Targeting thermogenesis in brown fat and muscle to treat obesity and metabolic disease

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Abstract | Brown fat is emerging as an interesting and promising target for therapeutic intervention in obesity and metabolic disease. Activation of brown fat in humans is associated with marked improvement in metabolic parameters such as levels of free fatty acids and insulin sensitivity. Skeletal muscle is another important organ for thermogenesis, with the capacity to induce energy-consuming futile cycles. In this Review, we focus on how these two major thermogenic organs — brown fat and muscle — act and cooperate to maintain normal body temperature. Moreover, in the light of disease-relevant mechanisms, we explore the molecular pathways that regulate thermogenesis in brown fat and muscle. Brown adipocytes possess a unique cellular mechanism to convert chemical energy into heat: uncoupling protein 1 (UCP1), which can short-circuit the mitochondrial proton gradient. However, recent research demonstrates the existence of several other energy-expending 'futile' cycles in both adipocytes and muscle, such as creatine and calcium cycling. These mechanisms can complement or even substitute for UCP1-mediated thermogenesis. Moreover, they expand our view of cold-induced thermogenesis from a special feature of brown adipocytes to a more general physiological principle. Finally, we discuss how thermogenic mechanisms can be exploited to expend energy and hence offer new therapeutic opportunities.

In the 16th century, the Swiss naturalist and physician Conrad Gessner first described brown adipose tissue (BAT)[1]; however, it was not until the 1960s that its physiological function was revealed[2]. BAT can convert the chemical energy from triglycerides stored in numerous lipid droplets directly into heat when activated by the sympathetic nervous system in response to cold exposure. This process has been called cold-induced thermogenesis or non-shivering thermogenesis, and results from an evocable short circuit in the tissue's abundant mitochondria, which is facilitated by uncoupling protein 1 (UCP1) — the hallmark of brown adipocytes. The development, differentiation and molecular function of BAT have been further elucidated in recent decades. Importantly, in 2009, it was unequivocally demonstrated that a majority of human adults possess active BAT and that it is associated with a favourable metabolic phenotype[3,4].

This 'rediscovery' has rekindled the scientific community's interest in human thermogenesis in general and specifically in BAT thermogenesis. However, a growing body of evidence indicates that adipose tissues other than BAT and factors other than UCP1 contribute substantially to cold-induced thermogenesis. This Review focuses on cold-induced thermogenesis as an important physiological adaptation to cold and outlines the current knowledge in this field both from a basic science and a clinical perspective. Moreover, we indicate how this adaptive mechanism might be exploited to improve human health and ameliorate metabolic disease.

Physiologic response to cold

Humans and other mammals register cooling of the skin with high sensitivity, especially on the trunk and neck[5]. Modest cooling of the skin below a temperature of 26 °C activates transient receptor potential cation channel subfamily M member 8 (TRPM8) in sensory neurons, which acts as a sensor for mild, non-noxious cold and mediates this sensation to the central nervous system (CNS)[6–9]. Within the CNS, the signal is transmitted to the primary somatosensory cortex, which leads to perception of cool temperature and voluntary actions to counteract cold exposure. In parallel, afferent pathways lead to the preoptic area (POA) of the hypothalamus, where the thermosensory signals are integrated[10]. Sympathetic outflow from the POA in turn stimulates shivering as well

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Key points

- Cold-induced thermogenesis is an important component of total energy expenditure and contributes to overall energy balance
- Brown adipose tissue (BAT) has been known to be the effector organ for cold-induced thermogenesis for decades
- The recent discovery of metabolically active BAT in human adults has made it clear that cold-induced thermogenesis is of physiologic and potentially therapeutic relevance
- Cold-induced thermogenesis is a fundamental physiologic principle helping the body adapt to environmental challenges and is not limited to BAT
- Recent research elucidated novel thermogenic mechanisms that contribute to cold-induced thermogenesis both in BAT and beige adipose tissue and in muscle
- Targeting thermogenesis in adipose tissue and muscle might be a promising therapeutic tool against obesity and associated metabolic diseases

as non-shivering thermogenesis[11,12]. Lesions in the hypothalamus in humans, such as those that occur in multiple sclerosis, lead to defective thermoregulation and hypothermia, thereby exemplifying the importance of the aforementioned mechanisms[13]. A detailed review of CNS integration of thermal sensation can be found elsewhere[14].

As the first step in autonomous defence against cold, sympathetic outflow from the POA and its primary neurotransmitter noradrenaline primarily mediate vasoconstriction of cutaneous vessels, which reduces the amount of heat lost via the skin[15,16]. If mild cold exposure persists, the body's resting energy expenditure increases without shivering as a second step to preserve a normal core body temperature, which is called non-shivering thermogenesis. If non-shivering thermogenesis does not sufficiently increase total energy expenditure to maintain core body temperature or when the cold stimuli are strong, shivering occurs. Shivering thermogenesis is defined as the involuntary contraction or twitching of muscles as a means to produce heat. The muscle contractions are caused by bursts of activity from the α-motor neurons that control the respective muscle fibres[17]. The brain regions that elicit shivering in response to skin cooling also facilitate non-shivering thermogenic responses, underscoring a unified response to cold exposure that uses different effectors[18].

In humans, extreme, cold-induced shivering can lead to an increase in thermogenesis of up to approximately five times the resting metabolic rate[19]. Different muscle groups are recruited during shivering, with continuous, low-intensity shivering being related to type I fibres and high-intensity bursts of shivering due to type II fibres[20]. If cold exposure persists for a long time or is repetitive, shivering gradually subsides and non-shivering thermogenesis increases, as has been demonstrated by classic cold-exposure experiments in both rodents[21,22] and humans[23]. Around the same time, it became apparent that a major source of non-shivering thermogenesis in rodents and human infants is BAT[2,24,25], which led to the stepwise elucidation of the molecular function of the tissue[26–28]. Indeed, during cold acclimatization, the density of noradrenergic fibres increases dramatically in murine BAT and white adipose tissue (WAT) depots, which supports the noradrenergic stimulation and induction of beige adipocytes[29]. Thus, the defence of normal body temperature relies on the integrated regulation of shivering and non-shivering thermogenesis, which involves both BAT and skeletal muscle. An overview of the physiologic reaction to cold is shown in FIG. 1.

BAT — a thermogenic tissue

Characteristics of BAT. BAT is characterized by a dense vasculature and sympathoadrenergic innervation that differs markedly from that of WAT. White adipocytes contain a single large lipid droplet surrounded by a narrow rim of cytosol and few mitochondria. By contrast, brown adipocytes store lipids in multiple small droplets and contain large numbers of mitochondria. A unique feature of BAT mitochondria is the presence of UCP1 in the inner mitochondrial membrane. UCP1 is a protonophore that can uncouple oxidative phosphorylation from ATP regeneration, thereby converting energy stored in the form of triglycerides into heat[30] (FIG. 2). Although the importance of BAT for maintaining body temperature in cool ambient temperatures was well known in small mammals such as human infants, the relevance for adult humans was disputed until recently[31–33]. It was only in 2009 that a series of papers unambiguously demonstrated the presence and metabolic activity of BAT in adult humans[3,34–36].

Different types of BAT and recruitment of brown adipocytes. In rodents and human infants, a prominent BAT depot can easily be identified in the interscapular region, which forms a 'BAT organ'. Acute cold exposure leads to activation of pre-existing brown adipocytes through the sympathetic nervous system within minutes and to a corresponding increase in thermogenesis, both in rodents[37] and in humans[38]. In response to long-term or repeated cold stimuli or pharmacological stimulation with β3-adrenergic receptor agonists, additional brown adipocytes are recruited, thus increasing the animal's[39,40] or human's[41–43] thermogenic capacity. Importantly, the newly recruited brown-like adipocytes also appear in previously WAT depots, in rodents predominantly in the inguinal subcutaneous adipose tissue depot and in humans in the supraclavicular region. The latter population of cells is now most commonly called 'beige' (also known as 'inducible', 'recruitable' or 'brite') adipocytes.

The brown adipocytes found in the interscapular depot are known as 'classic brown adipocytes'. Although classic BAT constitutively expresses a high level of UCP1 even at high ambient temperatures, UCP1 levels are low in beige fat depots unless they are stimulated by cold or catecholamines, which increase UCP1 levels dramatically to those found in classic BAT[44]. Whereas the transcriptional signature of brown and beige adipocytes differs and reflects the different origin of the cells[45,46], the morphology and especially the thermogenic capacity are similar in classic brown and beige adipocytes[47,48]; several crucial transcription factors such as peroxisome proliferator-activated receptor-γ co-activator 1α (PGC1α) and PR domain zinc-finger protein 16 (PRDM16) are common to both classic brown and beige adipocytes.

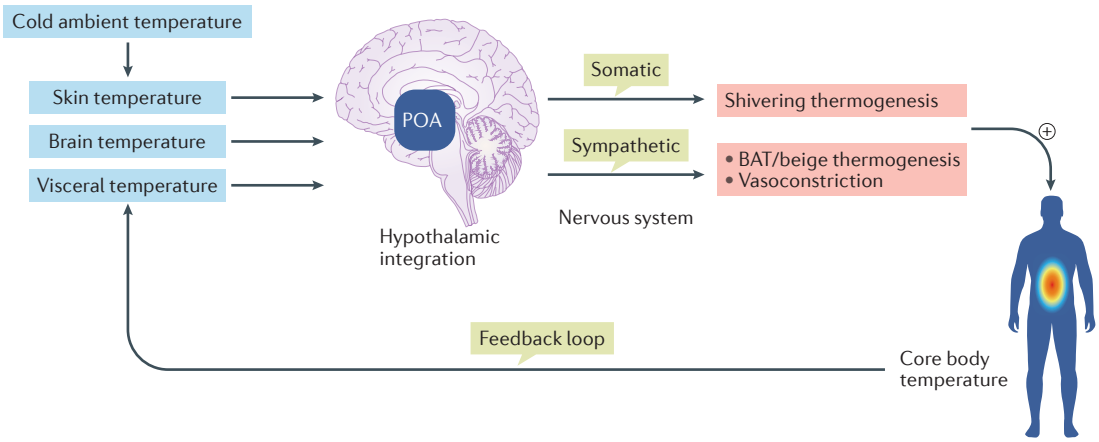

Figure 1 | **Schematic model of physiologic cold response.** Modest cooling of the skin below a temperature of 26 °C activates transient receptor potential cation channel subfamily M member 8 (TRPM8) in sensory neurons (not shown), which acts as a sensor for mild, non-noxious cold and mediates the sensation of cold to the central nervous system. The signals from the skin are integrated in the preoptic area (POA) of the hypothalamus together with temperature sensations from the brain and viscera. Sympathetic and somatic nervous system outflow from the POA stimulates shivering as well as non-shivering thermogenesis, thus pre-emptively counteracting cold ambient temperatures. BAT, brown adipose tissue.

The interscapular BAT depot of rodents is a very stable trait, but the capacity to induce beige adipocytes in response to cold or β3-adrenergic receptor agonists varies considerably between different mouse and rat strains. Notably, mouse strains with greater potential to induce beige adipocytes are less prone to develop obesity and insulin resistance when fed a high-fat diet[49–51]. Conversely, mice that cannot induce beige adipocytes to the same extent are more susceptible to obesity and insulin resistance.

Plasticity of WAT and BAT. During the past two decades, we have gained considerable knowledge about brown and beige adipocyte differentiation and development. Several lines of evidence indicate that brown adipocytes in interscapular BAT arise from the central dermomyotome and share a common MYF5$^+$ lineage with myocytes, whereas the beige adipocytes do not[52,53]. However, more recently, MYF5$^+$ precursors were shown to contribute to the pool of white and beige adipocytes, which changes in response to modifiable (for example, diet) and non-modifiable (for example, adipose tissue depot) factors[54]. As beige adipocytes emerge in response to cold exposure or β3-adrenergic receptor stimulation in otherwise WAT depots, the question arises of whether they transdifferentiate from white adipocytes or develop *de novo* from beige adipocyte precursors.

One line of evidence suggests that rodent beige adipocytes originate from bi-potential perivascular precursor cells[55] and that human beige adipocytes are derived from capillary networks[56]. In line with this observation, human obesity is associated with a low capillary density in subcutaneous adipose tissue depots, which potentially indicates a reduced ability to recruit beige adipocytes[57]. Using an inducible cellular tracing system, beige adipocytes were shown to arise *de novo* and not to be converted from pre-existing unilocular adipocytes[58]. In contrast to these results, another study using a different genetic tracing system demonstrated that all newly formed beige adipocytes in the inguinal fat pad were derived from pre-existing unilocular adipocytes[59].

Exposure of obese and diabetes-prone Zucker rats to β3-adrenergic receptor agonists increases the number of multilocular fat cells in WAT depots, the majority of which are converted from pre-existing unilocular cells[60]. Using a knockout model of the β3-adrenergic receptor in mice, white to beige transdifferentiation was shown to be due to β3-adrenergic receptor signalling and to be the dominant mechanism in cold-exposed mice. The number of preadipocytes in fat pads prone to beige conversion, however, is increased by stimulation of the β1-adrenergic receptor[61]. Further, in the classic interscapular BAT depot, *de novo* development of brown adipocytes is under the control of the β1-adrenergic receptor and can be abrogated by receptor knockout[59]. The sometimes conflicting results could thus be the consequence of different genetic tracing systems or differential stimulation of adrenergic receptors.

Importantly, recent data demonstrate that re-exposure to warm temperatures converts beige to white adipocytes[62]. In parallel, cells express a typical pattern of white adipocyte genetic markers, which suggests that beige adipocytes are a major source of white adipocytes in adipose tissue depots prone to 'browning' (REF. 62). In addition to warm temperatures, increasing age and obesity induce changes towards the white phenotype[63–65]. Ingestion of a high amount of lipids leads to an increase in the number of white adipocytes in BAT depots[66]. In humans, BAT can be found at various locations at birth and during infancy, and most importantly in the interscapular region. With age, the interscapular depot regresses, and in human adults the supraclavicular, para-aortal and retroperitoneal adipose tissue depots are the major depots[34,67,68]. Using gene expression analysis,

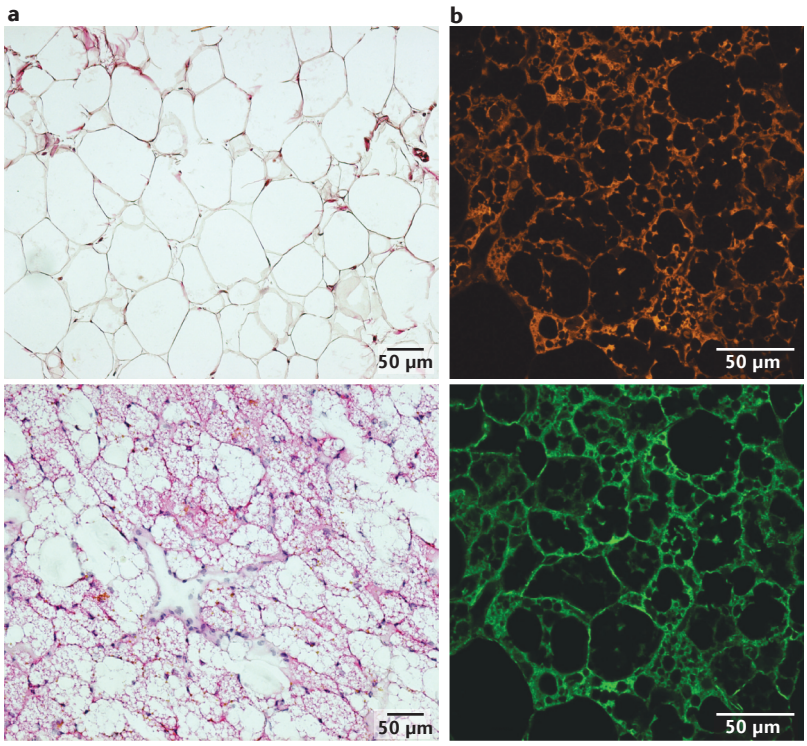

Figure 2 | **Human white adipose tissue (WAT) and brown adipose tissue (BAT).** Microphotographs of human adipose tissue. **a** | Haematoxylin and eosin staining of WAT (upper panel) and BAT (lower panel). White adipocytes are characterized by a large single lipid droplet, little cytoplasm and few mitochondria. Conversely, brown adipocytes are characterized by multiple small lipid droplets (storing triglycerides) and high numbers of mitochondria that harbour uncoupling protein 1 (UCP1). **b** | The same sections visualized by immunohistochemical staining for UCP1 (green; lower panel) and the mitochondrial marker cytochrome *c* (orange; upper panel).

BAT depots found in adult humans were shown to contain both beige and classic brown adipocytes[44], whereas the interscapular BAT depot of human infants consists solely of classic brown adipocytes[69,70].

It is tempting to speculate whether the beige fat depots found in human adults might also be subject to genetic variability and linked to risk of developing obesity and obesity-related diseases, as is the case in rodents. The evidence in this field is, however, currently very limited. In a prospective observational study from the Netherlands, healthy lean volunteers of South Asian descent had lower BAT volume and levels of cold-induced thermogenesis than matched white participants[71]. In a cohort of 190 healthy Japanese volunteers, polymorphisms in the genes encoding UCP1 and the β3-adrenergic receptor were associated with a large, age-related decrease in cold-induced BAT activity[72]. Indirect evidence includes a gene polymorphism in the *FTO* region, which is strongly associated with human obesity and impairs browning of WAT depots in cell culture[73]. Additionally, the great plasticity and expandability of beige adipose tissue depots could enable thermogenic capacity in humans to increase in order to increase cold-induced thermogenesis, which might contribute to an increase in total energy expenditure.

Thermogenic mechanisms

Although many biochemical processes, such as protein breakdown and re-synthesis, contribute to whole-body energy metabolism, we focus here on the molecular mechanisms that increase energy expenditure in response to cold exposure (TABLE 1).

UCP1. UCP1 is uniquely expressed in brown and beige adipocytes and is localized to the inner mitochondrial membrane. When activated, UCP1 uncouples mitochondrial respiration from the regeneration of ATP. The mitochondrial respiratory chain builds up a proton gradient across the inner membrane by actively transporting electrons from electron donors to electron acceptors via redox reactions. This electron transfer is coupled to the transfer of protons across the inner membrane into the intermembrane space. This proton gradient drives regeneration of ATP by ATP synthase[74]. Stimulation of β3-adrenergic receptors on the surface of brown adipocytes by noradrenaline is transduced via G_s proteins and the cAMP second messenger system, which results in lipolysis of the intracellular triglyceride stores that provide free fatty acids (FFAs) as fuel for the electron transport chain in mitochondria. Additionally, long-chain fatty acids increase the proton conductance of UCP1 in the inner mitochondrial membrane[75], thus uncoupling the respiratory chain from ATP regeneration. The ensuing increase in ADP in turn upregulates the activity of the respiratory chain, which leads to increased oxygen consumption and heat production[74] (FIG. 3a).

Active BAT takes up glucose avidly, which is the molecular basis for functional imaging with ^{18}F-fluorodeoxyglucose PET (^{18}F-FDG–PET). Glucose uptake increases after repeated cold exposure[41,76,77]. Elegant studies using different PET tracers have also demonstrated that BAT increasingly takes up fatty acids in response to acute cold exposure[78]. Repeated cold exposure, however, does not increase the uptake rate of fatty acids into BAT[79]. The question of whether BAT uses glucose as a direct substrate for thermogenesis or rather as a substrate for lipogenesis has not been studied in detail. Evidence from measurement of the respiratory quotient during cold exposure, CT and MRI favour fatty acids as the predominant primary fuel for thermogenesis, as the respiratory quotient decreases during cold exposure[35], radiodensity increases, and the fat content of BAT depots decreases, respectively[78,80]. Evidence from transgenic mouse models underscores that UCP1 is part of a very efficient thermogenic mechanism that evolved in order to maintain core body temperature during cold exposure at the lowest energy cost possible.

It should be noted that UCP1 did not primarily evolve as an anti-obesity protein but as a means to quickly generate heat. Gradual cold exposure of *Ucp1*-knockout mice reduced rather than increased metabolic efficiency and rendered animals resistant to obesity[81,82], probably due to the higher energy cost of alternative thermogenic mechanisms[83]. *Ucp1* knockout studies have not only confirmed the importance of UCP1 for thermogenesis in response to acute cold[84] but also indicated compensatory thermogenic mechanisms that could be

Table 1 | **Mediators of non-shivering thermogenesis in thermogenically active cells**

Mediator	Classic brown adipocytes	UCP1$^+$ beige adipocytes	UCP1$^-$ beige adipocytes	Myocytes
UCP1	+	+	–	–
Creatine cycling	(+)	+	+	Unknown
Glycerol-3-phosphate shuttle	(+)	+	Unknown	Unknown
Sarcolipin and SERCA	Unknown	Unknown	Unknown	+

+ denotes an important role. (+) denotes a limited role. SERCA, sarcoplasmic/endoplasmic reticulum calcium ATPase; UCP1, uncoupling protein 1.

activated by stepwise acclimation to colder temperatures. *Ucp1*$^{-/-}$ mice have more beige adipocytes in the inguinal subcutaneous fat pad and a greater induction of mitochondrial glycerol-3-phosphate dehydrogenase (mtGPD) and other markers characteristic of BAT such as PGC1α or type 2 iodothyronine deiodinase (DIO2) and sarcoplasmic/endoplasmic reticulum calcium ATPases (SERCAs) in this fat pad than wild-type littermates[85,86]. These studies prompted the investigation of alternative molecular mechanisms of thermogenesis that could complement or even replace UCP1-mediated thermogenesis.

mtGPD shuttle. The high expression of mtGPD observed in BAT[87,88] suggests that the GPD shuttle also contributes to thermogenesis. The shuttle enables electrons from cytosolic NADH to enter the mitochondrial electron transport chain. As the electrons are accepted by FAD rather than by NAD^+, the resulting yield in ATP is 1.5 molecules of ATP rather than 2.5 molecules per electron pair; the shuttle is thus less efficient and contributes to thermogenesis[74]. Although ablation of *Gpd2*, which encodes mtGPD, did not result in defective thermogenesis[89], double knockout of *Ucp1* and *Gpd2* synergistically increased energy expenditure during cold exposure and led to the browning of inguinal subcutaneous adipose tissue[90], thereby indicating the existence of additional mechanisms that complement UCP1-mediated thermogenesis.

Creatine cycling. Among the emerging mechanisms implicated in thermogenesis, creatine cycling has recently been reported to contribute markedly to thermogenesis in beige adipocytes[91]. Earlier work had already detected an increase in mitochondrial-type creatine kinase in BAT of cold-exposed rats[92]. Using proteomic analysis of mitochondria, Kazak *et al.* identified arginine–creatine metabolism as a specific signature of beige adipocytes in mice[91]. Short-term cold exposure (6 h) dramatically increased gene expression and protein levels of creatine kinase U-type, mitochondrial (CKMT1) and creatine kinase S-type, mitochondrial (CKMT2) primarily in beige adipose tissue. Moreover, the addition of creatine to mitochondria from beige adipocytes stimulated respiration when levels of ADP were limiting respiration. This effect was not seen in mitochondria from muscle or brown adipocytes. *In vivo* inhibition of creatine transport reduced catecholamine-stimulated whole-body oxygen consumption by decreasing beige adipose tissue respiration[91]. Moreover, *Ckmt1* gene expression was upregulated in the beige adipose tissue of *Ucp1*-knockout mice, which indicates a compensatory role for creatine-cycling-mediated thermogenesis in the absence of UCP1. Blocking creatine transport in cultured human brown adipocytes nearly halved oxygen consumption, and small interfering RNA-mediated knockdown of *Ckmt1* also diminished respiration[91].

These findings are in line with studies in human beige adipose tissue samples, which indicated increased expression of the mitochondrial creatine kinase CKMT1 (REF. 93), as well as with a more recent proteomic analysis of human brown/beige adipose tissue, which revealed an enrichment of the mitochondrial creatine kinases CKMT1A, CKMT1B and CKMT2 in parallel with UCP1 (REF. 94). More recently, it was demonstrated by patch clamp analysis of the internal mitochondrial membrane that UCP1-negative adipocytes within beige adipose tissue depots use creatine cycling as a thermogenic mechanism[95]. Thus, beige adipocytes could contribute to non-shivering thermogenesis even in the absence of UCP1. FIGURE 3b gives a conceptual overview of the proposed mechanisms of creatine cycling.

Sarcolipin and calcium cycling — non-shivering thermogenesis in muscle. Shivering is probably the most commonly known response to cold exposure. It is defined as an involuntary contraction or twitching of muscle as a means to produce heat. The muscle contractions are caused by bursts of activity from the α-motor neurons that control the respective muscle fibres[17]. The brain regions that elicit shivering in response to skin cooling also facilitate the non-shivering thermogenic responses to cold exposure, which underscores a unified response to cold exposure that uses different effectors in response to cold[18]. However, several lines of evidence indicate that muscle could also contribute substantially to non-shivering thermogenesis.

Much attention has been given to the fact that skeletal muscle fibres express mitochondrial proteins similar to UCP1, namely, UCP2 and UCP3. These proteins have been speculated to also function as uncoupling proteins and to contribute to non-shivering thermogenesis; however, high-level expression of UCP2 and UCP3 cannot compensate for the loss of UCP1 in brown adipocytes in *Ucp1*$^{-/-}$ mice[96]. Loss or overexpression of UCP2 in murine muscle does not change uncoupling activity but alters fatty acid oxidation, with higher levels

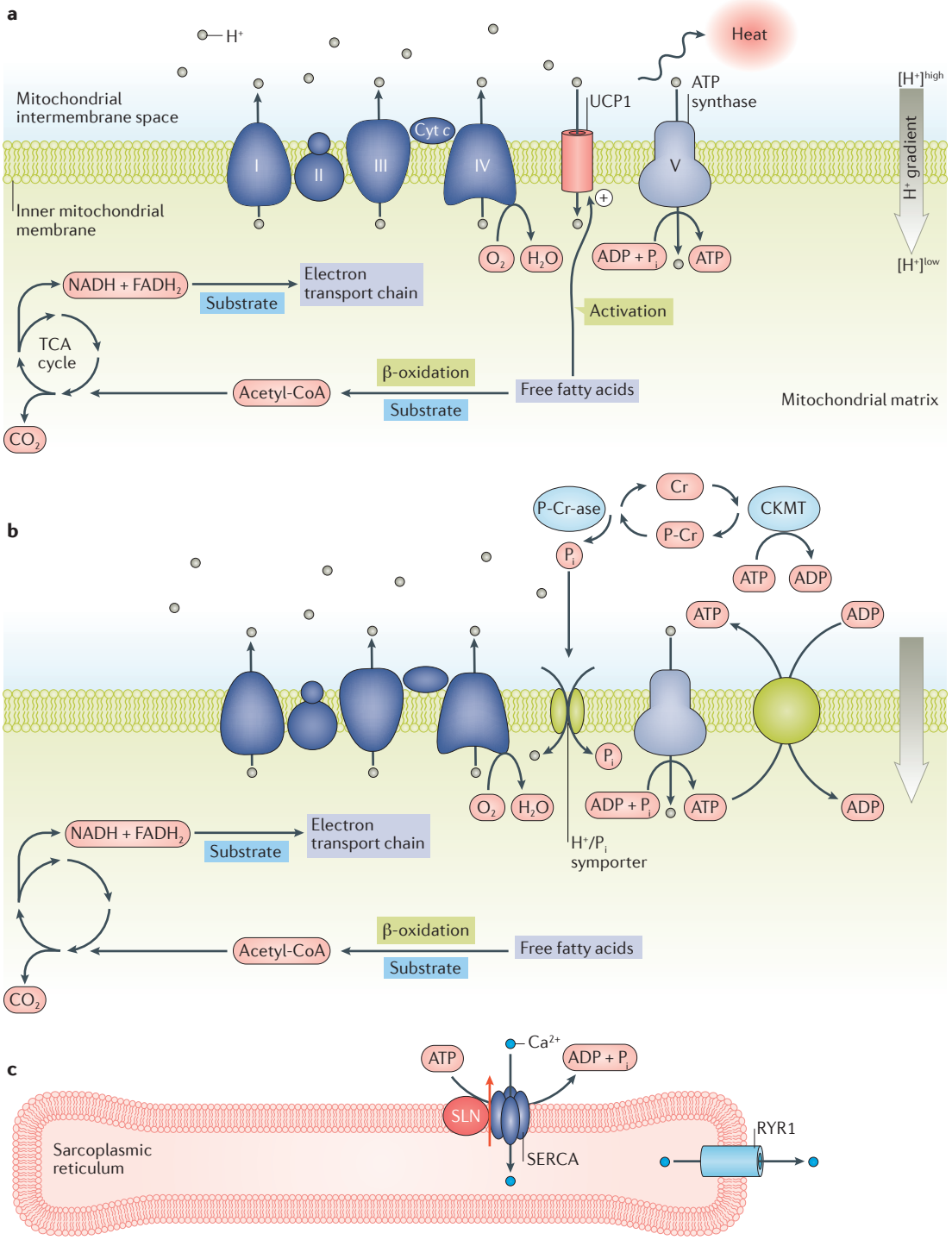

Figure 3 | **Mechanisms of thermogenesis. a** | Uncoupling protein 1 (UCP1). The respiratory chain pumps protons into the intermembrane space, which creates a gradient whose electromotive force drives ATP synthase. In brown adipocytes, this gradient can be short-circuited by activation of UCP1, which dissipates the energy stored in the gradient as heat. **b** | Creatine cycling (conceptual model). Mitochondrial creatine kinase (CKMT) transfers an activated phosphate from ATP to creatine (Cr), which forms phosphor-creatine (P-Cr). The resulting ADP is transferred into the mitochondrial matrix and drives oxidative phosphorylation. The P-Cr is hydrolysed by P-Cr-ase and is available for another futile cycle. **c** | Sarcolipin (SLN) and sarcoplasmic/endoplasmic reticulum calcium ATPases (SERCAs). SLN (a small transmembrane proteolipid) reduces the efficiency of SERCAs, which leads to increased availability of ADP and thus increased activity of oxidative phosphorylation and the tricarboxylic acid (TCA) cycle. This is probably an important mechanism of non-shivering thermogenesis in muscle. Cyt *c*, cytochrome *c*; RYR1, ryanodine receptor 1. Part **a** is adapted with permission from REF. 135, Elsevier (https://creativecommons.org/licenses/by/4.0/).

of UCP2 facilitating improved fatty acid utilization[97]. Fasting increases levels of UCP2 and UCP3 in skeletal muscle of mice and humans, which indicates a role for these proteins in metabolic adaptation and a shift towards lipid metabolism[98,99]. This finding is in line with reports suggesting a role for these proteins as mitochondrial carriers[100] and recent findings demonstrating a role for UCP2 in the mitochondrial transport of C4 metabolites[101]. Additionally, acute cold exposure decreases the expression of UCP3 in human muscle[102].

Despite being named similarly to UCP1, UCP2 and UCP3 seem to facilitate not uncoupling activity but rather metabolic adaptation and lipid metabolism. Skeletal muscle has been shown to contribute to cold-induced thermogenesis by mechanisms independent of shivering and not involving uncoupling proteins. This fact is exemplified by the finding that the core body temperature of *Ucp1*-ablated mice exposed to 4 °C fell to moderately hypothermic temperatures but not to those characteristic of profound hypothermia even when shivering was blocked by curare[103]. Non-shivering thermogenesis in muscle results from calcium cycling by SERCA, which resides in the sarcoplasmic reticulum of muscle cells and BAT and actively pumps Ca^{2+} ions into the sarcoplasmic reticulum during muscle relaxation. Calcium cycling has been described as an important part of non-shivering thermogenesis in rabbit BAT and muscle[104]. SERCA is regulated by phospholamban and the small peptide sarcolipin[105]. Sarcolipin uncouples the hydrolysis of ATP from Ca^{2+} transport and thus leads to futile cycling and thermogenesis[106] (FIG. 3c).

Mice deficient in both sarcolipin and UCP1 cannot maintain body temperature and become severely hypothermic upon cold exposure[107]. Single knockout of the gene encoding sarcolipin results in a compensatory increase in UCP1 in BAT and beige adipose tissue, whereas knockout of *Ucp1* is compensated for by increased levels of sarcolipin in muscle, which indicates synergism of both thermogenic mechanisms *in vivo*[107]. In line with these findings, muscle-specific overexpression of sarcolipin increased the basal metabolic rate and prevented diet-induced obesity in mice[108]. Ryanodine receptor 1 (RYR1) in muscle is essential for calcium release from the sarcoplasmic reticulum, which leads to coupling of excitation to contraction[109]. Treatment of wild-type mice with dantrolene, a blocker of RYR1, mimics the phenotype of mice lacking sarcolipin but inhibits non-shivering thermogenesis instead of muscular movements[103], thus providing evidence for a role of calcium cycling in this process.

Intriguingly, a feared complication of general anaesthesia in humans, malignant hyperthermia, is due to mutations in RYR1 and can be successfully treated with dantrolene[110]. Malignant hyperthermia is characterized clinically by massively increased metabolism and on a molecular level by excessive futile cycling and oxidative phosphorylation in mitochondria[111]. This emphasizes a close interrelationship between BAT and muscle as important and powerful organs for preserving normal body temperature on the basis of both shivering and non-shivering thermogenic mechanisms.

Interestingly, humans with thyroid hormone resistance caused by a mutation in thyroid hormone receptor β (TRβ) exhibit increased resting energy expenditure. The mutation causes elevated levels of thyroid hormones in plasma, which is paralleled by an increase in tricarboxylic acid cycle flux and increased uncoupling of oxidative phosphorylation in muscle mitochondria (determined by MRI)[112]. Although expression of UCP1 is dependent on the function of TRβ[113,114], the expression of SERCA in muscle is induced by stimulation of TRα1 (REF. 115). These findings are in line with the fact that large mammals, such as humans, possess small BAT depots in relation to body size compared with small mammals, such as rodents. Conversely, sarcolipin expression is high in muscle tissue of large mammals and present in all muscles. Non-shivering thermogenesis in BAT and muscle might thus complement each other[116]. Taken together, these data underscore an important contribution of muscle to non-shivering thermogenesis.

In addition, it is well known that fatty acids can induce uncoupling in muscle, which is driven by the mitochondrial protein adenine nucleotide translocator 1 (ANT1). Interestingly, this protein can be induced by endurance training in humans[117], even though endurance training is associated with lower amounts of active BAT in humans[118]. These findings are in line with MRI of muscle in individuals who were endurance-trained versus those who were sedentary, which also demonstrated substantial differences in mitochondrial uncoupling[119].

Although the amount of scientific evidence on UCP1-independent cold-induced thermogenesis is constantly growing, it is important to remember that we have far more knowledge and evidence about UCP1-mediated thermogenesis. This is especially true for the field of human physiology, in which mechanisms such as sarcolipin-mediated thermogenesis and creatine cycling still have to be unequivocally demonstrated. However, ongoing research in the field of thermogenesis impressively demonstrates that cold-induced thermogenesis is very important for mammals and relies on redundant mechanisms to protect its function.

Potential benefits in humans

Overweight and obesity and the diseases associated with them have become a major burden for affected individuals and health-care systems worldwide. It has been speculated that a reduction in cold-induced thermogenesis, for example, due to advances in home insulation and heating, has contributed to the increased prevalence of obesity[120]. Even if reduced cold-induced thermogenesis is not necessarily a major cause for overweight in humans, it might well be suitable to increase total energy expenditure in order to treat obesity and its associated diseases. Increasing energy expenditure instead of limiting energy intake could also improve metabolic flexibility[121], especially as BAT activity increases β-oxidation of fatty acids[122].

Therapeutic activation of thermogenesis in BAT. BAT found in adult humans exhibits molecular signatures of both classic and beige adipocytes[69,70,123]. In rodents, the number of beige adipocytes increases in response to

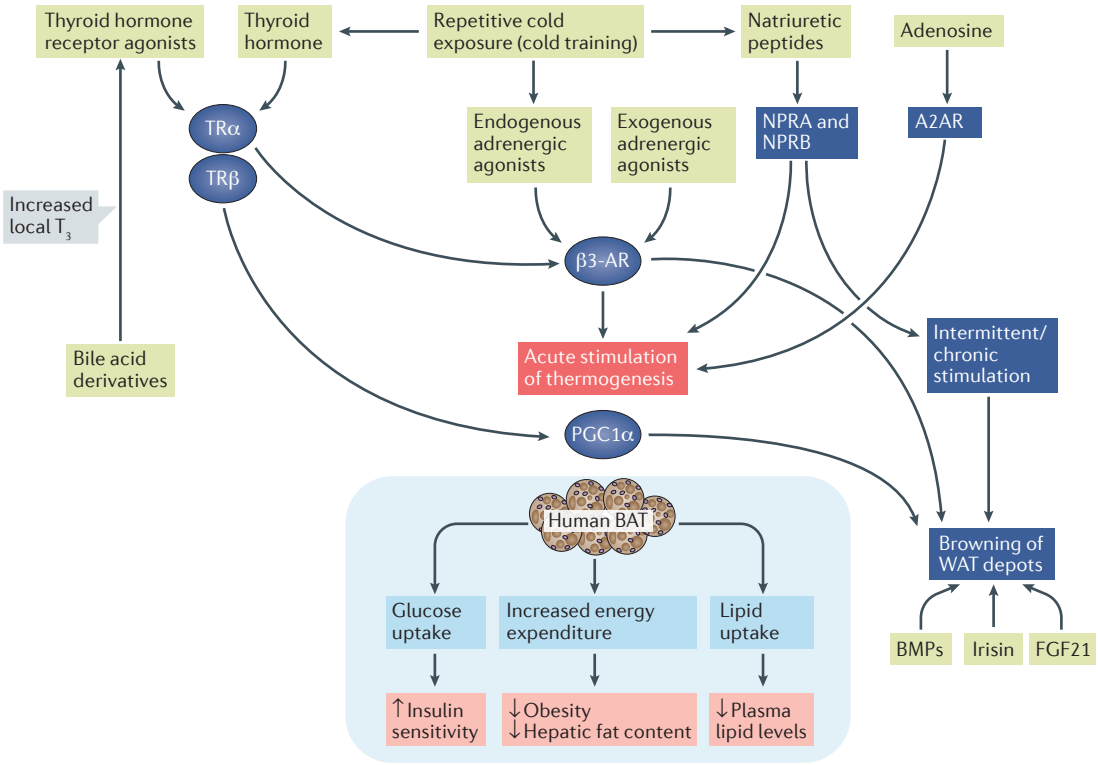

Figure 4 | **Potential therapeutic targets to expand brown adipose tissue (BAT).** Numerous hormones and other substances have been described in recent years that stimulate BAT. Although transmitters such as noradrenaline and adenosine acutely increase thermogenesis and lead in the long term to more brown adipocytes in white adipose tissue (WAT) depots, other substances such as bone morphogenetic proteins (BMPs) and fibroblast growth factor 21 (FGF21) act primarily as 'browning agents' by increasing the number of beige adipocytes in WAT depots. These newly recruited cells can then be stimulated by the sympathetic nervous system or exogenous β3 adrenergic receptor (β3-AR) agonists. A2AR, adenosine receptor 2A; NPR, natriuretic peptide receptor; PGC1α, peroxisome proliferator-activated receptor-γ co-activator 1α; TR, thyroid hormone receptor.

cold stimuli or treatment with adrenergic receptor agonists (discussed earlier) and could thus be expanded by pharmacological or non-pharmacological interventions such as repeated voluntary cold exposure. This possibility is underscored by the evident seasonal variability of human beige adipose tissue both in the subcutaneous[124] and in the retroperitoneal[125] fat depots, with a higher prevalence of BAT during the cold season. These changes in histology occur in parallel with an increased thermogenic response to cold during winter compared with summer[126,127] and uptake of ^{18}F-FDG in BAT depots[34]. Furthermore, BAT is the target of a multitude of endocrine and pharmacological agents, which could be used to increase its activity and mass (FIG. 4). Although studies on pharmacological or non-pharmacological activation of BAT in humans are currently limited, it is already evident that the amount and activity of human BAT can be augmented.

Increased cold exposure during the winter season can be simulated by repetitive voluntary cold exposure. Such cold training increases the amount of BAT and non-shivering thermogenesis in healthy volunteers and decreases the sensation of cold and shivering[76]. In a similar setting, cold exposure to 17 °C for 2 h per day over 6 weeks induced BAT in human adults in whom BAT was undetectable at baseline[41]. The increased amounts of BAT after cold training were associated with increased oxidative capacity[77] and insulin sensitivity[128], as well as decreased body fat mass[41]. Although cold exposure increases UCP1 mRNA expression within hours to a few days when studied in laboratory animals, it is important to remember that BAT thermogenesis is mediated by UCP1 protein and not by *UCP1* RNA. In most human BAT trials, BAT tissue was not sampled and thus UCP1 mRNA or protein levels were not reported. However, the aforementioned studies used functional methods to evaluate BAT activity (usually ^{18}F-FDG-PET) and metabolism (indirect calorimetry) and thus reported robust and reliable data on the effects of cold on human BAT.

Both retrospective[129] and prospective[34,64] studies in humans have demonstrated substantially decreased or abolished BAT activity in individuals with obesity, which indicates a potential role for BAT in the setting of human obesity. In comparison to the large amount of information on consequences of functional BAT (gained from experiments in rodents), the data from human studies are still very limited. For example, it is not yet clear whether reduced BAT activity in humans with obesity is a consequence or a cause of increased body weight. Weight loss after bariatric surgery led to an increase in

BAT activity in individuals who previously had morbid obesity[130], which suggests that reduced BAT activity might be caused by thicker layers of subcutaneous fat insulating better against lower temperatures. When lean individuals and those with obesity were exposed to cool ambient temperatures (15 °C), the individuals with obesity responded with a lower increase in energy expenditure and a larger drop in proximal skin temperature[131]. However, it should be noted that differences in the potential to recruit beige adipocytes might also be a cause of human obesity. A gene polymorphism in the *FTO* region, which is strongly associated with human obesity, impairs browning of both murine and human primary pre-adipocytes, which suggests an important role for beige adipocyte thermogenesis in the development or prevention of human obesity[73]. In patients who are overweight, short-term cold acclimation of 10 days increased the amount of active BAT[42]. Although this short study did not enable studying the consequences on body weight, it is still a very helpful proof of principle that should be pursued in long-term trials.

Therapeutic activation of thermogenesis in muscle. Compared with BAT, the amount of skeletal muscle is much larger in an average adult human. Therefore, futile cycling or uncoupling mechanisms in muscle in response to cold could substantially contribute to energy expenditure in humans. Before BAT was shown to be metabolically active and relevant in a majority of human adults in 2009 (REFS 4,34), the effects of cold on thermogenesis had been studied extensively. Adrenergic stimulation of human energy expenditure was shown to increase oxygen consumption in skeletal muscle markedly, and it was calculated that muscle accounted for 40% of the total increase in energy expenditure[132]. Cold-induced thermogenesis correlated with uncoupled respiration in skeletal muscle biopsy samples from human volunteers[133]. Short-term (10-day) repetitive cold exposure of patients with type 2 diabetes mellitus increased peripheral insulin sensitivity by 43%. Increased glucose uptake into skeletal muscle was shown to be due to a higher density of GLUT4 glucose transporters in the muscle membrane in response to cold exposure[43].

Whether cold-induced thermogenesis in humans is mainly a consequence of BAT or muscle activity is still not decided. In a study using several different PET tracers for glucose and fatty acids, oxidative metabolism in BAT, but not in adjacent skeletal muscle, was shown to increase in response to cold exposure[78]. Van der Lans *et al.* studied the effects of cold acclimation on BAT and muscle in healthy volunteers and found that neither the proton leak nor the fatty-acid-induced uncoupling in skeletal muscle increased[76]. In line with these results, 4 weeks of cold acclimation in healthy young men slightly reduced the amount of non-shivering thermogenesis in skeletal muscle while substantially increasing BAT oxidative metabolism (almost twofold) and volume (by 45%)[134]. It should be noted that the studies discussed were relatively short-term (2–6 weeks) and that longer cold training might lead to more pronounced results. Furthermore, the cold exposure used in these studies was mild and could be maintained for longer periods without adverse effects.

Conclusions

Non-shivering thermogenesis both from BAT and muscle can contribute substantially to human energy expenditure, and targeting it in order to increase its activity might serve as a promising therapeutic tool to treat obesity and associated diseases. Research has advanced considerably from animal models to studies in humans during the past few years, and a multitude of potential pharmaceutical targets in BAT have been discovered. Larger, prospective controlled trials are needed to corroborate these findings and to develop effective treatment strategies. Until a safe and specific thermogenesis-activating drug is developed, reducing ambient temperatures or repetitive 'cold exercises' could be used to stimulate human BAT activity.

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