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Human Brown Adipose Tissue: What We Have Learned So Far

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Brown adipose tissue (BAT) is a unique tissue that is able to convert chemical energy directly into heat when activated by the sympathetic nervous system. While initially believed to be of relevance only in human newborns and infants, research during recent years provided unequivocal evidence of active BAT in human adults. Moreover, it has become clear that BAT plays an important role in insulin sensitivity in rodents and humans. This has opened the possibility for exciting new therapies for obesity and diabetes. This review summarizes the current state of research with a special focus on recent advances regarding BAT and insulin resistance in human adults. Additionally, we provide an outlook on possible future therapeutic uses of BAT in the treatment of obesity and diabetes.

During recent years, brown adipose tissue (BAT) has regained the attention of biomedical research. While previously thought to be negligible in adult humans, its presence and metabolic activity in this population was unequivocally demonstrated by several articles published in 2009 (1–4). BAT is a thermogenic tissue that allows small mammals to keep body core temperature constant at cold ambient temperatures without shivering. It differs markedly from white adipose tissue (WAT). While white adipocytes mainly consist of a single large lipid droplet and possess only a few mitochondria, brown adipocytes contain multiple lipid droplets per cell and are packed with mitochondria. BAT is densely innervated by the sympathetic nervous system (SNS) and is highly vascularized. In rodents and human infants, a major depot is found between the scapulae, and more depots exist along the great vessels and in the retroperitoneum (5,6). In short, the unique thermogenic capacity of the tissue is due to its high content

of mitochondria and the expression of uncoupling protein 1 (UCP1) (Fig. 1). UCP1 is a proton channel within the inner mitochondrial membrane that, upon activation, short circuits the respiratory chain, thereby dissipating chemical energy as heat.

Cold receptors in the skin register cool ambient temperatures and relay the signal to hypothalamic centers that regulate body temperature (7). The efferent signal to BAT is conveyed by the SNS and its transmitter norepinephrine (8), which activates β 3-adrenergic receptors and rapidly stimulates intracellular lipolysis. The released fatty acids fuel the respiratory chain and open UCP1, which dissipates the mitochondrial proton gradient as heat (9). In addition to its thermoregulatory function, BAT has been described to be activated by certain nutrients and high-calorie diets. In a classic experiment, Rothwell and Stock (10) showed that high-calorie diets (so-called “cafeteria diets”) led to the activation of BAT in rodents and thus contributed to diet-induced thermogenesis. It was speculated that this mechanism would protect the rodents, at least in part, from diet-induced obesity (10). While the contribution of BAT to cold-induced thermogenesis is generally undisputed, the concept of diet-induced thermogenesis as a function of BAT has been challenged in recent years because of conflicting study results (11–13).

Stimulation of β 3-adrenergic receptors not only activates BAT thermogenesis in the short term, but also increases mitochondrial biogenesis and the expression of UCP1, and leads to growth of the tissue. Long-term adrenergic stimulation induces brown adipocytes in what otherwise were WAT depots, which contribute to thermogenesis (5). Thyroid hormone increases sensitivity toward adrenergic stimuli, and a high expression level of type 2 deiodinase (DIO2) is

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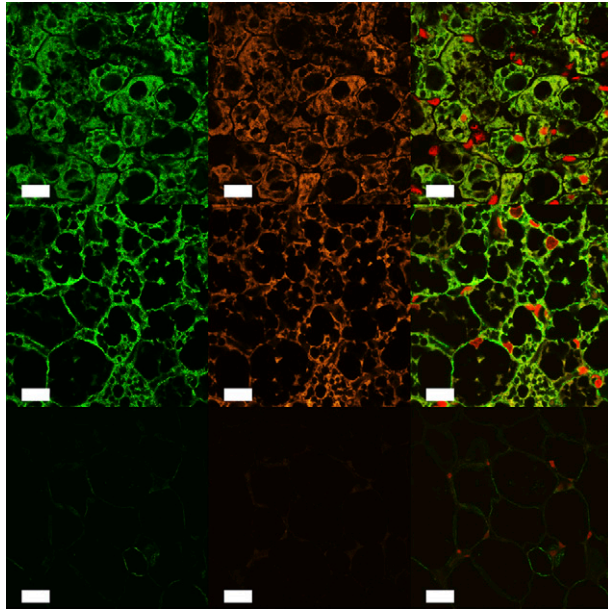


Figure 1—Immunohistochemistry of human interscapular (top row), periaxillary (middle row), and subcutaneous WAT (bottom row), demonstrating the high number of mitochondria and UCP1 in BAT as opposed to WAT. UCP1 is stained green, and cytochrome c oxidase is stained orange. The merged image (right column) shows the perfect colocalization of the two proteins, and nuclei are stained red.

one of the distinctive features of BAT. DIO2 converts thyroxine (T4) into triiodothyronine (T3), which has a considerably higher affinity for the thyroid hormone receptors (THRs) and is crucial for the thermogenic function of BAT (14).

While the physiology and function of the tissue were widely elucidated during the 1970s and 1980s, remarkable discoveries regarding the molecular development and differentiation of the tissue and its prevalence in human adults were made during the last decade.

ORIGINS OF BAT AND TWO TYPES OF BAT

Given the high amounts of intracellular lipids, a common lineage for white and brown adipocytes was suspected. Surprisingly, classic BAT was shown to develop from the central dermomyotome, which also gives rise to dorsal dermis and epaxial muscle (15). This finding was corroborated by lineage-tracing studies demonstrating that myocytes and brown adipocytes, but not white adipocytes, derive from precursor cells expressing *Myf5*, a gene encoding the myogenic regulatory factor MYF-5. Chronic adrenergic activation in cold-exposed rodents leads to the development of brown adipocytes in WAT depots (16), which contribute to the thermogenic capacity (17). While the animals initially need to rely on shivering in order to maintain normal body core temperature, BAT expansion leads to a gradual increase in nonshivering thermogenesis (5). These induced brown adipocytes have been called “recruitable,” “brite” (brown in white), or “beige” adipocytes, and the latter

currently is the most widely used term. Beige adipocytes exhibit the same cell morphology as classic brown adipocytes and appear to be functionally equal (18), but do not originate from *Myf5*-expressing progenitors (19) and exhibit unique gene signatures that differ from those of classic brown adipocytes found in the interscapular BAT (20).

Of the many transcription factors involved in BAT differentiation, two deserve special attention: peroxisome proliferator-activated receptor γ coactivator-1 α (PGC-1 α) and PR domain zinc finger protein 16 (PRDM16).

PGC-1 α stimulates mitochondrial biogenesis and oxidative metabolism, and is crucial for the adaptations to cold exposure (21,22). Its expression is considerably increased by cold exposure, and its knockout in brown preadipocytes considerably blunts the increase in UCP1 upon adrenergic signaling (23). PRDM16 is a zinc finger protein that was shown to be preferentially expressed in brown adipocytes compared with white adipocytes. It acts as a transcription factor in conjunction with PGC-1 α . The overexpression of PRDM16 in preadipocytes from WAT depots led to a full brown adipocyte phenotype (24), and its abrogation in preadipocytes from classic BAT depots causes the loss of the brown adipocyte phenotype and differentiation into myocytes (19). Moreover, it is also expressed in the subcutaneous WAT depot in which adrenergic stimuli can give rise to beige adipocytes, but not in epididymal WAT depots, which are resistant to “browning” (25).

(RE)DISCOVERY OF BAT IN HUMAN ADULTS

The relevance of BAT for human newborns and infants had been acknowledged and undisputed for decades (26,27), and even the presence of BAT in human adults was demonstrated in autopsy studies more than 40 years ago (28). However, even though UCP1 had already been shown to be expressed in human adult BAT in the 1980s and 1990s (29–31), its functional role was less clear-cut. Because of findings in rodent models and observations of increased energy expenditure in response to β 3-adrenergic agonists (32) and different responses to cold in lean versus obese humans (33), it was speculated that decreased BAT mass and activity might be implicated in the development of obesity and type 2 diabetes (34). At that time it was, however, not possible to unequivocally prove the metabolic activity in human adults (35–37).

The increasing clinical use of 2-deoxy-2-[fluorine-18]fluorodeoxyglucose integrated with computed tomography (^{18}F -FDG PET/CT) scanning for the detection and surveillance of cancer led to the observation of bilateral tracer uptake in supraclavicular area fat in a proportion of patients, especially during the cold season (38), which prompted the assumption that this tissue might in fact be active BAT (39). In 2009, three articles (1,3,4) published in parallel in the *New England Journal of Medicine* unequivocally demonstrated the presence of functional BAT in human adults. Cypess et al. (1) extensively reviewed almost 2,000 routine [^{18}F]-FDG PET/CT scans and identified bilateral tracer uptake in >5% of patients. In a different group of patients

undergoing surgery in the cervical region, they could identify UCP1⁺ multilocular adipocytes in the supraclavicular region (1). Using a different approach, van Marken Lichtenbelt et al. (3) demonstrated that the characteristic [¹⁸F]-FDG uptake in the supraclavicular region occurred in response to mild cold exposure in a group of healthy volunteers and was associated with an increase of thermogenesis, as determined by indirect calorimetry. A similar approach was used by Virtanen et al. (4), who additionally took biopsy specimens from the supraclavicular region, which had been PET positive in response to cold exposure and could unequivocally prove the BAT identity of this tissue. In the following years, these findings have been corroborated by numerous observational and prospective studies (40–48).

PET/CT imaging revealed active BAT in adult humans in the supraclavicular area, the retroperitoneum, and along the aorta, with the main depot in the supraclavicular area (2). In line with previous autopsy studies (28), there was no BAT depot in the interscapular area of adults. In analogy to the findings in animal models, it was speculated that the depots found in adult humans are of the beige BAT type and might thus not be equivalent to the classic interscapular BAT depot (49,50). Indeed, recent studies (51,52) using repetitive cold exposure demonstrate high plasticity of BAT in human adults. Additional evidence for a browning phenomenon comes from the fact that BAT was more likely to be found in the retroperitoneal tissue of patients undergoing adrenal surgery during the cold season (53).

Using a combination of MRI and molecular analysis, we could demonstrate that an interscapular BAT depot is found in human infants and consists of classic brown adipocytes, while the supraclavicular and retroperitoneal depots found in human adults show a molecular signature that is consistent with the beige type of brown adipocytes (54). Additionally, two other groups of investigators (55,56) independently identified both types of brown adipocytes in different depots of cervical BAT in human adults and thus corroborated our finding that human adults exhibit two types of BAT. Recently, a role for alternatively activated macrophages and eosinophils in the development of functional beige BAT has been reported in mice (57,58). Additionally, a population of brown adipocyte progenitors was identified in skeletal muscle in both mice (59) and humans (60,61). These cells could be differentiated into mature brown adipocytes by stimulation with bone morphogenetic protein (BMP) 7, thus presenting a potential target for pharmacological intervention in humans.

The presence of different brown adipocyte cell types in humans is conceptually important since they can possibly be targeted by different pharmacologic stimuli.

THE ROLE OF BAT IN INSULIN RESISTANCE AND OBESITY DIABETES IN HUMANS

The details of important studies on the role of BAT and obesity and insulin resistance in humans are given in Table 1.

Retrospective studies (1,42,46) using PET/CT data from large cohorts of humans demonstrated an inverse relationship between the presence of active BAT and obesity. However, the prevalence of BAT was low in these studies (5–10%) because BAT activity was not stimulated by cold exposure. Carefully designed studies (2,3) using mild cold exposure not only demonstrated active BAT in up to 95% of subjects, but could also corroborate the inverse association with BMI. Additionally, BAT prevalence and activity were much lower in patients with severe obesity (62), and BAT activity increased in this group after weight loss induced by bariatric surgery (63). Given the association of obesity and insulin resistance, it is not surprising that BAT activity was inversely associated with diabetes and fasting glucose level in observational studies (1,42,46).

It seems obvious that an energy-expending tissue—similar to exercising muscle—might have the potential to both counteract obesity and ameliorate insulin resistance. This notion is supported by several transgenic mouse models that exhibit increased BAT activity. Direct overexpression of UCP1 in WAT of C57BL/6J mice prone to obesity reduced body weight (64). Overexpression of the transcription factor FOXO2 in adipose tissue induced brown adipocytes in WAT depots. Transgenic animals were resistant to diet-induced obesity (65), intramuscular fatty acyl-CoA deposition, and diet-induced hepatic insulin resistance (66). While the interscapular BAT depot is a stable trait in rodents, the capacity to induce brown adipocytes in WAT depots when stimulated by cold or adrenergic agonists varies significantly between genetically different mouse strains. Interestingly, this ability is lowest in strains susceptible to obesity and diabetes (17,67,68). What exactly causes these variations and whether these observations can be translated to humans is yet to be revealed. Currently, few studies have investigated the association of BAT and genetic variation in humans. Single nucleotide polymorphisms in the human UCP1 and ADRB3 (adrenergic receptor 3 β) genes were associated with increased age-related decline of BAT activity, as measured by FDG PET in healthy Japanese volunteers (69) and increased visceral fat in the same population (70).

Human BAT expresses high levels of the glucose transporter GLUT4, and insulin stimulates glucose uptake into the tissue to a similar degree as in muscle. In analogy to the noninsulin-dependent glucose uptake in exercising muscle, cold exposure leads to higher glucose uptake in BAT than does insulin stimulation (71). Consistent with these findings, a short-term mild cold stimulus increased insulin sensitivity in healthy volunteers with active supraclavicular BAT (72). Conversely, both the cold- and the insulin-activated glucose uptake into BAT were significantly lower in obese humans than in control subjects of normal weight (73). Decreased BAT activity could be a consequence of obesity or insulin resistance or an underlying metabolic phenotype. Interventional studies aiming to increase BAT mass and activity in humans, however, demonstrated increased glucose disposal and

Table 1—Important studies investigating BAT and its relevance for obesity and insulin sensitivity/glucose metabolism in humans

Study	Design	Main findings
Lee et al. 2010 (46)	Retrospective analysis of 2,934 PET/CT scans; no cold stimulation used	Prevalence of BAT in 8.5% of all scans; detection of BAT inversely associated with age, BMI, and fasting glucose level
Ouellet et al. 2011 (42)	Retrospective analysis of 4,842 PET/CT scans; no cold stimulation used	Active BAT in 6.8% of patients; highly significant inverse association with outdoor temperature, age, BMI, and diabetes status
Orava et al. 2011 (71)	Prospective controlled study in 27 healthy volunteers (20 male, 7 female), using mild cold exposure (2 h at 17°C in light clothing) to stimulate BAT	Glucose uptake induced 12-fold in BAT by cold; induced fivefold in BAT by insulin
Ouellet et al. 2012 (40)	Prospective controlled study in 6 healthy men using cold exposure by liquid-cooled suit and PET/CT	Increased glucose and nonesterized fatty acid uptake in BAT upon cold exposure; reduction in BAT triglyceride content after cold-induced thermogenesis, indicating consumption of intracellular lipids
Vijgen et al. 2012 (63)	Prospective observational study in 10 patients undergoing bariatric surgery for morbid obesity (BMI 42 kg/m ² at baseline, 30 kg/m ² after weight loss); individual cooling by cold air, BAT detection by FDG PET	Active BAT in 2 of 10 patients at baseline and in 5 of 10 patients after weight loss; BAT activity increased significantly after weight loss in those positive for BAT at baseline
Orava et al. 2013 (73)	Prospective study in 27 lean (BMI 22.7 kg/m ²) and 36 obese (BMI 38.1 kg/m ²) individuals; mild cold exposure (2 h at 17°C in light clothing) to stimulate BAT PET/CT	BAT glucose uptake to cold and insulin stimulation were twice as large in lean as in obese subjects; weight loss (15% of body weight) did not increase BAT volume or activity
Yoneshiro et al. 2013 (52)	Prospective controlled study in 51 healthy men (24 years of age, BMI 22 kg/m ²); 22 subjects without detectable BAT at baseline underwent repeated cold exposure (2 h/day at 17°C) or control for 6 weeks; BAT activity was assessed by FDG PET	Increased BAT activity and decreased body fat mass after “cold training”
Chondronikola et al. 2014 (72)	Prospective controlled study in 12 male volunteers (7 BAT positive, 5 BAT negative), who underwent controlled cold exposure for 8 h using a water-cooled vest and blanket PET/CT	Cold exposure increased resting energy expenditure, whole-body glucose disposal, plasma glucose oxidation, and insulin sensitivity in BAT-positive subjects
Lee et al. 2014 (74)	Prospective study in 5 healthy men; cold acclimatization by exposure to a controlled temperature environment for 1 month, with mild cold exposure to air at 19°C; PET/CT	No change in cold-induced thermogenesis, but increased insulin sensitivity after cold acclimatization
Blondin et al. 2014 (51)	Prospective study in 6 healthy men; daily exposure to cold at 10°C using liquid-conditioned suit for 4 weeks, 2 h/day; PET/CT	45% increase in BAT volume, 2.2-fold increase in BAT oxidative metabolism, and 37% increase in BAT glucose uptake

insulin sensitivity after repetitive cold exposure designed to increase BAT activity (51,74). Together with data obtained from rodent models, this evidence suggests that reduced BAT activity contributes to the development of obesity and insulin resistance.

Apart from effects on glucose metabolism, experiments in rodents also provide evidence for beneficial effects of

active BAT on lipid metabolism. It should be pointed out that intracellular lipids are the main substrate of BAT thermogenesis during short-term cold exposure. In mice, the uptake of triglyceride-rich lipoproteins in BAT increased upon cold exposure through upregulation of lipoprotein-lipase and CD36 in the endothelium of its dense vasculature (75), thus contributing to triglyceride

homeostasis. Ouellet et al. (40) could demonstrate consumption of intracellular lipids and increased uptake of fatty acids into cold-activated BAT in healthy human volunteers. Interestingly, in a mouse model of human-like lipoprotein metabolism, metformin was able to enhance the clearance of VLDL particles in BAT and also to increase BAT mitochondrial content and activity, suggesting potential implications of BAT in the actions of this widely used antidiabetic drug (76).

In summary, a role for BAT in glucose and lipid metabolism, and thus in the pathogenesis of the metabolic syndrome and type 2 diabetes, is supported by an increasing amount of evidence. It might thus constitute a valuable therapeutic target with which to treat metabolic disease in humans.

BAT AS A POTENTIAL DRUG TARGET TO TREAT OBESITY AND DIABETES IN HUMANS

Obesity and its associated diseases lead to enormous human suffering and health care–related costs. Increasing energy expenditure by the expansion and activation of

BAT could potentially help reduce body weight and improve insulin sensitivity, especially in those who are not able to increase energy expenditure through muscular activity. Figure 2 summarizes the physiological pathway leading to BAT activation and expansion, effects on metabolism, and potential pharmaceutical approaches.

The most straightforward approach would be to use catecholamine derivatives to stimulate BAT. While norepinephrine and related substances cannot be used in the therapeutic setting because of cardiovascular side effects, specific β_3 -adrenergic receptor agonists were successfully used in rodents to increase BAT activity, and ameliorate obesity and insulin resistance (77,78). In lean men, a β_3 -adrenergic agonist increased fat oxidation and insulin sensitivity, but it failed to alter energy expenditure after 8 weeks of use (79). Another β_3 -adrenergic receptor agonist could increase resting energy expenditure in the short term in healthy obese men (80). Extended treatment over 28 days reduced plasma triglyceride levels, but thermogenesis or body weight did not change (81). However, these studies were performed before the presence of

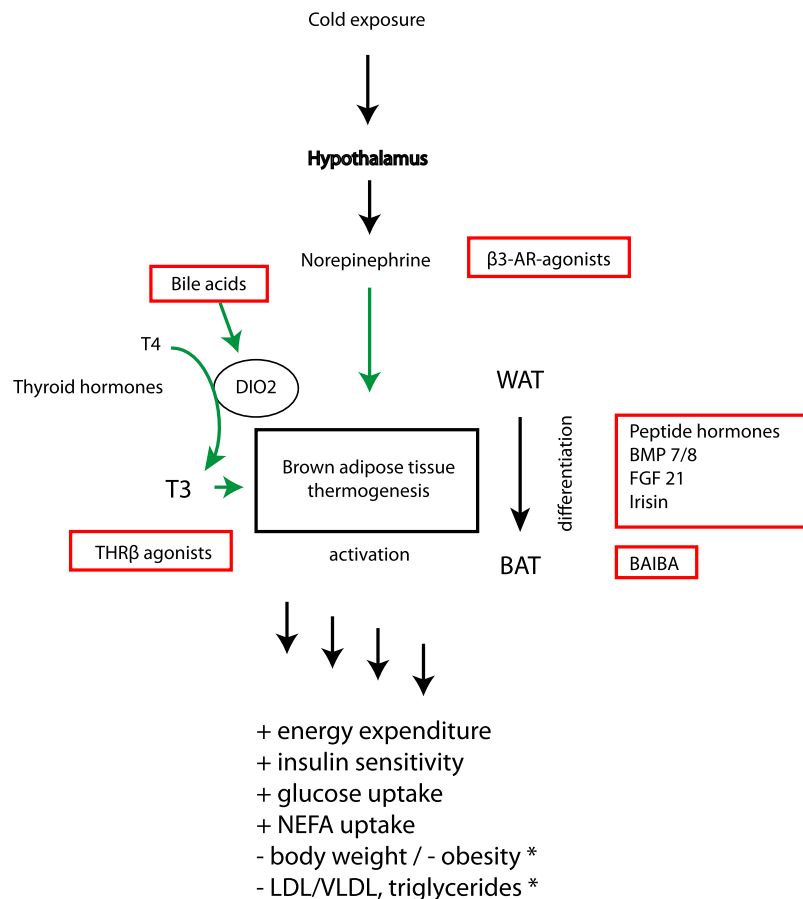


Figure 2—BAT is activated upon cold exposure via the SNS. Thyroid hormones play an important role in the upregulation of thermogenesis, and their local activity is dramatically increased by DIO2. Several peptide hormones have been demonstrated to induce differentiation of beige BAT in WAT depots in mice. Potential pharmaceutical substances for BAT activation are placed next to their physiological targets and framed in red. Activation of BAT leads to beneficial effects on whole-body metabolism; the effects, denoted by *, have currently only been demonstrated in animal models. β_3 -AR, β_3 -adrenergic receptor; FGF, fibroblast growth factor; NEFA, nonesterified fatty acid.

active BAT in adult humans was acknowledged, and therefore BAT activity was not assessed directly.

Using [^{18}F]-FDG PET/CT scanning, Cypess et al. (82) recently demonstrated that the short-term increase in energy expenditure due to a selective β_3 -adrenergic receptor agonist was due to BAT activity in healthy human volunteers. The equivocal results seen in previous studies might have been caused by the fact that BAT was not present in all studied individuals or by downregulation of adrenergic receptors in response to their long-term stimulation. The indirect sympathomimetic agent ephedrine was also shown to stimulate BAT thermogenesis in the short term in lean men (BMI 22 kg/m², $n = 9$), but not in obese men (BMI 36 kg/m², $n = 9$) (83). However, another study (84) in 10 healthy volunteers (mean BMI 24 kg/m², 4 males/6 females) could not detect an ephedrine-mediated increase of BAT activity. Importantly, long-term administration of ephedrine over 4 weeks reduced BAT activity even in healthy young men (mean BMI 24 kg/m², $n = 23$) (85), possibly as a result of adrenergic receptor downregulation.

BAT thermogenesis is markedly increased in humans who have hyperthyroidism (86). Supraphysiologic doses of thyroid hormones are associated with serious side effects, prohibiting their therapeutic use to reduce body weight. However, adverse symptoms such as tachycardia are mainly related to the activation of THR α . Selective targeting of THR β , on the other hand, conferred resistance to diet-induced obesity by increased BAT activity and was well tolerated in rats (87,88). Additionally, in mice, bile acids have been shown to increase DIO2 expression by binding to TGR5, a G $_s$ -protein-coupled transmembrane receptor, and thus to increase the local availability of triiodothyronine and energy expenditure (89,90). Data on BAT activity from human studies with THR agonists or bile acid derivatives are currently not available.

In recent years, numerous efforts have been made to identify substances and hormones that are able to expand and activate BAT, and a range of different hormones and molecules has been described, mainly in animal models. The overexpression of PGC-1 α in muscle led to the discovery of a novel peptide hormone secreted by muscle in response to exercise, which increases the number of beige adipocytes in murine WAT depots. Exogenous administration of this peptide hormone, which was named "Irisin," led to the browning of subcutaneous adipose tissue depots in mice (91). Irisin expression was shown to be correlated to exercise in humans in some, but not all, studies (92). A recent article (93) convincingly demonstrated, however, that shivering leads to an increase in Irisin levels in humans, and it might thus provide a link between shivering and nonshivering thermogenesis in response to cold. While the receptor for Irisin still needs to be discovered, it was recently shown that Irisin acts on adipocytes through mitogen-activated protein kinase p38 and the extracellular signal-related kinase-mitogen-activated protein kinase pathway (94). On the same lines, another

peptide named meteorin-like (Metrl) was identified as a factor induced in muscle by exercise and in adipose tissue by cold exposure (57). Its administration increased energy expenditure and improved glucose tolerance in mice through an eosinophil-dependent increase in interleukin-4 concentrations, resulting in adipose tissue macrophage activation.

β -Aminoisobutyric acid (BAIBA) is another substance secreted by myocytes overexpressing PGC-1 α , and its plasma levels rise in response to exercise training in humans. The administration of BAIBA increased the expression of BAT-specific genes in WAT depots of mice, and peroxisome proliferator-activated receptor α was identified as a potential target. Energy expenditure in BAIBA-treated animals was higher than in placebo-treated animals, and insulin sensitivity was improved (95). BMP7 and BMP8B (96–98) and fibroblast growth factor 21 (93,99) have also been described as peptide hormones influencing BAT function and development in mice. Using a different approach, a recent screen for small molecules able to transform human white adipocytes to brown adipocytes in vitro revealed that the inhibitors of Janus kinase, tofacitinib and R406, consistently increased UCP1 expression and mitochondrial gene expression (100).

Analogues of Irisin and other peptide hormones, as well as small molecules might be promising pharmacologic options to expand and activate BAT, but their efficacy in humans still needs to be tested.

On a physiological basis, BAT could also be expanded and activated by mild cold stimuli. Repeated intermittent exposure of healthy Japanese men without detectable BAT at baseline (mean BMI 22 kg/m², $n = 12$) to mild cold (17°C for 2 h/day) during 6 weeks recruited and expanded BAT, and led to a decrease in body fat mass (52). In a similar experiment, the reduction of ambient temperature from 24°C to 19°C resulted in a mean cold-induced increase in energy expenditure of 5%, and of BAT activity of 10% in 24 healthy volunteers (101). These environmental strategies are backed by the seasonal variation of BAT prevalence and UCP1 expression in human adults, with the highest amounts seen during the cold season (2,53). In analogy to the promotion of physical activity on a population-based level, slightly reducing indoor temperatures could potentially help the prevention of obesity.

In conclusion, increasing BAT mass and activity in order to increase energy expenditure represents a promising new therapeutic target for the treatment of both obesity and insulin resistance.

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