishes normal POMC mRNA levels (Pelletier *et al.* 2007). Interestingly, E₂ 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 homeostatic 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 (Rodríguez *et al.* 2002) and a concomitant reduction in PGC1- α (PPARGC1A; Rodríguez-Cuenca *et al.* 2007b), 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; Rodríguez-Cuenca *et al.* 2007a).

Progesterone seems to exert an important role in BAT physiology by stimulating adrenergic signaling. Indeed, progesterone stimulates *Ucp1* mRNA expression (Rodríguez *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 (Rodríguez-Cuenca *et al.* 2007a). 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 (Rodríguez-Cuenca *et al.* 2007b) 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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References

- Abelenda M, Nava MP, Fernandez A & Puerta ML 1992 Brown adipose tissue thermogenesis in testosterone-treated rats. *Acta Endocrinologica* **126** 434–437.
- Barrett-Connor E 1992 Lower endogenous androgen levels and dyslipidemia in men with non-insulin-dependent diabetes mellitus. *Annals of Internal Medicine* **117** 807–811.
- Bartelt A, Bruns OT, Reimer R, Hohenberg H, Itrich H, Peldschus K, Kaul MG, Tromsdorf UI, Weller H, Waurisch C *et al.* 2011 Brown adipose tissue activity controls triglyceride clearance. *Nature Medicine* **17** 200–205. (doi:10.1038/nm.12297)
- Bartness TJ & Wade GN 1984 Effects of interscapular brown adipose tissue denervation on body weight and energy metabolism in ovariectomized and estradiol-treated rats. *Behavioral Neuroscience* **98** 674–685. (doi:10.1037/0735-7044.98.4.674)
- Barud W, Palusinski R, Beltkowski J & Wojcicka G 2002 Inverse relationship between total testosterone and anti-oxidized low density lipoprotein antibody levels in ageing males. *Atherosclerosis* **164** 282–288. (doi:10.1016/S0021-9150(02)00069-2)
- Björntorp P 1997 Hormonal control of regional fat distribution. *Human Reproduction* **12** 21–25. (doi:10.1093/humrep/12.suppl_1.21)
- Brito MN, Brito NA, Baro DJ, Song CK & Bartness TJ 2007 Differential activation of the sympathetic innervation of adipose tissues by melanocortin receptor stimulation. *Endocrinology* **148** 5339–5347. (doi:10.1210/en.2007-0621)
- Brown LM, Gent L, Davis K & Clegg DJ 2010 Metabolic impact of sex hormones on obesity. *Brain Research* **1350** 77–85. (doi:10.1016/j.brainres.2010.04.056)
- Cannon B & Nedergaard J 2004 Brown adipose tissue: function and physiological significance. *Physiological Reviews* **84** 277–359. (doi:10.1152/physrev.00015.2003)
- Cederberg A, Grønning LM, Åhrén B, Taskén K, Carlsson P & Enerbäck S 2001 FOXC2 is a winged helix gene that counteracts obesity, hypertriglyceridemia, and diet-induced insulin resistance. *Cell* **106** 563–573. (doi:10.1016/S0092-8674(01)00474-3)
- Choi DK, Oh TS, Choi JW, Mukherjee R, Wang X, Liu H & Yun JW 2011 Gender difference in proteome of brown adipose tissues between male and female rats exposed to a high fat diet. *Cellular Physiology and Biochemistry* **28** 933–948. (doi:10.1159/000335807)
- Crandall DL, Busler DE, Novak TJ, Weber RV & Kral JG 1998 Identification of estrogen receptor beta RNA in human breast and abdominal subcutaneous adipose tissue. *Biochemical and Biophysical Research Communications* **248** 523–526. (doi:10.1006/bbrc.1998.8997)
- Cypess AM, Lehman S, Williams G, Tal I, Rodman D, Goldfine AB, Kuo CF, Palmer EL, Tseng YH, Doria A *et al.* 2009 Identification and importance of brown adipose tissue in adult humans. *New England Journal of Medicine* **360** 1509–1517. (doi:10.1056/NEJMoa0810780)
- Fan W, Yanase T, Nomura M, Okabe T, Goto K, Sato T, Kawano H, Kato S & Nawata H 2005 Androgen receptor null male mice develop late-onset obesity caused by decreased energy expenditure and lipolytic activity but show normal insulin sensitivity with high adiponectin secretion. *Diabetes* **54** 1000–1008. (doi:10.2337/diabetes.54.4.1000)
- Feldmann HM, Golozoubova V, Cannon B & Nedergaard J 2009 UCP1 ablation induces obesity and abolishes diet-induced thermogenesis in mice exempt from thermal stress by living at thermoneutrality. *Cell Metabolism* **9** 203–209. (doi:10.1016/j.cmet.2008.12.014)
- Ferré P, Burnol AF, Leturque A, Terretaz J, Penicaud L, Jeanrenaud B & Girard J 1986 Glucose utilization *in vivo* and insulin-sensitivity of rat brown adipose tissue in various physiological and pathological conditions. *Biochemical Journal* **233** 249–252.
- Gamberini A, Repaci A, Patton L, Grassi I, Pocognoli P, Cognigni GE, Pasqui F, Pagotto U & Pasquali R 2009 Prominent role of low HDL-cholesterol in explaining the high prevalence of the metabolic syndrome in polycystic ovary syndrome. *Nutrition, Metabolism, and Cardiovascular Diseases* **19** 797–804. (doi:10.1016/j.numecd.2009.01.007)
- Gao Q, Mezei G, Nie Y, Rao Y, Choi CS, Bechmann I, Leranth C, Toran-Allerand D, Priest CA, Roberts JL *et al.* 2007 Anorectic estrogen mimics leptin's effect on the rewiring of melanocortin cells and Stat3 signaling in obese animals. *Nature Medicine* **13** 89–94. (doi:10.1038/nm1525)
- Gautron L & Elmquist JK 2011 Sixteen years and counting: an update on leptin in energy balance. *Journal of Clinical Investigation* **121** 2087–2093. (doi:10.1172/JCI45888)
- Glavas MM, Joachim SE, Draper SJ, Smith MS & Grove KL 2007 Melanocortinergic activation by melanotan II inhibits feeding and increases uncoupling protein 1 messenger ribonucleic acid in the developing rat. *Endocrinology* **148** 3279–3287. (doi:10.1210/en.2007-0184)
- Grossmann M, Gianatti EJ & Zajac JD 2010 Testosterone and type 2 diabetes. *Current Opinion in Endocrinology, Diabetes, and Obesity* **17** 247–256. (doi:10.1097/MED.0b013e32833919cf)
- Haifei S, Seeley RJ & Deborah J 2009 Sexual differences in the control of energy homeostasis. *Frontiers in Neuroendocrinology* **3** 396–404. (doi:10.1016/j.yfme.2009.03.004)
- Heine PA, Taylor JA, Iwamoto GA, Lubahn DB & Cooke PS 2000 Increased adipose tissue in male and female estrogen receptor-alpha knockout mice. *PNAS* **97** 12729–12734. (doi:10.1073/pnas.97.23.12729)
- Jones ME, Thorburn AW, Britt KL, Hewitt KN, Wreford NG, Proietto J, Oz OK, Leury BJ, Robertson KM, Yao S *et al.* 2000 Aromatase-deficient (ArKO) mice have a phenotype of increased adiposity. *PNAS* **97** 12735–12740. (doi:10.1073/pnas.97.23.12735)
- Jones ME, Boon WC, McInnes K, Maffei L, Carani C & Simpson ER 2007 Recognizing rare disorders: aromatase deficiency. *Nature Clinical Practice. Endocrinology & Metabolism* **3** 414–421. (doi:10.1038/ncpendmet0477)
- Kontani Y, Wang Y, Kimura K, Inokuma KI, Saito M, Suzuki-Miura T, Wang Z, Sato Y, Mori N & Yamashita H 2005 UCP1 deficiency increases susceptibility to diet-induced obesity with age. *Aging Cell* **4** 147–155. (doi:10.1111/j.1474-9726.2005.00157.x)
- Kristensen K, Pedersen SB & Richelsen B 1999 Regulation of leptin by steroid hormones in rat adipose tissue. *Biochemical and Biophysical Research Communications* **259** 624–630. (doi:10.1006/bbrc.1999.0842)
- Landau IT & Zucker I 1976 Estrogenic regulation of body weight in the female rat. *Hormones and Behavior* **7** 29–39. (doi:10.1016/0018-506X(76)90002-7)
- Laudenslager ML, Wilkinson CW, Carlisle HJ & Hammel HT 1980 Energy balance in ovariectomized rats with and without estrogen replacement. *American Journal of Physiology* **238** R400–R405.
- LeBlanc J, Arvaniti K & Richard D 1998 Effect of dehydroepiandrosterone on brown adipose tissue and energy balance in mice. *Hormone and Metabolic Research* **30** 236–240. (doi:10.1055/s-2007-978873)
- Lee HC & Wei YH 2005 Mitochondrial biogenesis and mitochondrial DNA maintenance of mammalian cells under oxidative stress. *International Journal of Biochemistry & Cell Biology* **37** 822–834. (doi:10.1016/j.biocel.2004.09.010)
- Lobo RA 2008 Metabolic syndrome after menopause and the role of hormones. *Maturitas* **60** 10–18. (doi:10.1016/j.maturitas.2008.02.008)
- Machinal F, Dieudonne MN, Leneuve MC, Pecquery R & Giudicelli Y 1999 *In vivo* and *in vitro* ob gene expression and leptin secretion in rat adipocytes: evidence for a regional specific regulation by sex steroid hormones. *Endocrinology* **140** 1567–1574. (doi:10.1210/en.140.4.1567)
- Martin I, Giral M, Vinas O, Iglesias R, Mampel T & Villarroya F 1995 Co-ordinate decrease in the expression of the mitochondrial

- genome and nuclear genes for mitochondrial proteins in the lactation-induced mitochondrial hypotrophy of rat brown fat. *Biochemical Journal* **308** 749–752.
- Monjo M, Rodriguez AM, Palou A & Roca P 2003 Direct effects of testosterone, 17 β oestradiol and progesterone on adrenergic regulation in cultured brown adipocytes: a potential mechanism for gender-dependent thermogenesis. *Endocrinology* **144** 4923–4930. (doi:10.1210/en.2003-0537)
- Nedergaard J, Bengtsson T & Cannon B 2010 Three years with adult human brown adipose tissue. *Annals of the New York Academy of Sciences* **1212** E20–E36. (doi:10.1111/j.1749-6632.2010.05905.x)
- Nisoli E, Clementi E, Carruba MO & Moncada S 2007 Defective mitochondrial biogenesis: a hallmark of the high cardiovascular risk in the metabolic syndrome? *Circulation Research* **100** 795–806. (doi:10.1161/01.RES.0000259591.97107.6c)
- Ohlsson C, Hellberg N, Parini P, Vidal O, Bohlooly M, Rudling M, Lindberg MK, Warner M, Angelin B & Gustafsson JA 2000 Obesity and disturbed lipoprotein profile in estrogen receptor- α -deficient male mice. *Biochemical and Biophysical Research Communications* **278** 640–645. (doi:10.1006/bbrc.2000.3827)
- Ouellet V, Labbé SM, Blondin DP, Phoenix S, Guérin B, Haman F, Turcotte EE, Richard D & Carpentier AC 2012 Brown adipose tissue oxidative metabolism contributes to energy expenditure during acute cold exposure in humans. *Journal of Clinical Investigation* **122** 545–552. (doi:10.1172/JCI60433)
- Pedersen SB, Bruun JM, Kristensen K & Richelsen B 2001 Regulation of UCP1, UCP2, and UCP3 mRNA expression in brown adipose tissue, white adipose tissue, and skeletal muscle in rats by estrogen. *Biochemical and Biophysical Research Communications* **288** 191–197. (doi:10.1006/bbrc.2001.5763)
- Pelletier G, Li S, Luu-The V & Labrie F 2007 Oestrogenic regulation of pro-opiomelanocortin, neuropeptide Y and corticotrophin-releasing hormone mRNAs in mouse hypothalamus. *Neuroendocrinology* **19** 426–431. (doi:10.1111/j.1365-2826.2007.01548.x)
- Pfannenberger C, Werner MK, Ripkens S, Stef I, Deckert A, Schmadl M, Reimold M, Häring HU, Claussen CD & Stefan N 2010 Impact of age on the relationships of brown adipose tissue with sex and adiposity in humans. *Diabetes* **59** 1789–1793. (doi:10.2337/db10-0004)
- Quevedo S, Roca P, Pico C & Palou A 1998 Sex associated differences in cold-induced UCP1 synthesis in rodent brown adipose tissue. *Pflügers Archiv: European Journal of Physiology* **436** 689–695. (doi:10.1007/s004240050690)
- Richard D, Carpentier AC, Doré G, Ouellet V & Picard F 2010 Determinants of brown adipocyte development and thermogenesis. *International Journal of Obesity* **34** S59–S66. (doi:10.1038/ijo.2010.241)
- Roca P, Rodriguez AM, Oliver P, Bonet ML, Quevedo S, Pico C & Palou A 1999 Brown adipose tissue response to cafeteria dietfeeding involves induction of the UCP2 gene and is impaired in female rats as compared to males. *Pflügers Archiv: European Journal of Physiology* **438** 628–634. (doi:10.1007/s004240051086)
- Rodriguez E, Monjo M, Rodriguez-Cuenca S, Pujol E, Amengual B, Roca P & Palou A 2001 Sexual dimorphism in the adrenergic control of rat brown adipose tissue response to overfeeding. *Pflügers Archiv: European Journal of Physiology* **442** 396–403. (doi:10.1007/s004240100556)
- Rodriguez AM, Monjo M, Roca P & Palou A 2002 Opposite actions of testosterone and progesterone on UCP1 mRNA expression in cultured brown adipocytes. *Cellular and Molecular Life Sciences* **59** 1714–1723. (doi:10.1007/PL00012499)
- Rodriguez-Cuenca S, Pujol E, Justo R, Frontera M, Oliver J, Gianotti M & Roca P 2002 Sex-dependent thermogenesis, differences in mitochondrial morphology and function, and adrenergic response in brown adipose tissue. *Journal of Biological Chemistry* **277** 42958–42963. (doi:10.1074/jbc.M207229200)
- Rodriguez-Cuenca S, Monjo M, Frontera M, Gianotti M, Proenza AM & Roca P 2007a Sex steroid receptor expression profile in brown adipose tissue. Effects of hormonal status. *Cellular Physiology and Biochemistry* **20** 877–886. (doi:10.1159/000110448)
- Rodriguez-Cuenca S, Monjo M, Gianotti M, Proenza AM & Roca P 2007b Expression of mitochondrial biogenesis signalling factors in brown adipocytes is influenced specifically by 17 β oestradiol, testosterone and progesterone. *American Journal of Physiology. Endocrinology and Metabolism* **292** E340–E346. (doi:10.1152/ajpendo.00175.2006)
- Rothwell NJ & Stock MJ 1979 A role for brown adipose tissue in diet-induced thermogenesis. *Nature* **281** 31–35. (doi:10.1038/281031a0)
- Saito M, Okamatsu-Ogura Y, Matsushita M, Watanabe K, Yoneshiro T, Nio-Kobayashi J, Iwanaga T, Miyagawa M, Kameya T, Nakada K *et al.* 2009 High incidence of metabolically active brown adipose tissue in healthy adult humans: effects of cold exposure and adiposity. *Diabetes* **8** 1526–1531. (doi:10.2337/db09-0530)
- Seale P, Conroe HM, Estall J, Kajimura S, Frontini A, Ishibashi J, Cohen P, Cinti S & Spiegelman BM 2011 Prdm16 determines the thermogenic program of subcutaneous white adipose tissue in mice. *Journal of Clinical Investigation* **121** 96–105. (doi:10.1172/JCI44271)
- Sjogren J, Li M & Bjorntorp P 1995 Androgen hormone binding to adipose tissue in rats. *Biochimica et Biophysica Acta* **1244** 117–120. (doi:10.1016/0304-4165(94)00208-F)
- Song CK, Vaughan CH, Keen-Rhinehart E, Harris RB, Richard D & Bartness TJ 2008 Melanocortin-4 receptor mRNA expressed in sympathetic outflow neurons to brown adipose tissue: neuroanatomical and functional evidence. *American Journal of Physiology. Regulatory, Integrative and Comparative Physiology* **295** R417–R428. (doi:10.1152/ajpregu.00174.2008)
- Tagliaferro AR, Davis JR, Truchon S & Van Hamont N 1986 Effects of dehydroepiandrosterone acetate on metabolism, body weight and composition of male and female rats. *Journal of Nutrition* **116** 1977–1983.
- Traish AM, Miner MM, Morgentaler A & Zitzmann M 2011 Testosterone deficiency. *American Journal of Medicine* **124** 578–587. (doi:10.1016/j.amjmed.2010.12.027)
- Trayhurn P, Douglas JB & McGuckin MM 1982 Brown adipose tissue thermogenesis is 'suppressed' during lactation in mice. *Nature* **298** 59–60. (doi:10.1038/298059a0)
- Van Marken Lichtenbelt WD, Vanhomerig JW, Smulders NM, Drossaerts JM, Kemerink GJ, Bouvy ND, Schrauwen P & Teule GJ 2009 Cold activated brown adipose tissue in healthy men. *New England Journal of Medicine* **360** 1500–1508. (doi:10.1056/NEJMoa0808718)
- Virtanen KA, Lidell ME, Orava J, Heglind M, Westergren R, Niemi T, Taittonen M, Laine J, Savisto NJ, Enerbäck S *et al.* 2009 Functional brown adipose tissue in healthy adults. *New England Journal of Medicine* **360** 1518–1525. (doi:10.1056/NEJMoa0808949)
- Wabitsch M, Blum WF, Mucic R, Braun M, Hube F, Rascher W, Heinze E, Teller W & Hauner H 1997 Contribution of androgens to the gender difference in leptin production in obese children and adolescents. *Journal of Clinical Investigation* **100** 808–813. (doi:10.1172/JCI119595)
- Wade GN & Gray JM 1978 Cytoplasmic 17 beta-[3H]estradiol binding in rat adipose tissues. *Endocrinology* **103** 1695–1701. (doi:10.1210/endo-103-5-1695)
- Yanase T, Fan W, Kyoya K, Min L, Takayanagi R, Kato S & Nawata H 2008 Androgens and metabolic syndrome: lessons from androgen receptor knock out (ARKO) mice. *Journal of Steroid Biochemistry and Molecular Biology* **109** 254–257. (doi:10.1016/j.jsmb.2008.03.017)

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