MECHANISMS IN ENDOCRINOLOGY

Brown adipose tissue in humans: regulation and metabolic significance

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Abstract

The recent discovery that functional brown adipose tissue (BAT) persists in adult humans has enkindled a renaissance in metabolic research, with a view of harnessing its thermogenic capacity to combat obesity. This review focuses on the advances in the regulation and the metabolic significance of BAT in humans. BAT activity in humans is stimulated by cold exposure and by several factors such as diet and metabolic hormones. BAT function is regulated at two levels: an acute process involving the stimulation of the intrinsic thermogenic activity of brown adipocytes and a chronic process of growth involving the proliferation of pre-existing brown adipocytes or differentiation to brown adipocytes of adipocytes from specific white adipose tissue depots. BAT activity is reduced in the obese, and its stimulation by cold exposure increases insulin sensitivity and reduces body fat. These observations provide strong evidence that BAT plays a significant role in energy balance in humans and has the potential to be harnessed as a therapeutic target for the management of obesity.

*European Journal of
Endocrinology*
(2016) **175**, R11–R25

Introduction

Brown adipose tissue (BAT) is a thermogenic organ that dissipates nutrient energy as heat, protecting animals from hypothermia and obesity. The thermogenic property of BAT is conferred by a unique protein, called uncoupling protein 1 (UCP1), located in the inner mitochondrial membrane of brown adipocytes (1). By mediating the

proton conductance into the mitochondrial matrix, UCP1 uncouples mitochondrial oxidation from ATP synthesis, resulting in the generation of heat instead of ATP (1).

It was traditionally believed that BAT was present only in infants. However, over the last decade, metabolic imaging based on the use of an isotopic glucose analogue,

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has provided conclusive evidence that BAT exists in adult humans (2, 3, 4). This technique of positron emission tomography combined with computed tomography (PET–CT), has demonstrated the avid uptake of fluoro-deoxy-glucose (FDG), into tissue of fat density which on biopsy displayed the characteristics of BAT (2, 4, 5). BAT depots are located in the supraclavicular, neck, paravertebral and perinephric regions of adult humans.

Much research effort has gone into investigating the biology of BAT in humans. This paper will review recent advances in the regulation and metabolic significance of BAT in humans.

Two types of BAT

Studies in rodents have provided strong evidence for two types of BAT, ‘classical’ and ‘brite’, which differ in developmental origin but display similar biological properties (6, 7, 8). Classical BAT, located in the interscapular region, originates from stem cells of muscle lineage (9). Brite cells (brown in white; also termed ‘beige’) are brown adipocyte-like cells derived from white adipose cell origin, and the formation of brite cells within the white adipose depot (WAT) is referred to as ‘browning’ (10, 11, 12). Molecular/gene signatures have been identified for classical brown, brite and white adipocytes in rodents and humans (8, 13, 14), the details of which are out of scope for this review. In rodents, brite adipocytes are located in the inguinal and retroperitoneal WAT depots (7, 12). BAT depots around the neck and in the supraclavicular regions of adult humans consist of a mixed population of both classical brown and brite cells (8, 13, 15). In this review, these depots will be simply referred to as BAT and identification of increased UCP1 expression in typical WAT depots will be reported as browning.

Two processes have been proposed for browning. Some researchers report that the cells arise directly from trans-differentiation of mature white adipocytes (10, 16) while others postulate they arise from a distinct precursor cell population within the WAT depot (17, 18). The browning of white adipose tissue is regarded as a process of huge potential for harnessing the metabolic capacity of BAT.

Regulation of BAT in humans

BAT is regulated by environmental and nutritional factors that are mediated by neural and endocrine mechanisms. Some of these factors control the thermogenic activity, others regulate BAT mass and some may affect both processes. For example, in rodents, cold exposure stimulates

BAT function acutely (3, 4) but over a protracted period increases BAT mass, a process involving proliferation of classical brown adipocytes (19, 20) and expansion of brite depots from the browning phenomenon (10, 11, 12).

Assessment of BAT and browning

PET imaging allows for non-invasive study of human BAT activity. The avidity of FDG uptake on PET imaging reflects the UCP1 content on tissue analysis (2). PET–CT also allows for the quantification of the volume of active BAT. Some investigators have interpreted an increase in active BAT volume on PET–CT as ‘recruitment’ of BAT (21, 22, 23, 24). This terminology implies that the intervention has increased the abundance or mass of BAT. However, the increase in BAT volume on a scan may simply reflect the stimulation of BAT depot function beyond a detectable threshold of quantification. Thus, PET–CT cannot differentiate BAT activity from abundance as the mechanism underlying a change in BAT volume. Quantification of BAT mass will require a technique which can identify BAT tissue mass/volume, independent of activity. To date, such a technique has not been established non-invasively. Similarly, determination of the browning process is only possible by direct tissue examination at this stage.

The following sections cover regulation of BAT in humans.

Cold exposure ► Cold exposure stimulates BAT activity. There is a higher prevalence of BAT in scans undertaken in winter than in summer (25, 26, 27, 28). BAT FDG uptake is increased when subjects are precooled before scanning (3, 4, 29) and lower with pre-warming (30, 31, 32). Cold-stimulated increase in glucose uptake is accompanied by a parallel increase of oxidative capacity (21, 33), oxygen consumption (34) and blood flow (34, 35) in human BAT.

In addition to stimulating activity, prolonged cooling increases BAT mass and induces browning of WAT in rodents (10, 11, 12, 19, 20). The evidence as to whether prolonged cold exposure promotes BAT mass and browning in humans is not established conclusively. Cold exposure for 2–6 weeks markedly increases the avidity of FDG uptake and volume of active BAT (21, 22, 23, 24). However, it is not possible to determine from these studies whether the increase in detectable volume arises from enhanced activity or from an expanded cell mass. Indirect evidence that prolonged cold stimulus can increase BAT mass in humans comes from the necropsy finding of a high amount of BAT around the neck of Finnish outdoor workers (36).

Three studies have investigated whether browning occurs in humans after cold acclimatisation (22, 23, 37). Two studies employed cooling at 15 °C for 6 h per day for 10 days (23, 37), while the other at 19 °C for at least 10 h per day for 1 month (22). None of the studies found evidence of browning in biopsies of subcutaneous abdominal WAT under these cooling conditions (22, 23, 37). However, subcutaneous abdominal WAT is not recognised as a depot harbouring brite precursors in humans and examination of other depots is warranted.

Cold stimulates BAT activity via the sympathetic nervous system (SNS). SNS activation is reflected by an increase in plasma and urinary noradrenaline levels in cold-exposed subjects (35, 38, 39, 40, 41). Denervation of sympathetic nerves abrogated the cold-induced changes in BAT activity in animals (42), providing strong evidence that SNS plays a pivotal role in mediating the stimulatory effect of cold on BAT.

SNS and catecholamines ► BAT is innervated by the sympathetic nerves (43), under central control of the hypothalamus (1). The SNS regulation of BAT is mediated by the release of noradrenaline (NA) from the nerve endings (1). In addition to locally released noradrenaline, circulating catecholamines also regulate BAT activity. The evidence comes from studies of patients with pheochromocytoma who exhibit widespread uptake of glucose in many depots not normally seen in routine imaging (44, 45). BAT activity correlates with the levels of circulating catecholamines (45, 46). Tissue analysis (47, 48, 49) shows scattered brown adipocytes in omental white fat, strongly suggesting that sustained catecholamine stimulation can induce the browning of WAT in humans.

Noradrenaline regulates brown adipocyte function via beta adrenergic receptors (β -AR) (50). Activation of β -AR triggers a cascade of intracellular changes via the cyclic AMP-phosphokinase A signalling pathway (1, 50). These include transcriptional activation and expression of the *UCP1* gene (50), increased glucose uptake (51) and lipolysis (1), resulting in increase in thermogenic capacity and substrate availability of the tissue.

There are three types of β -ARs (β_1 , β_2 and β_3), all of which are expressed in BAT (52, 53, 54, 55). β_3 is the predominant β -AR in BAT of rodents (54, 55). In contrast, β_1 and β_2 are more abundant than β_3 in human brown adipocytes (52, 53). Propranolol, a beta-blocker with predominant action on β_1 and β_2 and poor efficacy for β_3 , abolishes BAT activity seen on FDG-PET scan in humans (56, 57). This observation suggests that β_1 and β_2 receptors are more important than β_3 in regulating

human BAT activity. However, a recent study found that human BAT activity can be stimulated by a β_3 -AR-agonist (mirabegron) at high dose (58). Mirabegron caused significant cardiovascular effects (58), indicating that the effects observed may not be β_3 exclusive, and may in part be β_1 -AR mediated. Further work is needed to understand the action of each beta-adrenergic receptor subtype in regulating BAT in humans.

Thyroid hormone ► Thyroid hormone plays an important role in the regulation of thermogenesis (59). Thyroid hormone regulates BAT function via peripheral and central mechanisms. Type 2 deiodinase (DiO2), which converts T_4 to bioactive T_3 , is highly expressed in BAT (4). The resultant T_3 directly stimulates UCP1 expression and mitochondrial biogenesis in human adipocytes *in vitro* (60). Rodent studies reveal that T_3 also interacts synergistically with the SNS, by upregulating β -AR expression (61) and noradrenaline signalling (62). Moreover, T_3 acts centrally on the hypothalamus. Intracerebral administration of T_3 causes marked stimulation of BAT function, causing weight loss in rats (63).

Thyroid hormone also stimulates BAT activity in humans *in vivo*. BAT FDG uptake in a hypothyroid subject increased with thyroid hormone replacement and fell on its withdrawal (64). Lahesmaa *et al.* (65) reported a threefold higher BAT activity by FDG-PET-CT in hyperthyroid patients than in healthy subjects and that BAT activity decreased after treatment of hyperthyroidism.

There is evidence that thyroid hormone also induces the browning of WAT in humans. Brown adipocyte-like cells with increased UCP1 expression were found within the periumbilical WAT after therapy with a supraphysiological dose of thyroid hormone (64).

Effects of thyroid hormone are mediated by thyroid hormone receptors (TR). There are two isoforms of TR, TR α and TR β , with different tissue distributions. The cardiac effects of thyroid hormone are predominantly mediated via TR α while its metabolic effects are mediated via TR β (66). In rodent brown adipocytes, the binding of thyroid hormone to TR β upregulates UCP1 expression (67, 68). A TR β -agonist, GC-1, enhances BAT UCP1 content (68), increases metabolic rate, reduces body weight and lowers serum cholesterol level (69) without cardiac adverse effects (70) in animals. If these findings are replicable in humans, selective activation of TR β may be a promising therapeutic strategy to exploit the beneficial metabolic effects of thyroid hormone while avoiding the detrimental cardiac side effects.

Glucocorticoids ► Glucocorticoid excess causes obesity, typically observed in patients with Cushing's syndrome (71). The mechanisms by which glucocorticoids induce adiposity are not well understood but are likely multifactorial. Glucocorticoids enhance the recruitment of preadipocytes to mature white adipocytes and interact with other hormones such as insulin to activate a program of white adipocyte differentiation (72). Glucocorticoids also stimulate appetite centrally (73). The possibility that suppression of BAT function may mediate in part the weight gain induced by glucocorticoids has received little attention in humans.

BAT contains high affinity glucocorticoid receptors (74). Rodent studies show that glucocorticoids inhibit the expression of UCP1 (75, 76). Glucocorticoids also down regulate β -adrenergic receptors (77, 78), reducing tissue responsiveness to adrenergic and cold stimulation (76, 79). Pharmacological inhibition of glucocorticoid action by mifepristone, a glucocorticoid receptor antagonist, activates BAT and reduces body weight gain without change in food intake (80). This observation suggests that the lesser gain in body weight arose from stimulation of energy expenditure. Collectively, these animal data provide strong evidence that glucocorticoids cause weight gain by suppressing BAT activity.

Evidence for a regulatory role of glucocorticoids in human BAT is sparse. Using primary brown preadipocytes derived from supraclavicular fat biopsies, Barclay *et al.* (81) observed that glucocorticoids stimulated the proliferation and differentiation of the cells into mature brown adipocytes, but attenuated the adrenergic-stimulation of brown adipocytes function. These *in vitro* observations indicate that glucocorticoids exert complex effects on development and function of brown adipocytes in humans and that BAT is involved in mediating the obesogenic consequences of glucocorticoid excess. The *in vivo* effect of glucocorticoids on BAT activity in humans has not yet been reported.

Mineralocorticoid ► Mineralocorticoid receptors (MCRs) are expressed in many tissues including adipose tissue (82). The prevalence of metabolic syndrome is increased in primary aldosteronism (83, 84), and correction of mineralocorticoid excess in subjects with primary hyperaldosteronism improves the metabolic abnormality (85), suggesting that mineralocorticoid system may play a role in metabolic regulation.

Rodent studies have provided evidence that aldosterone affects BAT function (86, 87). Aldosterone inhibits the expression and function of UCP1 in a rodent brown

adipose cell line (87, 88). MCR antagonists such as spironolactone and drospirenone induce the emergence of brown adipocyte-like cells within the white adipose tissue, improve glucose tolerance and prevent weight gain in mice fed a high-fat diet (89). These observations provide evidence that mineralocorticoid system regulates BAT in rodents. It is unknown whether mineralocorticoid system exerts a similar effect on BAT in humans.

Sex hormones ► Sex hormones regulate body fat mass (90). Brown adipocytes from male and female rats express sex steroid receptors (91). Animal studies provide some evidence that oestrogen and androgen regulate BAT function. Ovariectomy causes atrophy of BAT depots, an effect reversed by oestrogen replacement (92), indicating that oestrogen promotes BAT mass. The effect of testosterone on BAT is less clear. Some studies have observed negative effect (93, 94) while others have reported positive (95) or no apparent effect (96).

Role of sex hormones in the control of human BAT is suggested by the observation of gender dimorphism in the prevalence of BAT in several retrospective analyses of the scans taken under ambient conditions (25, 27, 97, 98). Women exhibit approximately two to threefold higher prevalence of BAT than men (97, 98). Women also have a higher level of BAT FDG uptake activity and a greater BAT volume than men (27, 97). This striking gender dimorphism may suggest that oestrogen stimulates while androgen suppresses BAT function in humans. However, a gender difference has not been observed in prospective studies employing cold stimulation of subjects (3, 29, 99), suggesting that a difference in sensitivity to environmental temperature might explain the gender difference observed in the retrospective studies. Studies investigating the specific effects of oestrogen and androgen on BAT activity in humans have not yet been reported.

Insulin ► Another candidate regulating BAT is insulin. The role of insulin in the regulation of BAT is complex. The effect seems to be time-dependent. This is supported by the findings from studies of animals rendered insulin depleted by streptozocin treatment. BAT thermogenic response was unaffected acutely but reduced significantly after 7 days of insulin depletion, in parallel with a progressive decline in the tissue weight and the protein content (100). Replacement of insulin restored BAT function and mass over a few days (100, 101, 102). These findings suggest that insulin has a role in maintaining BAT mass and function over time.

Insulin regulates BAT via a direct action on brown adipocytes as well as indirectly via the SNS. Insulin stimulates BAT glucose uptake and lipogenesis in isolated brown adipocytes (103, 104). In rodents, BAT is lost when the insulin receptor is knocked out, suggesting that insulin regulates BAT growth (105).

The regulation of UCP1 expression and thermogenic function in BAT by insulin seems to be mediated via its augmentation of the SNS (106, 107). In streptozocin-treated diabetic mice, insulin replacement restores UCP1 content of BAT with intact SNS. However, denervation of SNS to BAT prevents the restoration of the UCP1 content by insulin (108), indicating that the regulation of UCP1 and BAT thermogenic function by insulin requires sympathoactivation.

In humans, Orava *et al.* (35) have investigated the regulatory role of insulin using the hyperinsulinaemic euglycaemic clamp in a warm environment to minimise the contributing effect of the SNS. Under these conditions, insulin enhanced FDG uptake in BAT by fivefold, an extent similar to that in skeletal muscle. However, the increase in glucose uptake was not accompanied by an increase in thermogenesis nor blood flow to the BAT (35). These findings, in agreement with those from the animal studies, demonstrated that insulin, by itself, increased glucose uptake in BAT without stimulating its thermogenic function. Taken together, it is likely that insulin plays a permissive role in the regulation of BAT mass and function by SNS, especially in the long term.

Dietary factors ► Food intake induces thermogenesis (109). The caloric content of a meal correlates with the thermogenic response (110). The response also varies with the type of nutrient ingested; dietary protein induces higher thermogenic response than fat or carbohydrate (111).

BAT has been proposed to mediate the thermic effect of food. This is based on the findings from animal studies that prolonged overfeeding results in BAT hyperplasia (112, 113) while caloric restriction reduces BAT mass and function (114). Although the issue as to whether BAT contributes to diet-induced thermogenesis is controversial (115), there is evidence suggesting that some dietary supplements exert thermic effects through BAT in humans.

Capsaicin is a component of chilli peppers which stimulates thermogenesis and reduces body fat in both animals (116) and humans (117, 118). Capsaicin stimulates BAT thermogenesis via indirect and direct mechanisms involving vanilloid subtype 1 of transient receptor potential (TRPV1) receptors (119). The indirect

effect is mediated by TRPV1 receptors in the gastrointestinal tract. Activation of gastrointestinal TRPV1 receptors by capsaicin increases central sympathetic stimulation of BAT (120). Capsaicin also acts directly on TRPV1 receptors in adipose tissue (121), increasing expression of brown adipocytes marker genes in differentiating 3T3-L1 pre-adipocytes *in vitro* (122).

Capsinoids are non-pungent counterparts of capsaicin possessing effects similar to capsaicin on energy metabolism, but without the pungent taste (123, 124). The involvement of human BAT in the thermic effect of these compounds was reported by Yoneshiro *et al.* (125). Capsinoids acutely increased resting energy expenditure significantly only in BAT-positive subjects but not in BAT-negative subjects (125). Another dietary ingredient sharing a similar structure to capsaicin is grains of paradise (GP), also known as guinea pepper or alligator pepper. Sugita *et al.* (126) showed that a single oral ingestion of GP extract stimulated energy expenditure in BAT-positive subjects while no effect was observed in BAT-negative subjects. These findings suggest that the thermogenic effects induced by capsinoids and GP are mediated by BAT.

Rodent studies have provided some evidences that the thermogenic response to a meal involves the activation of BAT (112, 113). The question as to whether meal-induced thermogenesis in humans is also mediated by BAT was investigated by Vosselman *et al.* (41) by comparing FDG uptake after a standardised meal to that observed under cool stimulation during the fasted state. They found that BAT activity after a meal was less than that observed during cool stimulation. They also observed greater FDG uptake into muscle after a meal than during cool stimulation (41). Vrieze *et al.* (127) addressed the same question by comparing BAT activity 90 min after a meal to that after an overnight fast. They observed that FDG uptake was significantly lower during the meal study. The results from both these studies are problematic and difficult to interpret because of the competing effects of meal-derived glucose with FDG uptake into BAT and the confounding stimulatory effects of variable concentrations of insulin on glucose uptake into tissues. Thus, evidence for a role of BAT in mediating diet-induced thermogenesis is not established in humans.

Exercise and irisin ► The notion that exercise can induce the browning of WAT emerged after the discovery of a myokine termed irisin. Irisin is a peptide cleaved from fibronectin type III domain containing protein 5 (FNDC5), released by the exercising muscle, and mediates browning of WAT in mice (128). In mice, 3 weeks of exercise

increased *UCP1* mRNA level in subcutaneous inguinal fat depot by more than 20-fold, and led to a twofold rise in plasma irisin level. Intravenous administration of irisin-expressing adenoviral particles also replicated a browning effect, causing a significant reduction in body weight and an improvement in glucose tolerance (128). These findings suggest that the metabolic effects of exercise may have been mediated by irisin through the browning of adipose tissue.

In humans, the evidence supporting an effect of exercise on irisin and adipose tissue browning is inconclusive. Some studies have observed an acute increase in plasma irisin level after exercise (40, 129, 130, 131) whereas others have not (132, 133). With regards to chronic exercise, Bostrom *et al.* (128) reported a twofold rise in plasma irisin level after 10 weeks of endurance training (128) while Norheim *et al.* (131) found an opposite effect after 12 weeks of combined endurance and strength training. In a randomised controlled trial of 26 weeks evaluating the effect of two training programs (aerobic endurance and strength endurance training) on serum irisin level, Hecksteden *et al.* (134) observed no significant effect of either of the exercise regimens. Huh *et al.* (129) and Pekkala *et al.* (133) also found no effect of long-term training on irisin level. Browning of WAT has not been observed after irisin/FNDC5 treatment *in vitro* (40, 135) in humans. A study investigating the effect of FNDC5 treatment on human adipose cells found that *UCP1* mRNA expression increased predominantly in classical brown adipocytes rather than white adipocytes (40).

The discrepancies in the findings may arise from methodological differences in the exercise regimens, timing of blood collection and assays used for measuring irisin. In summary, the effect of exercise on irisin and brown fat genes program remains to be substantiated in humans.

Others ► Several peptides have recently been identified as promoters of browning in humans: fibroblast growth factor-21 (FGF21), bone morphogenic proteins (BMPs) and cardiac natriuretic peptides (ANP and BNP).

FGF21, a member of FGF family, is produced mainly by the liver but is also expressed in adipocytes, skeletal muscle and pancreas. It is a regulator of substrate metabolism and body weight in animals (136). Systemic administration of FGF21 to obese mice lowers blood glucose level, enhances fatty acid oxidation and increases energy expenditure, leading to weight loss (136, 137, 138). Recently, Lee *et al.* (139) reported that human BAT is a source of FGF21. FGF21 was secreted by brown adipocytes from human neck fat and promoted a BAT thermogenic

program in white adipocytes (139). This group also showed in healthy subjects that cold exposure increased plasma FGF21, the levels of which correlated with the thermogenic responses (140). These results suggest that FGF21 may be a mediator of WAT browning induced by cold. These findings are supported by a study in mice in which genetic ablation of FGF21 attenuated the molecular and morphological features of browning induced by cold stimulation (141).

BMPs belong to the transforming growth factor beta (TGF β) superfamily which is involved in the formation of mesenchymal cells (142). BMP4, BMP7 and BMP6 induce the development of brite and brown adipocytes from human primary adipose stem cells (143) and from skeletal muscle precursor cells (144) respectively. Interestingly, the findings from Elsen *et al.* study (143) also suggested that BMP4 is an adipokine secreted by the adipose stem cells, acting as an autocrine regulator of brown adipogenesis.

Natriuretic peptides play a role in food intake and energy expenditure in mice (145). A possible regulatory role of ANP and BNP in human brown fat was investigated by Bordicchia *et al.* (146) using differentiated human multipotent adipose-derived stem cells culture, the authors found that the peptides induced the expression of brown fat genes, which was accompanied by an increase in oxygen consumption, an effect potentiated by β -agonists.

These findings highlight the complex interactions between BAT and several systems, regulating energy homeostasis.

Metabolic significance of BAT in humans

While it is well established that BAT contributes significantly to energy balance in animals (147, 148, 149), evidence that BAT is metabolically significant in humans has only emerged recently.

BAT and adiposity

Observational studies ► Several large observational studies have reported that active BAT is more frequently detected in lean than in obese individuals (28, 97, 98, 150, 151). BAT mass and activity negatively correlates with BMI (27). These studies are limited in being retrospective, with results drawn from scans performed under non-standardised ambient conditions. Subsequent prospective studies have however confirmed a negative relationship between the BAT activity and adiposity under standardised scanning conditions (3, 29, 99, 152) (Fig. 1). Some studies

Figure 1

Relationship between BMI and BAT activity measured on FDG-PET-CT scans after acute cold exposure (Reproduced with permission from van Marken Lichtenbelt WD, Vanhommerig JW, Smulders NM, Drossaerts JM, Kemerink GJ, Bouvy ND, Schrauwen P & Teule GJ. Cold-activated brown adipose tissue in healthy men. *New England Journal of Medicine* 2009 **360** 1500–1508).

have employed a precooling protocol aimed at enhancing BAT function maximally without inducing shiver (3, 99). These observations suggest that impaired BAT function may predispose to obesity in humans. However, cause and effect cannot be determined from association studies.

Interventional studies ► Several groups have addressed the metabolic significance of BAT in adult humans by determining i) whether resting energy expenditure is increased when BAT is activated (33, 34, 35, 38, 39, 153),

ii) whether prolonged BAT activation results in a loss of body fat that is related to the degree of BAT activation (24). BAT 'positive' subjects show a greater increase in resting EE during cold stimulation than BAT 'negative' subjects. The increment in energy expenditure from cold stimulation is termed cold-induced thermogenesis (CIT). CIT correlates with BAT activity (153). The differences in CIT between BAT-positive and BAT-negative subjects ranged from 120 to 368 kcal per day. Table 1 summarises the studies reporting the contribution of BAT to energy expenditure in humans on cold stimulation.

It is estimated that on average, ~50 g of BAT is present in adult humans and, when continually activated, contributes ~170 kcal to daily energy expenditure (34, 35). Energy deficit of 500 kcal per day equates to 0.4 kg of fat loss per week, based on the energy density of fat (9 kcal per 1 g). Therefore, an increase in energy expenditure of 170 kcal per day from continuous activation of BAT translates into ~7 kg of fat loss in 1 year, assuming no change in energy intake. However, given the complex feedback mechanisms of energy metabolism and the lack of a realistic way for constantly stimulating BAT, caution should be exercised in extrapolating the extent of clinical benefit.

The evidence that activating BAT can indeed control body fat comes from a study in which subjects with low or undetectable BAT activity at baseline were randomly assigned to undergo 6 weeks of intermittent cold exposure at 17 °C for 2 h daily or to continue their usual daily living (24). Those assigned to the cold intervention lost 0.7 kg of mean body fat in 6 weeks in parallel with an ~1.5-fold increase in BAT activity whereas those who continued with usual daily living had no change in BAT activity or body fat (Fig. 2) (24).

Taken together, these controlled interventional studies provide unequivocal evidence that BAT in human is metabolically significant.

Table 1 Summary of studies reporting detected amount of BAT and associated CIT after acute cold stimulation in adult humans.

References	Number of subjects	Cooling temperature (°C)	BAT amount	CIT (kcal/day)
(33)	6	18	Mean 168 ml	Mean 2000
(34)	9 with high BAT 15 with low BAT	15	Mean 59.1 g Mean 2.2 g	Mean 237 Mean 39
(35)	19 BAT positive 8 BAT negative	17 ± 1	Mean 34 g	Mean 287 ^a Mean 167 ^a
(38)	24	19	Mean 63 ml	Mean 88
(39)	10	14	Median 15 ml	Mean 79
(153)	6 BAT positive 7 BAT negative	19	No data	Mean 410 Mean 42

^aConverted from Megajoule using 1 MJ=239 kcal, normalised for fat free mass.

Figure 2

Effect of chronic cold exposure on BAT activity, CIT and body fat mass in humans. (A) BAT activity plotted in standardised uptake value (SUV), measured on FDG–PET–CT scans before and after 6 weeks of daily cold exposure. (B) CIT expressed in kcal/day in subjects who underwent the cold intervention (cold group) and those who continued their usual daily living (control group). (C) Change in body fat mass from baseline after 6 weeks in the cold group and control group. Expressed in mean $\pm$ s.e.m.; $*P < 0.05$ (Adapted with permission from (24)).

BAT and substrate metabolism

BAT utilises free fatty acids and glucose as metabolic fuels (1). It is therefore conceivable that activated BAT may induce beneficial changes in metabolic profile, by reducing blood triglyceride and glucose levels. In rodents, chronic BAT activation by cold-acclimatisation markedly reduces circulating triglyceride and glucose concentrations (154). Compared to rodents, the amount of BAT corrected for body mass is much lower in humans and the absolute ability of BAT to clear metabolic substrates from the circulation could be less in humans.

In humans, cross-sectional studies have reported that blood glucose levels of BAT-positive subjects are significantly lower than that of BAT-negative individuals (28, 29, 97, 98). The metabolic significance cannot be deduced from these studies because it is not clear whether the glucose difference is a cause or consequence of reduced BAT activity.

Convincing evidence that activation of BAT in humans can improve the metabolic status comes from recent longitudinal studies reporting improvements in glucose metabolism in subjects undergoing intermittent cold stimulation (21, 22, 37). The participants underwent a few hours of cooling daily for varying periods (10 days (37)–1 month (21, 22)). Stimulation of BAT function by cold over time was associated with a significant fall in circulating glucose (21) and improvements in insulin sensitivity (22, 37). Table 2 summarises the studies addressing the role of BAT in adiposity and substrate metabolism in humans. Interestingly, one study (37) found enhanced skeletal muscle GLUT4 translocation after cold acclimatisation and attributed the improvement in glucose metabolism mainly to the skeletal muscle glucose uptake. However, parallel tissue analysis was not performed in BAT. The intensity of FDG uptake in the cold-acclimatised BAT was comparable to that in skeletal muscle on PET scan (37), indicating that BAT also contributed to the glucose clearance (Fig. 3). Moreover, another study (22) reported no change in skeletal muscle GLUT4 expression after cold acclimatisation. Orava *et al.* (35) have also showed that the avidity of glucose uptake in BAT during cold stimulation in humans is comparable with that of insulin-stimulated skeletal muscle. Thus, on balance, the evidence supports a significant and beneficial role of BAT in glucose metabolism.

Future perspectives

An understanding of the physiology of BAT in humans is evolving. Development of therapeutic approaches to

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Table 2 Summary of cold acclimatisation studies addressing the role of BAT in adiposity and substrate metabolism in humans.

References	Number of subjects	Acclimatisation protocol	Δ BAT activity from baseline	Δ Metabolism from baseline
(24)	22 randomised to cold intervention or usual living (control)	17 °C, 2 h/day for 6 weeks	58% increase (no significant change in the control group)	5.2% or 0.7 kg decrease in body fat mass (no significant change in the control group)
(21)	6	10 °C, 2 h/day for 4 weeks	45% increase	6.2% decrease in plasma glucose
(22)	5	19 °C overnight for 1 month	54% increase	> 50% increase in insulin sensitivity
(37)	10	14–15 °C for 10 days	~50% increase	43% increase in insulin sensitivity

harness BAT will require a comprehensive appreciation of the pathways regulating BAT function and mass and of the implication of altered BAT function and mass on energy metabolism in humans.

Cold exposure, despite being a powerful promoter of BAT mass and function, resulting in metabolic benefits, is unlikely to be widely adopted by individuals at the expense of personal comfort. However, controlling indoor temperature of buildings to allow a modest cooling of occupants is a realistic and acceptable public health measure at a population level to help tackle the obesity pandemic. Studies on its effectiveness are warranted.

An alternative way to mimic the stimulatory effect of cold is activation of the sympathetic β -adrenergic system. The results of studies using non-specific sympathomimetics to activate human BAT (155, 156) so far have not been promising with only one study showing BAT-enhancing effect using high dose ephedrine (157). This likely reflects the dose limitation of nonspecific sympathomimetics from adverse cardiac effects. The positive result from a recent study of a β_3 -selective agonist, mirabegron (58), brings some hope for possible pharmacological activation of BAT although the drug appears to induce cardiovascular side effects with the dose used. The potential of more highly selective β -adrenergics devoid of β_1 cardiac risks merits further exploration. One such agent is formoterol. Formoterol is a long-acting, potent, highly selective β_2 -agonist, approved for treatment of bronchial asthma and obstructive airway diseases. In an open label study of healthy individuals, oral formoterol stimulated fat oxidation and augmented resting energy expenditure by 15% without inducing tachycardia in therapeutic doses (158). These metabolic changes imparted by formoterol are similar to the changes expected from the activation of BAT, suggesting that formoterol may be BAT-activating.

The discovery of other novel, non-adrenergic pathways such as FGF21 and irisin and thyroid hormone analogue is exciting. It remains to be tested whether this could lead to new therapeutic utility in humans.

Figure 3

Effect of cold acclimatisation on FDG uptake in BAT and insulin sensitivity in humans. (A) FDG uptakes expressed as SUV_{mean} measured in the supraclavicular BAT, upper-body skeletal muscle (SM), subcutaneous WAT (WAT_{subc}), visceral WAT (WAT_{visc}), liver and brain on FDG-PET-CT scans taken before and after 10 days of cold acclimatisation. (B) Glucose infusion rate (GIR), corrected for body weight, during hyperinsulinemic euglycaemic clamp, before and after the cold intervention. Expressed in mean $\pm$ S.E.M.

* $P < 0.05$ (Adapted with permission from (37)).

In addition to its role in protection against cold, BAT also protects against diet-induced obesity in small mammals through mediation of the thermic effect of food (147, 148, 149). Whether this is true in humans is not yet established, but the findings of the studies using capsinoids (125, 126) support the contribution of BAT to the thermogenic effect of food supplements. Macronutrients differ in their thermogenic potential (111), and whether this is explained by differences in BAT activation awaits further investigation.

Finally, it is desirable to develop non-invasive techniques that can distinguish changes in BAT function from mass. This is important for developing therapeutic strategies; for instance, agents that affect only BAT mass should be complemented by those that activate BAT function, in order to achieve optimal therapeutic benefits. Currently, non-invasive assessment of BAT function is confined to PET-CT scan. In addition to the radiation safety constraints, FDG-PET-CT cannot provide information on BAT function during meal studies. Preliminary results on the use of thermal imaging (infrared thermography, IRT) in detecting BAT activity in humans are promising. IRT detects a higher temperature on the skin overlying BAT in the supraclavicular region compared with the skin overlying other area (159, 160, 161). This calls for further study to develop the use of IRT in assessing changes in BAT activity.

With regards to quantifying BAT mass, a few encouraging reports on the application of magnetic resonance imaging have emerged, albeit in a small number of adult humans (162, 163). The technique is based on the notion that BAT and WAT display different water-to-fat ratios or signal intensities, with the BAT having a higher water-to-fat ratio (162, 163). More research is warranted to verify its reliability and reproducibility.

Conclusion

Research in the past few years have substantiated the concept that BAT in adult humans is regulated by multiple mechanisms and is metabolically significant. This has given insights into its physiology in humans providing the basis for exploiting its thermogenic capacity in management of obesity and metabolic diseases.

Declaration of interest

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

Funding

M Thuzar is supported by a Research Fellowship from the Princess Alexandra Hospital Research Support Scheme.

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Received 15 December 2015

Revised version received 25 January 2016

Accepted 9 February 2016