Brown Adipose Tissue: A Human Perspective

Mariëtte R. Boon and Wouter D. van Marken Lichtenbelt

Contents

Abstract

Since 2009, the presence of brown adipose tissue (BAT) in adult humans has been irrefutably proven. It is estimated that active BAT can contribute up to 2.5–5% of

M.R. Boon • W.D. van Marken Lichtenbelt (✉)
Maastricht University, Maastricht, The Netherlands
e-mail: markenlichtenbelt@maastrichtuniversity.nl

S. Herzig (ed.), *Metabolic Control*, Handbook of Experimental Pharmacology 233,
DOI 10.1007/164_2015_11

resting metabolic rate in humans, suggesting that sustained activation of BAT may alleviate obesity and associated disorders. In the current chapter, the discovery of BAT in adult humans will be discussed. Furthermore, the characteristics of human BAT, methods to visualize the tissue as well as physiological and pharmacological methods to enhance its activity will be stressed.

Keywords
Beige adipocytes · Brown adipocytes · Cold acclimation · Energy expenditure · PET-CT scans · Pharmacology

1 Human Thermoregulation

1.1 Components of Thermogenesis in Humans

Body temperature is the net result of heat production (thermogenesis) and heat loss. Thermogenesis is the consequence of the body's energy metabolism, while heat loss, i.e., heat transport from the body to the environment, depends on conduction, convection, radiation, and evaporation (insensible and sweat). Body temperature can thus be regulated on the level of thermogenesis, e.g., shivering and nonshivering thermogenesis, and heat loss, e.g., sweat production in the heat and during exercise. Crucial in effective thermoregulation is the amount of heat

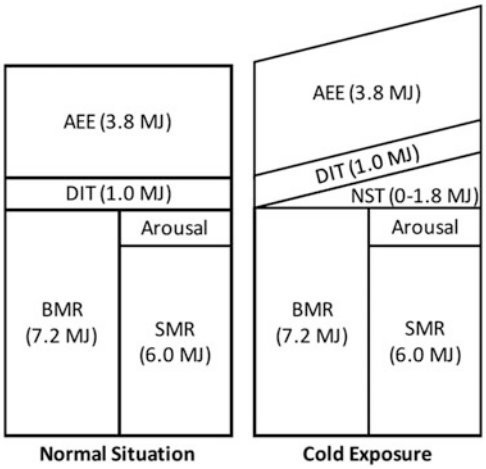

Fig. 1 Building blocks of total daily energy expenditure. Subdivision of daily energy expenditure in an individual with a usual energy expenditure of 12 MJ/day in a normal situation (*left pane*). During mild cold exposure (*right pane*), when no shivering occurs, large interindividual differences in NST exist, ranging from 0 to 30% (normal situation adapted from vMLAJP). *BMR* basal metabolic rate, *SMR* sleeping metabolic rate, *DIT* diet-induced thermogenesis, *AEE* activity-induced energy expenditure, *NST* nonshivering thermogenesis

transport from the core to the skin by blood perfusion, regulated by peripheral vasoconstriction and vasodilation. This also determines the heat distribution of the body. There are significant individual differences in the amount of insulation (due to relative low skin and peripheral tissue temperatures), which may affect energy metabolism. Indeed, an increase in insulation is negatively related to the amount of cold-induced thermogenesis (van Marken Lichtenbelt et al. 2002).

In this chapter on brown fat, we will mainly focus on the thermogenesis side of temperature regulation. Thermogenesis (or total daily energy expenditure, ADMR) can be divided in different components (see Fig. 1): basal metabolic rate (BMR, roughly 55–65% of ADMR), diet-induced thermogenesis (DIT, about 10% of ADMR), and energy expenditure for physical activity (AEE). BMR is measured under strictly defined conditions in the morning in fasted state in a (semi-)supine position and in a thermoneutral environment (see for more details (van Marken Lichtenbelt and Schrauwen 2011)). Resting metabolic rate (RMR) is often used instead of BMR, as this is measured under less strict conditions, for instance, in the afternoon, after food intake, or even sitting in a chair. In many human brown fat studies, RMR is used. Therefore, a clear description of the actual measurement conditions is needed in order to be able to compare different studies.

An alternative division in components of total daily energy expenditure is the use of obligatory and facultative thermogenesis. Obligatory thermogenesis refers to the energy expenditure needed for daily body functions, i.e., needed for the cells and organs to maintain the daily living functions. This also includes parts of DIT and AEE that are not needed for extra heat production. Facultative thermogenesis, on the other hand, is highly variable and consists of extra heat production in response to cold and diet, e.g., cold-induced thermogenesis (CIT) and DIT, respectively. DIT therefore can consist of both an obligatory part and a facultative part. In rodents, it has been shown that unbalanced diets can increase the facultative thermogenesis in order to be able to eat more to obtain enough valuable nutrients without gaining too much weight. Whether facultative DIT exists in humans is still under debate (Kozak 2010), although ingestion of a large meal increased DIT together with BAT activity (Vosselman et al. 2013). Most important in relation to human brown fat is the CIT. This consists of shivering thermogenesis (ST) and nonshivering thermogenesis (NST). ST can increase human energy expenditure by as much as 3–5 times BMR. Shivering, however, is generally experienced uncomfortable, leads to fatigue, and negatively affects the coordination of our movements. NST is more modest, ranges from 0 to 30% of RMR, but can be sustained without appreciable discomfort. Therefore, NST seems to be a way to increase energy expenditure with the potential to create a negative energy balance. This may have large health implications, especially for targeting disorders that are linked to overweight and obesity.

1.2 Adaptation to Long-Term Cold Acclimation

There is a large individual variation in NST in response to mild cold (Celi et al. 2010; van Ooijen et al. 2004; Warwick and Busby 1990). Some subjects

may increase NST by more than 30%, while others even drop their energy expenditure. The latter occurs most likely because of no or very small amounts of NST combined with a reduction of the energy expenditure in the cool peripheral tissue (Arrhenius law). Van Ooijen, however, also noted that NST is subjected to seasonal variation (van Ooijen et al. 2004). Twenty subjects were measured in both summer and winter season and under the same test conditions; NST was significantly higher in winter. Moreover, those individuals showing high NST in summer also did so in winter, indicating that individual differences persisted. In conclusion, there is individual variation in NST, but the level of NST is not fixed and can be increased. The latter was elegantly shown already in 1961 in a study by Davis (1961) who demonstrated that daily frequent cold exposure over time reduced shivering in humans without giving in on total energy expenditure, pointing to increased NST capacity.

In animals like rodents, it is well established that the tissue responsible for NST is brown adipose tissue (BAT), which combusts fatty acids toward heat in a process called mitochondrial uncoupling (Cannon and Nedergaard 2004). This uncoupling process is executed by uncoupling protein 1 (UCP1), a unique inner membrane mitochondrial protein for BAT. Briefly, UCP1 uncouples the electron transport chain, causing an increased proton leakage over the mitochondrial inner membrane. This results in production of extra heat instead of ATP. Indeed, in rodents, intermittent cold exposure results in both enhanced NST and enhanced uncoupling protein 1 (UCP1) content and BAT mass, underscoring that BAT contributes to the enhanced NST (Davis 1961). However, in humans, the tissue responsible for NST remained an enigma until 2009. Though early anatomical studies identified the presence of BAT in humans (Heaton et al. 1978; Huttunen et al. 1981), physiological experiments could not identify a functional role of BAT (Astrup et al. 1984). Therefore, it was generally agreed that, although present and functional in newborns, during maturing, the amount of BAT decreases to become physiologically insignificant in adults.

2 The Discovery of BAT in Adult Humans

2.1 FDG PET-CT Scans Lead to Discovery of Human BAT

It was not until the clinical application of the FDG PET-CT scans by nuclear medicine that functional BAT was identified in humans. This technique makes use of a labeled glucose analog (^{18}F-FDG) that is taken up by metabolically active tissues without being metabolized and therefore becomes trapped in cells. While the PET scan can visualize and quantify uptake of this radioactive tracer, the CT is needed for anatomical information. The ^{18}F-FDG PET-CT scan is used in oncology for localization of metabolically active tumors that exhibit relatively high glucose uptake (e.g., for glycolysis). Since active BAT also takes up high amounts of glucose (i.e., for de novo lipogenesis, see below), the FDG PET-CT can be used to visualize BAT. One of the earliest reported images of BAT in a patient was by Barrington and Maisey (1996) in 1996 who by the way classified the tissue as tense muscle. After this, several other

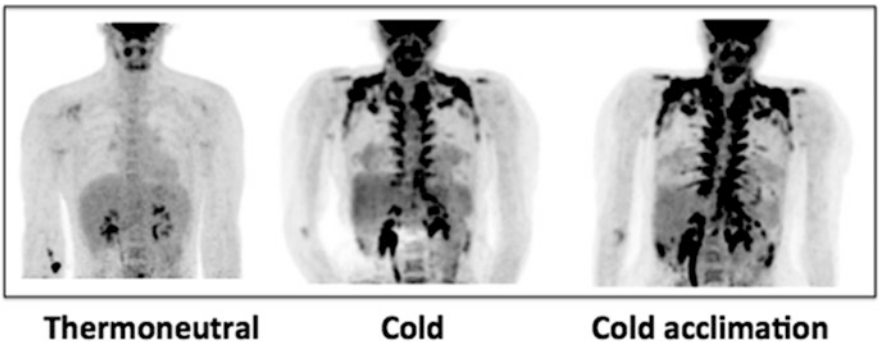

Fig. 2 ^{18}F-Fluorodeoxyglucose (FDG) PET-CT scan visualizing brown adipose tissue in adult humans under thermoneutral conditions after short-term cold exposure and after 10 days of cold acclimation. Brown adipose tissue (BAT) can be visualized by the use of an FDG PET-CT scan. To this end, the subjects (middle and right pane) are exposed to cold (16°C) for 2 h in order to activate BAT. After 1 h of cold induction, the radioactive tracer ^{18}F-FDG is injected intravenously. ^{18}F-FDG, a glucose analog, is taken up by organs which have a high glucose metabolism, especially the brain, heart, and BAT. After 2 h of cold induction, the uptake of ^{18}F-FDG is visualized by means of a low-dose CT scan, immediately followed by a PET scan. The CT scan is used for localization of the uptake areas. The activity and volume of the BAT are quantified by autocontouring the areas of FDG uptake, by the use of a previously set threshold. The left pane shows FDG uptake after 2 h of exposure to thermoneutral temperature (22°C). The right pane shows FDG uptake after 2 h of cold exposure (16°C) in a subject that has been cold acclimated (15–16°C, 6 h/day) for 10 consecutive days

studies from the field of nuclear medicine reported BAT in cancer patients, where it was regarded an artifact potentially obscuring the image in search for tumors. It was Nedergaard et al. (2007) in 2007 who reviewed these nuclear medicine retrospective studies in the context of metabolic implications for human brown fat physiology. In 2009, several research groups independently identified functional brown fat in adult humans after performance of dedicated cold exposure experiments (Saito et al. 2009; van Marken Lichtenbelt et al. 2009; Virtanen et al. 2009) together with retrospective patient studies (Cypess et al. 2009).

More specifically, the FDG PET-CT scans revealed increased glucose uptake upon cold exposure in fat tissue located in the supraclavicular, neck, paravertebral, mediastinal, para-aortic, and suprarenal areas (see Fig. 2). However, direct evidence that the tissue was indeed BAT came from biopsy material obtained from the supraclavicular and neck region, in which the presence of UCP1 was shown (van Marken Lichtenbelt et al. 2009; Virtanen et al. 2009; Zingaretti et al. 2009). Since UCP1 is the bona fide marker of BAT (Cannon and Nedergaard 2004), this information together with the imaging proved the presence of functional BAT in adult humans.

2.2 Other Methods of BAT Visualization

Strictly, FDG PET-CT scans only visualize tissue glucose uptake (static scan) or glucose uptake rate (dynamic scan) (van der Lans et al. 2014). However, high

glucose uptake does not necessarily mean that the tissue is metabolically active. A first indication that BAT is indeed metabolically active comes from the study of Orava et al. (2011), who used [^{15}O]H_2O to show that BAT activation results in enhanced blood flow in the tissue. However, even increased blood flow is not the final proof of metabolic activity because blood flow may also increase in tissues that are not metabolically active. The final proof came from Ouellet et al. (2012) who determined oxidative metabolism in human BAT indirectly using ^{11}C-acetate PET imaging. ^{11}C-acetate is rapidly taken up by BAT and other tissues (i.e., myocardium, muscle) and metabolized to $^{11}CO_2$ and H_2O after intravenous injection. The rate of clearance of ^{11}C-acetate from the tissue reflects oxidative metabolism, with higher oxidative metabolism resulting in faster clearance from the tissue. Indeed, based on ^{11}C-acetate tissue kinetics, cold exposure markedly increased oxidative metabolism in BAT in all subjects ($n = 6$), which has recently been confirmed (Blondin et al. 2014). In a recent study, oxygen consumption was measured in BAT during cold exposure by means of dynamic oxygen ($^{15}O_2$) PET imaging, a possibly very adequate marker for BAT metabolism (Muzik et al. 2013).

It is well known from animal studies that not glucose but fatty acids (FA) are the main source of fuel in BAT (see below). Therefore, Ouellet also used the fatty acid tracer ^{18}F-fluoro-thiaheptadecanoic acid (FTHA) in their cold exposure tests, next to ^{18}F-FDG and ^{11}C-acetate (Ouellet et al. 2012). They showed both substantial FA and glucose uptake upon cold exposure. However, a drawback of the use of a FA tracer as compared to a glucose tracer is that the uptake is rather nonspecific since it is also largely taken up by other organs such as the liver and intestine. Thus, the FA tracer needs to be optimized more before it can be used on a large scale.

Though these tracer studies reveal important new information on BAT metabolism and fuel utilization, an important aspect is not yet sufficiently covered. That is the fact that BAT, when activated, also uses its own internal triglyceride (TG) stores. In this respect, CT scans can provide useful information, since the radiodensity expressed in Hounsfield units reveals the water fat ratio of the tissue. Indeed, cold-exposed subjects show an increase in BAT radiodensity indicating reduced BAT TG after cold exposure (Ouellet et al. 2012).

3 Human BAT: Brown or Beige?

As described in Chapter 4a, from rodent studies, it is evident that besides brown adipocytes, also beige adipocytes exist (Seale et al. 2008; Wu et al. 2012). In rodents, these beige adipocytes lie mainly dispersed between WAT but can also be found in muscle. A recent topic of debate is on how “brown” human BAT actually is. Distinction between classical brown and beige adipocytes cannot be simply made on the basis of an FDG PET-CT scan, as both types of brown adipocytes take up high amounts of glucose when stimulated (Bartelt and Heeren 2013). Recent genotyping of human BAT biopsies, obtained from the supraclavicular area from subjects who showed FDG uptake in this area, demonstrated that human BAT more closely resembles the beige fat found in WAT depots in mice rather than the classical murine BAT (Wu et al. 2012).

Therefore, a former vision was that human BAT solely consists of "beige" adipocytes. A recent study by Cypess et al. (2013) refuted this vision. Different depots of neck adipose tissue were isolated from adult human volunteers, and gene expression, differentiation capacity, and basal oxygen consumption were compared to different mouse adipose depots. Although the variation in the properties of human neck adipose tissue was substantial between subjects, they showed that some human samples have many similarities with the classical BAT found in rodents. Intriguingly, it appeared that the unstimulated energy expenditure of the human BAT samples is similar to that of mouse interscapular BAT, underscoring the energy-combusting potential of this adipose tissue in humans (Okamatsu-Ogura et al. 2013). Moreover, we (Nascimento et al., unpublished) and others (Wu et al. 2012) have recently shown that human brown adipocytes respond to noradrenalin by markedly enhancing uncoupled respiration.

4 Contribution of BAT to Energy Expenditure and Lipid and Glucose Metabolism in Humans

4.1 Estimation: What Is the Potential Contribution of BAT Thermogenesis to Whole-Body Energy Expenditure in Humans?

As shown above, the combined information of different scanning techniques, together with tissue characterization including UCP1 staining, revealed metabolically active BAT in adult humans. However, the actual contribution of BAT (and beige) metabolism to whole-body NST is not yet determined. In rodents, it is very likely that BAT is the only tissue responsible for the classical nonshivering thermogenesis and that upon cold exposure, BAT can contribute to up to 60% of RMR (van Marken Lichtenbelt and Schrauwen 2011; Cannon and Nedergaard 2004; Feldmann et al. 2009). In humans, a large body of evidence points toward BAT as a contributor to nonshivering thermogenesis, possibly in conjunction with mitochondrial uncoupling in other tissues (Wijers et al. 2008). With proper standardized cold exposure tests, it has been shown that cold-induced BAT activity (based on glucose uptake) is significantly related to NST (Bakker et al. 2014; van der Lans et al. 2013). Unfortunately, from correlation studies, quantification is not possible. The abovementioned study that used the ^{15}O tracer technique could show oxygen consumption by BAT, but the used cooling protocol prevented calculation of the actual (maximal) BAT contribution during cold (Muzik et al. 2013). Alternatively, from estimates of the amount of BAT present in the body and potential tissue-specific metabolism, one could estimate the total oxidative capacity. However, several problems arise. The first is the volume quantification. PET volume can easily be overestimated, because of the partial volume effect (see for more details (van der Lans et al. 2014)). On the other hand, estimation based on CT may reveal an underestimation. Moreover, both scanning techniques may miss small more dispersed brown and beige fat depots because of the limited resolution of the scans. Nevertheless, a volume estimate can be made and has been made: 50–

100 g (van Marken Lichtenbelt and Schrauwen 2011; Virtanen et al. 2009). The second problem is that the tissue is very inhomogeneous. As described above, BAT in humans is very likely a mix of both white and brown (beige) adipocytes in contrast to BAT in rodents. This means that estimates of tissue-specific metabolic rate from animals may not apply to humans. Third is from which animal can tissue-specific metabolism be calculated. From limited animal data, it is revealed that the maximal heat-producing capacity of BAT is 300 W/kg (Rothwell and Stock 1983). However, it is well known that small mammals have higher tissue-specific metabolic rates than larger animals. Therefore, based on allometric corrections and a volume of BAT of 50 g, a careful estimation revealed a contribution of BAT of 2.5–5% of RMR. Virtanen et al. (2009) also came to approximately the same results. It should be noted that these are just estimations and in future studies this quantification requires much more attention.

4.2 Differences in BAT Volume/Activity

There is a striking individual variation in brown fat activity, which appears to be related to the level of NST. In addition, there are group differences. For instance, BAT presence and activity are reduced in obesity compared to lean adults (van Marken Lichtenbelt et al. 2009; Wang et al. 2011) and almost absent in morbid obesity (Vijgen et al. 2011). Also the elderly have reduced cold-induced BAT activity (Yoneshiro et al. 2011a), and from retrospective studies, it appears that diabetes is characterized by a diminished amount of BAT (Ouellet et al. 2011), but dedicated studies are needed to confirm this. Retrospective studies also hinted toward higher BAT presence in women compared to men. However, dedicated cold exposure studies showed no gender-specific BAT activity (van der Lans et al. 2013).

Though BAT studies have been carried out in many different ethnic groups (Caucasian, Chinese, Japanese), hardly any comparative studies have been carried out. In fact, only two studies have been carried out so far on South Asian and Caucasian populations. Relative to Caucasians, the South Asian population is characterized by a high risk of developing type 2 diabetes. Moreover, type 2 diabetes occurs at a younger age and lower BMI in South Asians than Caucasians (Mukhopadhyay et al. 2006; Razak et al. 2007), and the risk of complications related to diabetes is increased in this group (Chandie Shaw et al. 2002). The first study did not find differences in BAT activity between the two groups (Admiraal et al. 2013), while the second study did observe a significant difference (Bakker et al. 2014). The main difference between the two studies was the cooling protocol: while it was "fixed" in study one (e.g., constant environmental temperature of 16–18°C) and cooling was performed by air cooling, a standard individual attuned water cooling protocol that maximizes NST was used in study two. Interestingly, in the latter study, the lower BAT activity in South Asians did go hand in hand with differences in NST. RMR was significantly lower in SA, and NST only significantly increased in Caucasians. In conclusion, it appears that South Asians do have reduced BAT availability. Whether this is mainly due to an actual ethnic difference

that affects BAT availability or whether the lifestyle (culture, thermal behavior) is also involved remains to be investigated.

4.3 Involvement of BAT in Lipid Metabolism

As mentioned above, animal studies have shown that triglyceride-derived FA are the main fuel for BAT thermogenesis. The activation of BAT results in a fast induction of intracellular lipolysis, induced by the activation of adipose triglyceride lipase (ATGL) and hormone-sensitive lipase (HSL), resulting in the release of FA from TG-filled lipid droplets. FA are directed to the mitochondria where they either allosterically activate uncoupling protein 1 (UCP1) present in the inner membrane of the mitochondrion or can undergo oxidation (Cannon and Nedergaard 2004). Accordingly, after intracellular lipolysis, the intracellular TG stores of the brown adipocyte need to be replenished. This is mediated via three mechanisms: (1) uptake of glucose followed by de novo lipogenesis (see below), (2) uptake of albumin-bound free FA, and (3) uptake of TG-derived FA from very-low-density lipoproteins and chylomicrons in the plasma.

The magnitude of the TG clearance capacity of BAT became clear only recently when Bartelt et al. (Bartelt et al. 2011) demonstrated that mice that are housed at 4°C for 24 h, a major trigger for BAT activation, show a massive lowering of plasma TG levels. Furthermore, BAT activation by means of cold exposure is able to correct hyperlipidemia in hyperlipidemic ApoA5 knockout mice (Bartelt et al. 2011). In contrast, animals in which BAT is surgically denervated become rapidly obese and hypertriglyceridemic (Dulloo and Miller 1984). These findings underscore the involvement of BAT in total energy expenditure and TG clearance, at least in mice.

Also in humans, BAT likely contributes to TG metabolism. As mentioned above, exposure of humans to cold for 2 h resulted in enhanced FA uptake by BAT as compared to muscle and WAT. It is likely that human BAT also utilizes FA from circulating lipoproteins, though this has not been investigated yet. Furthermore, 2 h of cold exposure results in a rapid increase in BAT CT radiodensity, suggestive of lowering of intracellular TG stores in BAT. Indeed, a depletion of intracellular lipid in BAT was found at necropsy in newborn infants and adults who died from hypothermia (Aherne and Hull 1966). Thus, a fast initial combustion of intracellular TG upon acute BAT activation may well explain why short-term cold exposure does not result in acute lowering of plasma TG in human subjects, but does result in increased fat oxidation, while glucose oxidation is not changed (Bakker et al. 2014). Likely, prolonged BAT activation will result in lowering of plasma TG levels in human subjects as a consequence of increased clearance from the plasma toward BAT.

4.4 Involvement of BAT in Glucose Metabolism

Murine studies have shown that BAT expresses both GLUT-4 and GLUT-1, suggesting both insulin-dependent and insulin-independent uptake of glucose by the tissue (Cannon and Nedergaard 2004). As mentioned, while fatty acids are the main substrate used for oxidation and uncoupling, glucose is mainly used for de novo lipogenesis (e.g., to replenish intracellular lipid droplets) and ATP generation (e.g., via glycolysis) rather than oxidation. Still, murine studies suggest that plasma clearance of glucose by BAT can substantially contribute to whole-body glucose metabolism. For instance, transplantation of extra BAT in mice results in improved glucose tolerance due to higher uptake of glucose by the tissue (Stanford et al. 2013). Furthermore, long-term BAT activation by means of a β3 adrenergic agonist lowered plasma glucose levels (Wang and Li 2013). Of note, also induction of white adipose tissue "browning" by means of the recently identified hormone irisin improved glucose tolerance suggesting that also beige adipocytes have the capacity to contribute to whole-body glucose metabolism, at least in mice (Bostrom et al. 2012).

In humans, it is well established that upon cold exposure, BAT takes up high amounts of FDG, underscoring the large glucose clearance capacity of the tissue. Whether uptake of glucose is mainly mediated via the GLUT-1 or GLUT-4 transporter remains to be determined. However, Orava et al. (2011) showed that insulin stimulation markedly enhances FDG uptake by BAT to an extent comparable to muscle, suggesting that the GLUT-4 transporter is at least in part involved in glucose uptake by human BAT and that the tissue is insulin sensitive. Despite the large glucose uptake capacity of human BAT, the question remains whether active BAT is sufficient to impact on whole-body glucose metabolism in humans. An association study in which different plasma parameters were measured in healthy humans with and without BAT (BAT status was determined via FDG PET-CT scans) showed that BAT was a significantly independent determinant of glucose and HbA1c levels, suggesting that BAT could impact on glucose metabolism (Matsushita et al. 2014). However, whether long-term BAT activation indeed results in improvement of glucose metabolism in obese subjects with impaired glucose tolerance remains to be determined and is an interesting and relevant topic for future studies.

5 Involvement of BAT in Human Pathology

5.1 Pheochromocytomas

Pheochromocytomas are neuroendocrine tumors that secrete excessive amounts of noradrenalin, an important activator of BAT. Indeed, on ^{18}F-FDG PET-CT scans in patients with this tumor, an increased mass and activity of BAT are seen, accompanied by increased energy expenditure (Dong et al. 2013; Petrak et al. 2013). Moreover, after resection of the tumor, FDG uptake and energy

expenditure decrease dramatically (Kuji et al. 2008), supporting that the increased energy expenditure typical for this condition is likely due to increased BAT-mediated NST. Furthermore, pheochromocytoma patients exhibit increased browning of visceral WAT (Frontini et al. 2013), which may also contribute to the enhanced energy expenditure. Whether this browning is due to increased transdifferentiation of white into brown adipocytes or differentiation of brown preadipocytes present in the visceral WAT toward mature brown adipocytes remains to be determined.

5.2 Hyperthyroidism/Hypothyroidism

Mouse studies have shown that, in addition to cold, thyroid hormone is also involved in the activation of BAT. After uptake of T3 and T4 by the brown adipocyte and intracellular conversion of T4 into T3 by the enzyme type 2 deiodinase (D2), the active thyroid hormone T3 is translocated into the nucleus and binds to a thyroid hormone-responsive element located on the promoter of the UCP1 gene (Branco et al. 1999). This leads to increased transcription of UCP1 and ultimately to increased uncoupling. Furthermore, T3 is able to stabilize the UCP1 mRNA, thereby reducing its degradation in the cell (Cannon and Nedergaard 2004). During cold induction, the activity of D2 is increased in BAT, leading to locally increased amounts of T3 (Bianco and McAninch 2013). This is an additional and necessary mechanism to stimulate thermogenesis by BAT. In addition, thyroid hormone also activates BAT indirectly by enhancing sympathetic nervous system outflow toward the tissue (Lopez et al. 2010).

Interestingly, energy expenditure is increased in patients with hyperthyroidism and decreased in patients with hypothyroidism. A recent study showed that hyperthyroidism in human patients increases glucose uptake in BAT independently of BAT perfusion (Lahesmaa et al. 2013). Therefore, the weight loss and excessive transpiration in hyperthyroidism, and the weight gain and reduced cold tolerance in hypothyroidism, can at least be partly attributed to an increased and decreased activity of BAT, respectively. Furthermore, recent studies have shown that treatment of human stem cells with T3 results in the development of UCP1-positive cells within white adipose tissue, pointing to "browning" of white adipose tissue (Lee et al. 2012). This may also contribute to the enhanced energy expenditure seen in hyperthyroidism.

5.3 Obesity, Dyslipidemia, and Type 2 Diabetes

When energy intake exceeds energy expenditure (i.e., positive energy balance), TG is stored in WAT. In addition, TG may be stored ectopically in organs such as the skeletal muscle and liver, resulting in malfunction of these organs. A prolonged positive energy balance may result in development of overweight and obesity. Currently, in the USA, over 69% of the adult population is overweight

($25 < \text{BMI} < 30$ kg/m^2), and more than 35% is already obese (BMI > 30 kg/m^2) (CDC 2014). Obesity is strongly associated with the development of other disorders and diseases, such as dyslipidemia, type 2 diabetes, cardiovascular disease, and cancer (World Health Organ 2000).

Interestingly, recent studies point toward a role of disturbed BAT function in the development of obesity and related disorders. In human adults, the amount of BAT is inversely correlated with BMI and percentage of body fat (van Marken Lichtenbelt et al. 2009). More specifically, BAT volume is inversely correlated with parameters of central obesity, such as visceral fat volume on CT scan and waist circumference (Wang et al. 2011). These findings suggest that obesity is associated with a low level of BAT activity. Indeed, excision of BAT or sympathetic denervation of BAT in mice results in hypertriglyceridemia and obesity (Dulloo and Miller 1984). Thus, in humans, a reduced activity of BAT may predispose to obesity and obesity-related diseases such as dyslipidemia and type 2 diabetes by accumulation of TG in the blood and subsequent storage in WAT as well as in ectopic fat depots such as skeletal muscle and the liver. This is associated with reduced insulin sensitivity of these organs and eventually type 2 diabetes. Furthermore, since BAT is also involved in clearance of plasma glucose (i.e., for de novo lipogenesis) (Stanford et al. 2013), BAT could also contribute to glucose homeostasis, particularly in resting conditions when glucose utilization by skeletal muscle is minimal. A low activity of BAT might thus predispose to T2DM not only via the above described relation to obesity but also via reduced glucose uptake at rest (Nedergaard and Cannon 2010). However, the lower BAT activity found in overweight and obese human subjects may also at least in part be a consequence of their increased subcutaneous white fat layer, which may substantially contribute to the maintenance of body temperature, making active BAT redundant (Vijgen et al. 2011).

6 BAT as a Novel Target to Combat Obesity and Associated Disorders

When considering BAT as a novel therapeutic tool to enhance energy expenditure thereby lowering obesity and related diseases in humans, it is highly relevant to assess the actual contribution of human BAT to total daily energy expenditure. As mentioned above, BAT may contribute to 2.5–5% of RMR in humans when the tissue is maximally stimulated. BAT has the potential to contribute even more to total daily energy expenditure when its mass and activity are enhanced due to catecholamine excess. This appears from patients with pheochromocytomas, as discussed above. ^{18}F-FDG PET-CT scans in patients with such tumors show an increased mass and activity of BAT (Dong et al. 2013; Yoneshiro et al. 2011b), accompanied by increased energy expenditure up to twofold in a recent case report (Sondergaard et al., unpublished). Thus, when further stimulated due to endogenous or exogenous factors, BAT has the potential to even more substantially contribute to total daily energy expenditure.

All together, these data unequivocally demonstrate that BAT contributes to NST in humans and that BAT activation is a promising novel therapeutic modality to combat obesity. Therefore, identification of novel therapeutic targets that may activate BAT is highly warranted.

6.1 Physiological Activation of BAT

6.1.1 Cold Exposure

The most well-known stimulus to activate BAT is cold exposure. Cold exposure results in activation of transient receptor potential (TRP) channels in the skin, mediating signaling in the hypothalamic temperature center and subsequent sympathetic outflow toward BAT (Whittle et al. 2013). Short-term cold exposure results in a massive induction of FDG uptake by BAT and a concomitant increase in nonshivering thermogenesis of up to 70%. But does long-term cold acclimation impact on BAT activity and NST and even metabolic parameters? Several recent studies have investigated this issue. Acclimation of healthy young adults to 15–16°C for 10 consecutive days for 6 h/day resulted in recruitment and enhanced activity of BAT as well as enhanced NST (van der Lans et al. 2013). However, resting energy expenditure was not affected after the cold acclimation period, nor were effects found on body weight and plasma glucose and lipid levels. In contrast, another study in which healthy young adults were acclimated to 17°C for 6 weeks for 2 h/day did find that fat mass was significantly reduced in addition to positive effects on BAT (Yoneshiro et al. 2013). Whether cold acclimation is indeed a novel tool to improve obesity and associated disorders (e.g., dyslipidemia and insulin resistance) remains to be determined, for instance, by investigating the effect of cold acclimation in obese subjects.

6.1.2 Food Ingredients

The effect of cold exposure on BAT activation and recruitment relies on its potential to enhance sympathetic outflow toward the tissue. Of note, also bioactive food ingredients such as methylxanthines (caffeine or theophylline), ephedrine, and polyphenols (catechins, resveratrol, quercetin, kaempferol) are effective at increasing energy expenditure and lowering body weight (Dulloo 2011; Finer et al. 2000; Hansen et al. 1998), likely by enhancing activation of BAT. Of special interest are the capsinoids (nonpungent capsaicin analogs), the active compound found in chili pepper. Capsinoids can bind to and activate TRP 1 channels located in the upper digestive tract, leading to increased sympathetic nerve activity to BAT (Ono et al. 1985). Capsinoids have been shown to increase BAT activity in rodents (Kawabata et al. 2009). Of note, capsinoids could be effective in activating BAT in humans, without inducing unwanted side effects. Treatment of healthy young adults with daily capsinoid tablets increased energy expenditure more in subjects with BAT (as based on PET-CT) compared to subjects without BAT. Moreover, in subjects with BAT, capsinoids tended to reduce fat mass (Yoneshiro et al. 2013).

Direct evidence for the effect of capsinoids on BAT in humans is needed to draw definite conclusions.

6.2 Pharmacological Activation of BAT

6.2.1 β3-Agonism

Cold exposure stimulates murine BAT via activation of the β3-AR located on the membrane of the brown adipocytes (Cannon and Nedergaard 2004). Although β3-AR agonists have been shown to be potent inducers of BAT activation in mice, resulting in lowering of fat mass, plasma triglyceride, cholesterol, and glucose levels (Wang and Li 2013), the role of the β3-AR for human BAT remains elusive. Although both human and rodent isoforms of the β3-AR respond to specific agonists in adipocytes in vitro (Soeder et al. 1999), in vivo studies with β3 agonists in humans showed disappointing results. Whereas some agonists such as L796568 were able to enhance energy expenditure after a single dose (van Baak et al. 2002), none had beneficial effects on metabolic parameters (Whittle et al. 2013). This has been suggested to be due to the small amount of β3-expressing tissues in humans, downregulation of the receptor following stimulation, or perhaps due to the fact that the β3-AR is not responsible for BAT activation in humans (Larsen et al. 2002). Possibly, the β1-AR and/or β2-AR could be responsible for BAT activation in humans. Indeed, propranolol, a β-AR antagonist with low β3-AR efficacy compared to β1-AR and β2-AR, decreases 18F-FDG uptake by BAT visualized by PET-CT scans (Agrawal et al. 2009). In this respect, it is surprising that broad β-adrenergic receptor agonism with isoprenaline or the sympathomimetic ephedrine did not simulate BAT glucose uptake similar to cold exposure (Cypess et al. 2012; Vosselman et al. 2012). This may be due to the fact that local (e.g., within BAT) availability of both compounds did not reach the same extent as noradrenalin at nerve endings does in the case of cold exposure. Recently a study showed that a specific B3 agonist, Mirabegron, activates brown adipose tissue (Cypess et al. 2015).

All in all, future studies are evidently needed to illuminate the role of β-AR in human BAT activation. More recent studies have focused on the identification of cytokines and hormones that enhance BAT activity, either via enhancing sympathetic outflow of the tissue (i.e., central mechanism) or via direct activation of the brown adipocyte.

6.2.2 FGF21

Fibroblast growth factor 21 (FGF21) is a hormone that is excreted upon fasting, feeding a ketogenic diet (high fat, low carbohydrate), or after amino acid deprivation (Gimeno and Moller 2014). It is predominantly secreted by the liver, but also by other tissues such as WAT, BAT, skeletal muscle, and pancreatic β cells. FGF21 regulates both carbohydrate and lipid metabolism by impacting on different tissues. Interestingly, FGF21 administration results in increased energy expenditure (EE), enhanced thermogenesis in BAT, and lowering of plasma lipid levels and obesity in mice (Emanuelli et al. 2014). Furthermore, stimulation of isolated human preadipocytes with FGF21 induces the formation of beige cells (Lee et al. 2014a,

b), and administration of FGF21 in obese humans with type 2 diabetes lowers fat mass and dyslipidemia (Gaich et al. 2013). Although it is not known whether these effects in humans are due to BAT activation, FGF21 is considered a promising new therapy to activate BAT thereby lowering obesity and associated disorders.

6.3 Promising Novel BAT Activators

Animal studies have identified various other promising compounds that activate BAT in vivo. Although a thorough description of these compounds is beyond the scope of this chapter, a few will be shortly mentioned. The anti-diabetes drug metformin was recently shown to exhibit its triglyceride-lowering effect by enhancing BAT activation through a mechanism involving activation of AMP-activated protein kinase (AMPK) in the tissue (Geerling et al. 2014). As metformin is also associated with slight weight loss in type 2 diabetes patients (Golay 2008), this may be – at least in part – attributed to activation of BAT. Furthermore, irisin has been identified as a signaling peptide that is released by muscle upon exercise (Bostrom et al. 2012), providing a possible mechanism between the well-established links between exercise and enhanced energy expenditure. In mice, irisin treatment resulted in massive browning of WAT and improved insulin sensitivity. Whether irisin also exhibits beneficial metabolic effects in humans remains to be investigated. However, a recent study by Lee et al. (2014a) did show that upon shivering, irisin levels increased in human subjects and that the induction of irisin secretion was proportional to shivering intensity. Thus, during cold acclimation, release of irisin following shivering may be involved in BAT recruitment.

References

Admiraal WM, Holleman F, Bahler L, Soeters MR, Hoekstra JB, Verberne HJ (2013) Combining 123I-metaiodobenzylguanidine SPECT/CT and 18F-FDG PET/CT for the assessment of brown adipose tissue activity in humans during cold exposure. J Nucl Med 54:208–212

Agrawal A, Nair N, Baghel NS (2009) A novel approach for reduction of brown fat uptake on FDG PET. Br J Radiol 82:626–631

Aherne W, Hull D (1966) Brown adipose tissue and heat production in the newborn infant. J Pathol Bacteriol 91:223–234

Astrup A, Bulow J, Christensen NJ, Madsen J (1984) Ephedrine-induced thermogenesis in man: no role for interscapular brown adipose tissue. Clin Sci (Lond) 66:179–186

Bakker LE, Boon MR, van der Linden RA, Arias-Bouda LP, van Klinken JB, Smit F, Verberne HJ, Jukema JW, Tamsma JT, Havekes LM, van Marken Lichtenbelt WD, Jazet IM, Rensen PC (2014) Brown adipose tissue volume in healthy lean south Asian adults compared with white Caucasians: a prospective, case-controlled observational study. Lancet Diabetes Endocrinol 2:210–217

Barrington SF, Maisey MN (1996) Skeletal muscle uptake of fluorine-18-FDG: effect of oral diazepam. J Nucl Med 37:1127–1129

Bartelt A, Heeren J (2013) Adipose tissue browning and metabolic health. Nat Rev Endocrinol 10 (1):24–36

Bartelt A, Bruns OT, Reimer R, Hohenberg H, Ittrich H, Peldschus K, Kaul MG, Tromsdorf UI, Weller H, Waurisch C, Eychmuller A, Gordts PL, Rinninger F, Bruegelmann K, Freund B,

Nielsen P, Merkel M, Heeren J (2011) Brown adipose tissue activity controls triglyceride clearance. Nat Med 17:200–205

Bianco AC, McAninch EA (2013) The role of thyroid hormone and brown adipose tissue in energy homoeostasis. Lancet Diabetes Endocrinol 1:250–258

Blondin DP, Labbe SM, Tingelstad HC, Noll C, Kunach M, Phoenix S, Guerin B, Turcotte EE, Carpentier AC, Richard D, Haman F (2014) Increased brown adipose tissue oxidative capacity in cold-acclimated humans. J Clin Endocrinol Metab 99:E438–E446

Bostrom P, Wu J, Jedrychowski MP, Korde A, Ye L, Lo JC, Rasbach KA, Bostrom EA, Choi JH, Long JZ, Kajimura S, Zingaretti MC, Vind BF, Tu H, Cinti S, Hojlund K, Gygi SP, Spiegelman BM: A PGC1-alpha-dependent myokine that drives brown-fat-like development of white fat and thermogenesis. *Nature* 481:463-468, 2012

Branco M, Ribeiro M, Negrao N, Bianco AC (1999) 3,5,3′-Triiodothyronine actively stimulates UCP in brown fat under minimal sympathetic activity. Am J Physiol 276:E179–E187

Cannon B, Nedergaard J (2004) Brown adipose tissue: function and physiological significance. Physiol Rev 84:277–359

Celi FS, Brychta RJ, Linderman JD, Butler PW, Alberobello AT, Smith S, Courville AB, Lai EW, Costello R, Skarulis MC, Csako G, Remaley A, Pacak K, Chen KY (2010) Minimal changes in environmental temperature result in a significant increase in energy expenditure and changes in the hormonal homeostasis in healthy adults. Eur J Endocrinol 163:863–872

Chandie Shaw PK, Vandenbroucke JP, Tjandra YI, Rosendaal FR, Rosman JB, Geerlings W, de Charro FT, van Es LA (2002) Increased end-stage diabetic nephropathy in Indo-Asian immigrants living in the Netherlands. Diabetologia 45:337–341

Cypess AM, Lehman S, Williams G, Tal I, Rodman D, Goldfine AB, Kuo FC, Palmer EL, Tseng YH, Doria A, Kolodny GM, Kahn CR (2009) Identification and importance of brown adipose tissue in adult humans. N Engl J Med 360:1509–1517

Cypess AM, Chen YC, Sze C, Wang K, English J, Chan O, Holman AR, Tal I, Palmer MR, Kolodny GM, Kahn CR (2012) Cold but not sympathomimetics activates human brown adipose tissue in vivo. Proc Natl Acad Sci U S A 109:10001–10005

Cypess AM, White AP, Vernochet C, Schulz TJ, Xue R, Sass CA, Huang TL, Roberts-Toler C, Weiner LS, Sze C, Chacko AT, Deschamps LN, Herder LM, Truchan N, Glasgow AL, Holman AR, Gavrila A, Hasselgren PO, Mori MA, Molla M, Tseng YH (2013) Anatomical localization, gene expression profiling and functional characterization of adult human neck brown fat. Nat Med 19:635–639

Cypess AM, Weiner LS, Roberts-Toler C et al (2015) Activation of human brown adipose tissue by a beta3-adrenergic receptor agonist. Cell Metab 21:33–38

Davis TR (1961) Chamber cold acclimatization in man. J Appl Physiol 16:1011–1015

Dong A, Wang Y, Lu J, Zuo C (2013) Hypermetabolic mesenteric brown adipose tissue on dual-time point FDG PET/CT in a patient with benign retroperitoneal pheochromocytoma. Clin Nucl Med 39(3):e229–32

Dulloo AG (2011) The search for compounds that stimulate thermogenesis in obesity management: from pharmaceuticals to functional food ingredients. Obes Rev 12:866–883

Dulloo AG, Miller DS (1984) Energy balance following sympathetic denervation of brown adipose tissue. Can J Physiol Pharmacol 62:235–240

Emanuelli B, Vienberg SG, Smyth G, Cheng C, Stanford KI, Arumugam M, Michael MD, Adams AC, Kharitonenkov A, Kahn CR (2014) Interplay between FGF21 and insulin action in the liver regulates metabolism. J Clin Invest 124:515–527

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 Metab 9:203–209

Finer N, Bloom SR, Frost GS, Banks LM, Griffiths J (2000) Sibutramine is effective for weight loss and diabetic control in obesity with type 2 diabetes: a randomised, double-blind, placebo-controlled study. Diabetes Obes Metab 2:105–112

Frontini A, Vitali A, Perugini J, Murano I, Romiti C, Ricquier D, Guerrieri M, Cinti S (2013) White-to-brown transdifferentiation of omental adipocytes in patients affected by pheochromocytoma. Biochim Biophys Acta 1831:950–959

Gaich G, Chien JY, Fu H, Glass LC, Deeg MA, Holland WL, Kharitonenkov A, Bumol T, Schilske HK, Moller DE (2013) The effects of LY2405319, an FGF21 analog, in obese human subjects with type 2 diabetes. Cell Metab 18:333–340

Geerling JJ, Boon MR, van der Zon GC, van den Berg SA, van den Hoek AM, Lombes M, Princen HM, Havekes LM, Rensen PC, Guigas B (2014) Metformin lowers plasma triglycerides by promoting VLDL-triglyceride clearance by brown adipose tissue in mice. Diabetes 63:880–891

Gimeno RE, Moller DE (2014) FGF21-based pharmacotherapy–potential utility for metabolic disorders. Trends Endocrinol Metab 25:303–311

Golay A (2008) Metformin and body weight. Int J Obes (Lond) 32:61–72

Hansen DL, Toubro S, Stock MJ, Macdonald IA, Astrup A (1998) Thermogenic effects of sibutramine in humans. Am J Clin Nutr 68:1180–1186

Heaton GM, Wagenvoord RJ, Kemp A Jr, Nicholls DG (1978) Brown-adipose-tissue mitochondria: photoaffinity labelling of the regulatory site of energy dissipation. Eur J Biochem 82:515–521

Huttunen P, Hirvonen J, Kinnula V (1981) The occurrence of brown adipose tissue in outdoor workers. Eur J Appl Physiol Occup Physiol 46:339–345

Kawabata F, Inoue N, Masamoto Y, Matsumura S, Kimura W, Kadowaki M, Higashi T, Tominaga M, Inoue K, Fushiki T (2009) Non-pungent capsaicin analogs (capsinoids) increase metabolic rate and enhance thermogenesis via gastrointestinal TRPV1 in mice. Biosci Biotechnol Biochem 73:2690–2697

Kozak LP (2010) Brown fat and the myth of diet-induced thermogenesis. Cell Metab 11:263–267

Kuji I, Imabayashi E, Minagawa A, Matsuda H, Miyauchi T (2008) Brown adipose tissue demonstrating intense FDG uptake in a patient with mediastinal pheochromocytoma. Ann Nucl Med 22:231–235

Lahesmaa M, Orava J, Schalin-Jantti C, Soinio M, Hannukainen JC, Noponen T, Kirjavainen A, Iida H, Kudomi N, Enerback S, Virtanen KA, Nuutila P (2013) Hyperthyroidism increases brown fat metabolism in humans. J Clin Endocrinol Metab 99(1):E28–35

Larsen TM, Toubro S, van Baak MA, Gottesdiener KM, Larson P, Saris WH, Astrup A (2002) Effect of a 28-d treatment with L-796568, a novel beta(3)-adrenergic receptor agonist, on energy expenditure and body composition in obese men. Am J Clin Nutr 76:780–788

Lee JY, Takahashi N, Yasubuchi M, Kim YI, Hashizaki H, Kim MJ, Sakamoto T, Goto T, Kawada T (2012) Triiodothyronine induces UCP-1 expression and mitochondrial biogenesis in human adipocytes. Am J Physiol Cell Physiol 302:C463–C472

Lee P, Linderman JD, Smith S, Brychta RJ, Wang J, Idelson C, Perron RM, Werner CD, Phan GQ, Kammula US, Kebebew E, Pacak K, Chen KY, Celi FS (2014a) Irisin and FGF21 are cold-induced endocrine activators of brown fat function in humans. Cell Metab 19:302–309

Lee P, Werner CD, Kebebew E, Celi FS (2014b) Functional thermogenic beige adipogenesis is inducible in human neck fat. Int J Obes (Lond) 38:170–176

Lopez M, Varela L, Vazquez MJ, Rodriguez-Cuenca S, Gonzalez CR, Velagapudi VR, Morgan DA, Schoenmakers E, Agassandian K, Lage R, Martinez de Morentin PB, Tovar S, Nogueiras R, Carling D, Lelliott C, Gallego R, Oresic M, Chatterjee K, Saha AK, Rahmouni K, Dieguez C, Vidal-Puig A (2010) Hypothalamic AMPK and fatty acid metabolism mediate thyroid regulation of energy balance. Nat Med 16:1001–1008

Matsushita M, Yoneshiro T, Aita S, Kameya T, Sugie H, Saito M (2014) Impact of brown adipose tissue on body fatness and glucose metabolism in healthy humans. Int J Obes (Lond) 38:812–817

Mukhopadhyay B, Forouhi NG, Fisher BM, Kesson CM, Sattar N (2006) A comparison of glycaemic and metabolic control over time among South Asian and European patients with Type 2 diabetes: results from follow-up in a routine diabetes clinic. Diabet Med 23:94–98

Muzik O, Mangner TJ, Leonard WR, Kumar A, Janisse J, Granneman JG (2013) 15O PET measurement of blood flow and oxygen consumption in cold-activated human brown fat. J Nucl Med 54:523–531

Nedergaard J, Cannon B (2010) The changed metabolic world with human brown adipose tissue: therapeutic visions. Cell Metab 11:268–272

Nedergaard J, Bengtsson T, Cannon B (2007) Unexpected evidence for active brown adipose tissue in adult humans. Am J Physiol Endocrinol Metab 293:E444–E452

Okamatsu-Ogura Y, Fukano K, Tsubota A, Uozumi A, Terao A, Kimura K, Saito M (2013) Thermogenic ability of uncoupling protein 1 in beige adipocytes in mice. PLoS One 8:e84229

Ono K, Tsukamoto-Yasui M, Hara-Kimura Y, Inoue N, Nogusa Y, Okabe Y, Nagashima K, Kato F (2011) Intragastric administration of capsiate, a transient receptor potential channel agonist, triggers thermogenic sympathetic responses. J Appl Physiol (1985) 110:789–798

Orava J, Nuutila P, Lidell ME, Oikonen V, Noponen T, Viljanen T, Scheinin M, Taittonen M, Niemi T, Enerback S, Virtanen KA (2011) Different metabolic responses of human brown adipose tissue to activation by cold and insulin. Cell Metab 14:272–279

Ouellet V, Routhier-Labadie A, Bellemare W, Lakhal-Chaieb L, Turcotte E, Carpentier AC, Richard D (2011) Outdoor temperature, age, sex, body mass index, and diabetic status determine the prevalence, mass, and glucose-uptake activity of 18F-FDG-detected BAT in humans. J Clin Endocrinol Metab 96:192–199

Ouellet V, Labbe SM, Blondin DP, Phoenix S, Guerin 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. J Clin Invest 122:545–552

Petrak O, Haluzikova D, Kavalkova P, Strauch B, Rosa J, Holaj R, Brabcova VA, Michalsky D, Haluzik M, Zelinka T, Widimsky J Jr (2013) Changes in energy metabolism in pheochromocytoma. J Clin Endocrinol Metab 98:1651–1658

Razak F, Anand SS, Shannon H, Vuksan V, Davis B, Jacobs R, Teo KK, McQueen M, Yusuf S (2007) Defining obesity cut points in a multiethnic population. Circulation 115:2111–2118

Rothwell NJ, Stock MJ (1983) Luxuskonsumption, diet-induced thermogenesis and brown fat: the case in favour. Clin Sci (Lond) 64:19–23

Saito M, Okamatsu-Ogura Y, Matsushita M, Watanabe K, Yoneshiro T, Nio-Kobayashi J, Iwanaga T, Miyagawa M, Kameya T, Nakada K, Kawai Y, Tsujisaki M (2009) High incidence of metabolically active brown adipose tissue in healthy adult humans: effects of cold exposure and adiposity. Diabetes 58:1526–1531

Seale P, Bjork B, Yang W, Kajimura S, Chin S, Kuang S, Scime A, Devarakonda S, Conroe HM, Erdjument-Bromage H, Tempst P, Rudnicki MA, Beier DR, Spiegelman BM (2008) PRDM16 controls a brown fat/skeletal muscle switch. Nature 454:961–967

Soeder KJ, Snedden SK, Cao W, Della Rocca GJ, Daniel KW, Luttrell LM, Collins S (1999) The beta3-adrenergic receptor activates mitogen-activated protein kinase in adipocytes through a Gi-dependent mechanism. J Biol Chem 274:12017–12022

Stanford KI, Middelbeek RJ, Townsend KL, An D, Nygaard EB, Hitchcox KM, Markan KR, Nakano K, Hirshman MF, Tseng YH, Goodyear LJ (2013) Brown adipose tissue regulates glucose homeostasis and insulin sensitivity. J Clin Invest 123:215–223

van Baak MA, Hul GB, Toubro S, Astrup A, Gottesdiener KM, DeSmet M, Saris WH (2002) Acute effect of L-796568, a novel beta 3-adrenergic receptor agonist, on energy expenditure in obese men. Clin Pharmacol Ther 71:272–279

van der Lans AA, Wierts R, Vosselman MJ, Schrauwen P, Brans B, van Marken Lichtenbelt WD (2014) Cold-activated brown adipose tissue in human ADU. Am J Physiol Regul Integr Comp Physiol 307(2):R103–13

van der Lans AA, Hoeks J, Brans B, Vijgen GH, Visser MG, Vosselman MJ, Hansen J, Jorgensen JA, Wu J, Mottaghy FM, Schrauwen P, van Marken Lichtenbelt WD (2014b) Cold acclimation recruits human brown fat and increases nonshivering thermogenesis. J Clin Invest 123:3395–3403

van Marken Lichtenbelt WD, Schrauwen P (2011) Implications of nonshivering thermogenesis for energy balance regulation in humans. Am J Physiol Regul Integr Comp Physiol 301:R285–R296

van Marken Lichtenbelt WD, Schrauwen P, van De KS, Westerterp-Plantenga MS (2002) Individual variation in body temperature and energy expenditure in response to mild cold. Am J Physiol Endocrinol Metab 282:E1077–E1083

van Marken Lichtenbelt WD, Vanhommerig JW, Smulders NM, Drossaerts JM, Kemerink GJ, Bouvy ND, Schrauwen P, Teule GJ (2009) Cold-activated brown adipose tissue in healthy men. N Engl J Med 360:1500–1508

van Ooijen AM, van Marken Lichtenbelt WD, van Steenhoven AA, Westerterp KR (2004) Seasonal changes in metabolic and temperature responses to cold air in humans. Physiol Behav 82:545–553

Vijgen GH, Bouvy ND, Teule GJ, Brans B, Schrauwen P, van Marken Lichtenbelt WD (2011) Brown adipose tissue in morbidly obese subjects. PLoS One 6:e17247

Virtanen KA, Lidell ME, Orava J, Heglind M, Westergren R, Niemi T, Taittonen M, Laine J, Savisto NJ, Enerback S, Nuutila P (2009) Functional brown adipose tissue in healthy adults. N Engl J Med 360:1518–1525

Vosselman MJ, van der Lans AA, Brans B, Wierts R, van Baak MA, Schrauwen P, van Marken Lichtenbelt WD (2012) Systemic beta-adrenergic stimulation of thermogenesis is not accompanied by brown adipose tissue activity in humans. Diabetes 61:3106–3113

Vosselman MJ, Brans B, van der Lans AA, Wierts R, van Baak MA, Mottaghy FM, Schrauwen P, van Marken Lichtenbelt WD (2013) Brown adipose tissue activity after a high-calorie meal in humans. Am J Clin Nutr 98:57–64

Wang ZH, Li YF (2013) Guo YQ: beta3-Adrenoceptor activation attenuates atherosclerotic plaque formation in ApoE(-/-) mice through lowering blood lipids and glucose. Acta Pharmacol Sin 34:1156–1163

Wang Q, Zhang M, Ning G, Gu W, Su T, Xu M, Li B, Wang W (2011) Brown adipose tissue in humans is activated by elevated plasma catecholamines levels and is inversely related to central obesity. PLoS One 6:e21006

Warwick PM, Busby R (1990) Influence of mild cold on 24 h energy expenditure in 'normally' clothed adults. Br J Nutr 63:481–488

Whittle A, Relat-Pardo J, Vidal-Puig A (2013) Pharmacological strategies for targeting BAT thermogenesis. Trends Pharmacol Sci 34:347–355

Wijers SL, Schrauwen P, Saris WH, van Marken Lichtenbelt WD (2008) Human skeletal muscle mitochondrial uncoupling is associated with cold induced adaptive thermogenesis. PLoS One 3:e1777

World Health Organ (2000) Obesity: preventing and managing the global epidemic. Report of a WHO consultation. Tech Rep Ser 894:i–xii, 1–253

Wu J, Bostrom P, Sparks LM, Ye L, Choi JH, Giang AH, Khandekar M, Virtanen KA, Nuutila P, Schaart G, Huang K, Tu H, van Marken Lichtenbelt WD, Hoeks J, Enerback S, Schrauwen P, Spiegelman BM (2012) Beige adipocytes are a distinct type of thermogenic fat cell in mouse and human. Cell 150:366–376

Yoneshiro T, Aita S, Matsushita M, Okamatsu-Ogura Y, Kameya T, Kawai Y, Miyagawa M, Tsujisaki M, Saito M (2011a) Age-related decrease in cold-activated brown adipose tissue and accumulation of body fat in healthy humans. Obesity (Silver Spring) 19:1755–1760

Yoneshiro T, Aita S, Matsushita M, Kameya T, Nakada K, Kawai Y, Saito M (2011) Brown adipose tissue, whole-body energy expenditure, and thermogenesis in healthy adult men. Obesity (Silver Spring) 19:13–16

Yoneshiro T, Aita S, Matsushita M, Kayahara T, Kameya T, Kawai Y, Iwanaga T, Saito M (2013) Recruited brown adipose tissue as an antiobesity agent in humans. J Clin Invest 123:3404–3408

Zingaretti MC, Crosta F, Vitali A, Guerrieri M, Frontini A, Cannon B, Nedergaard J, Cinti S (2009) The presence of UCP1 demonstrates that metabolically active adipose tissue in the neck of adult humans truly represents brown adipose tissue. FASEB J 23:3113–3120