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Brown adipose tissue as an anti-obesity tissue in humans

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Summary

During the 11th Stock Conference held in Montreal, Quebec, Canada, world-leading experts came together to present and discuss recent developments made in the field of brown adipose tissue biology. Owing to the vast capacity of brown adipose tissue for burning food energy in the process of thermogenesis, and due to demonstrations of its presence in adult humans, there is tremendous interest in targeting brown adipose tissue as an anti-obesity tissue in humans. However, the future of such therapeutic approaches relies on our understanding of the origin, development, recruitment, activation and regulation of brown adipose tissue in humans. As reviewed here, the 11th Stock Conference was organized around these themes to discuss the recent progress made in each aspect, to identify gaps in our current understanding and to further provide a common groundwork that could support collaborative efforts aimed at a future therapy for obesity, based on brown adipose tissue thermogenesis.

Keywords: Brown adipose tissue, energy expenditure, sympathetic nervous system, uncoupling protein-1.

Abbreviations: ¹⁸F-FDG, 18 fluoro-deoxyglucose; BAT, brown adipose tissue; BDNF, brain-derived neurotrophic factor; BMP, bone morphogenetic protein; BMPR1, bone morphogenetic protein type 1 receptor; BMPR2, bone morphogenetic protein type 2 receptor; CITDEA1, Cbp/p300-interacting transactivator, with Glu/Asp-rich carboxy-terminal domain, 1; Ehmt1, euchromatin histone methyltransferase 1; MC4R, melanocortin 4 receptor; Myf5, myogenic factor 5; PET/CT, positron emission tomography/computed tomography; PRDM16, PRD1-BF1-RIZ1 homologous domain containing 16; SNS, sympathetic nervous system; UCP1, uncoupling protein-1; WAT, white adipose tissue.

Introduction

Global estimates by the World Health Organization indicate that the epidemic of obesity is on the rise worldwide. In 2008, more than 1.4 billion adults were overweight, of which 200 million men and 300 million women could be classified as obese. Additionally, more than 40 million children under the age of five were overweight (1). It is further estimated that 44% of the diabetes burden, 23% of ischaemic heart disease burden and 7–41% of the burden of certain cancers are attributable to the global epidemics of overweight and obesity (1). The inability of current approaches to curb the obesity epidemic calls for a reappraisal of these approaches and points towards a need to find alternative means for the prevention and management of obesity and related diseases.
The development of obesity represents a state of energy imbalance where energy intake exceeds energy expenditure, leading to storage of excess energy as fat. Continuous loading of excess energy results in hypertrophy of adipose tissue, and often in ectopic fat deposition as well as insulin resistance and metabolic disorders (2). Targeting a reduction in energy intake and/or an increase in energy expenditure represent the inescapable ways to combat obesity. Brown adipose tissue (BAT) has been long known for its capacity to enhance energy expenditure significantly in rodents upon activation (3). However, a role for this tissue in adult human energy metabolism was largely ignored until 2007, when a comprehensive review by Nørgaard et al. (4) of radiological data from clinical cancer studies, pointing to the presence of BAT in adult humans, rung the bell among obesity and energy metabolism researchers. This review was followed by a series of observations in 2009 (5–9) that not only verified the presence of genuine brown adipocytes in adult humans, but also opened avenues for researching its thermogenic potential and utility as an anti-obesity tissue in humans.

To consolidate recent progress made in the area of BAT biology by providing a platform for exchanging the opinions of world leaders, the International Association for the Study of Obesity organized the 11th Stock Conference, devoting it to BAT. With kind support from Novo Nordisk and Merck, the conference was held in Montreal, Quebec, Canada, 2–4 November 2012 and was co-chaired by Drs Denis Richard and Jan Nørgaard. The conference hosted 13 internationally renowned speakers who presented their findings to an enthusiastic group of keen participants. The conference sessions were organized to provide an overview of the cellular, developmental and regulatory aspects of BAT in both rodents and humans, with an objective of channeling future efforts towards the development of a plausible therapy for obesity based on BAT thermogenesis. Here, we summarize the presentations and discussions held during the 11th Stock Conference. The summary reflects the viewpoint of the speakers, the current state of knowledge in each domain (including some updates) and identifies important research questions that would need to be addressed in the future.

**Uncoupling protein-1 as the prerogative of brown adipocytes**

Brown adipocytes are unique in their ability to become thermogenic upon stimulation in a way not involving synthesis and breakdown of adenosine triphosphate (i.e. uncoupling oxygen consumption and substrate combustion from adenosine triphosphate synthesis). This unique property is conferred upon the brown adipocytes by the uncoupling protein-1 (UCP1), a member of the mitochondrial carrier protein family, which is exclusively expressed in brown and brown-like adipocytes (10). By increasing the proton conductance of the inner mitochondrial membrane, UCP1 diminishes the transmembranal electrical gradient, thereby weakening the brake on substrate oxidation caused by a high membrane potential. This thus leads to heat production as a result of enhanced substrate oxidation (10,11). Acknowledging the essentiality of UCP1 for BAT thermogenesis, the first session was opened by Barbara Cannon from the The Wenner-Gren Institute, Stockholm University, Sweden, who provided an overview of the structure, function and regulation of UCP1. As a member of the mitochondrial carrier protein family, UCP1 shares many points of homology, including its tripartite structure, with other members of this large family. However, there are two sequences, one in middle of central loop (likely facing the matrix) and another being the last part of the C-terminus (facing the cytosol), which are unique to UCP1 protein and are fully conserved in all UCP1-carrying species (12). Their unique and conserved presence points towards a specific role for these sequences in UCP1 function, i.e. in non-shivering thermogenesis (3).

That UCP1 is absolutely critical for non-shivering thermogenesis or for adaptive norepinephrine-induced thermogenesis was demonstrated by Matthias et al. (13) using brown adipocytes obtained from UCP1 knockout mice. Such brown adipocytes show no thermogenesis upon norepinephrine treatment (13). Similarly, an UCP1-dependent stimulatory role for fatty acids in BAT thermogenesis was demonstrated using UCP1 knockout mice (13). These fatty acids are derived from the lipolysis of the lipid droplets present within brown adipocytes upon adrenergic activation (3). Shabalina et al. (14) further demonstrated that UCP1 by itself is not leaky, but needs activation (plausibly via the released fatty acids) before acting as a proton conductor to execute thermogenesis. Fatty acids are known to be innate uncouplers in any kind of mitochondria. However, fatty acids are much more potent ‘uncouplers’ in the presence of UCP1, which supports either a catalytic or regulatory role for fatty acids in UCP1 function (the model presented by Cannon would favour a regulatory rather than a catalytic role). The affinity and efficacy of fatty acids in inducing thermogenesis depend upon fatty acid chain length, such that medium- and long-chain fatty acids are more potent inducers of UCP1 relative to the short-chain fatty acids ((15), unpublished observations).

In their possible catalytic role, fatty acids have been proposed to be localized to binding sites in the proton conduction channel within UCP1, such that their acidic moieties can serve as ‘stepping stone’ for protons as they pass through the membrane (16). This model, however, does not explain the interaction between the stimulatory effect of fatty acids and the inhibitory effect of purine nucleotides on UCP1 activity. Alternatively, fatty acids...
have been proposed to serve as proton shuttles, where protonated fatty acids undergo uncatalyzed trans-bilayer movement (flip-flop) from cytosolic to the matrix side of the mitochondrial inner membrane, get dissociated inside the matrix and then re-exit the mitochondria in their anionic form, catalyzed by UCP1, through this shuttling of protons over the membrane (17,18). However, unpublished observations from Cannon's group show that flip-flop ability is not a necessary property of UCP1-activating fatty acids. A variation of this model was recently proposed by Fedorenko et al. (19) in the long-chain fatty acid shuttle model, where UCP1 was proposed to serve as the long-chain fatty acid/H\(^+\) symporter that simultaneously transports one long-chain fatty acid anion and one H\(^+\) into the mitochondria, and that the long-chain fatty acid then swings back towards the cytosol, now not carrying a proton (19).

In a regulatory role, fatty acids have been proposed to either compete directly with purine nucleotides for an activating site on UCP1 or bind to an allosteric site on UCP1 that induces conformational changes in protein structure promoting UCP1’s protonophoric abilities (20). In the native state of UCP1, i.e. in mitochondria isolated from brown adipocytes, free fatty acids and purine nucleotides display a simple competitive kinetics, which tend to support the notion of a single binding site for purine nucleotides and fatty acids on UCP1 protein (21). However, fatty acids and purine nucleotides have very different structures. Correspondingly, no or only low-affinity competition between fatty acids and guanosine diphosphate for guanosine diphosphate-binding sites has been reported in brown fat mitochondria (22). A hypothesis proposing an allosteric binding of fatty acids to UCP1 protein leading to a conformational change that overcomes the purine nucleotide-mediated inhibition remains plausible, but has to be further investigated in additional experimental settings.

In conclusion, it must be realized that despite more than three decades of analyzing the function of UCP1, neither the molecular functional mechanics nor the manner of acute regulation have become fully established.

**Cellular properties and types of brown adipocytes**

In addition to UCP1, brown adipocytes exhibit other cellular properties that contribute to their identity and thermogenic function while simultaneously distinguishing them from white adipocytes. An overview of the cellular properties and physiological plasticity of white and brown adipocytes was provided by Saverio Cinti from the University of Ancona, Italy. Cinti proposed that, in both rodents and humans, there is a large adipose organ that possesses a discrete anatomy, specific vascular and nerve supplies, complex cytology and physiological plasticity (23). Indeed, both white and brown adipocytes represent important aspects of the adipose organ. While white adipocytes are characterized by single large lipid droplet and the presence of only a few mitochondria that are elongated and have less defined cristae, a ‘squeezed’ nucleus, brown adipocytes are multilocular cells that carry numerous spherical mitochondria with well-defined cristae. In addition, clusters of brown adipocytes or BAT are highly vascularized and innervated by sympathetic nervous system (SNS) fibres to a much greater extent than white adipocytes in white adipose tissue (WAT) depots (24). Anatomically, white adipocytes can be located in the adipose tissue depots that are found under the skin (i.e. subcutaneous) or in the trunk (i.e. visceral), whereas brown adipocytes (i.e. UCP1-positive adipocytes) are found clustered as classical BAT depots in the interscapular, cervical and peri-aortic regions in rodents and in the cervical-supraclavicular, peri-aortic and peri-renal areas in humans (7,25). In addition, brown adipocytes can be found dispersed within various WAT depots in rodents, especially upon cold exposure or treatment with \(\beta\)-adrenergic agonists (26,27). These adipocytes have in the literature been described as beige (28,29), brite (BRown-like adipocytes in whITE adipose tissue) (30) or recruitable (31,32). The distinct characteristics of these transforming WAT depots could be attributed to the abundance of adipocytes with intermediate features (paucilocular adipocytes) (27).

Although the exact developmental routes of beige/brite/recruitable adipocytes remain to be determined, Cinti presented the concept of transdifferentiation (33,34). According to this hypothesis, if required physiologically, mature white adipocytes can transdifferentiate into brown adipocytes – and vice versa – to support the overall plasticity of adipose organ. For instance, it has been shown that upon cold exposure, the number of brown cells in WAT depots is enhanced, which would be physiologically required for enhanced heat production. On the contrary, unilocular white adipocytes can be seen in the interscapular BAT of rodents fed with a high-fat diet since there is a physiological need to store energy (33). Beige/brite adipocytes would thus represent the transdifferentiated version of mature white adipocytes. The support for the transdifferentiation hypothesis is derived from observations that include (i) both white and brown adipocytes are derived from vascular endothelial cells of the adipose organ (35), (ii) cold exposure neither induces an increase in DNA content nor an increase in the number of adipocytes in murine WAT, despite the emergence of brown adipocytes (36,37) and (iii) paucilocular cells exhibit features that are intermediate between white and brown adipocytes, including mitochondrial pleomorphism during electron microscopic analysis (27). Additionally, the observations of a progressive decrease in the number of white adipocytes...
paralleled by an increase in the number of epithelial cells that form the functional adenomeres of the mammary glands during pregnancy and lactation in rodents, support the plasticity of the adipose organ and the transdifferentiation hypothesis (38,39). At the molecular level, transdifferentiation of white adipocytes into brown is strictly regulated by β-adrenergic receptors and the SNS since removal of β-adrenergoreceptors has been shown to largely suppress this phenomenon (23,40).

**Developmental origins of classical brown and beige/brite adipocytes**

Contrary to the principles of the transdifferentiation proposal for the origin of beige/brite adipocytes, the ‘classical’ or ‘constitutive’ brown adipocytes (32) (found in classical BAT depots such as interscapular BAT in mice) do not share their developmental origins with white adipocytes. It is now established that classical brown adipocytes originate from engrailed-1-expressing cells of the central dermomyotome and from myogenic factor 5 (Myf5)-positive precursors, much like myocytes do, whereas white and beige/brite adipocytes originate from Myf5-negative precursors (30,41–44). The ultimate cellular fate of these precursors is determined by a variety of cell-intrinsic and -extrinsic factors. For instance, expression of myoD and myogenin by β- extrinsic factors. For instance, expression of myoD and myogenin by β-negative Myf5+ precursors leads to the development of myocytes, whereas expression of PRD1-BF1-RIZ1 homologous domain containing 16 (PRDM16) by Myf5-positive precursors leads to the development of brown adipocytes (44,45). An overview of factors involved in brown adipocyte fate determination was provided by Shingo Kajimura from the University of California San Francisco Diabetes Center, USA, who highlighted that PRDM16 acts as a switch regulating the bidirectional cellular fate of myoblasts and brown adipocyte precursors. Depletion of PRDM16 was shown to induce loss of brown adipocyte characteristics and to promote overt skeletal muscle differentiation in brown adipocyte precursors, while its ectopic overexpression induced brown adipogenesis in committed myoblasts (45). PRDM16 has also been reported to be a cell-autonomous factor required for the development of beige/brite adipocytes in the murine subcutaneous WAT (46). Furthermore, Kajimura et al. (47) have reported that the binding of PRDM16 with the active form of CCAAT-enhancer-binding protein-β results in the formation of a molecular unit that is critical for the process of initiating the conversion of myoblasts into brown adipocytes. It is likely that PRDM16 has more binding partners that influence its capacity to induce brown adipogenesis.

The euchromatin histone methyltransferase 1 (Ehmt1)/GLP is an evolutionary conserved protein that targets lysine 9 of histone 3 of chromatin core to write the epigenetic code in mammals (48,49). Ehmt1/GLP haploinsufficiency has been shown to cause 9q34.4 subtelomeric deletion syndrome that is associated with childhood obesity among other anomalies such as mental retardation, brachycephaly and conotruncal heart defects in kids (50,51). Kajimura presented data indicating that Ehmt1/GLP interacts with the ZF domain of the PRDM16 to form an active complex that is required for PRMD16-mediated repression of myogenesis (unpublished observations). Loss of Ehmt1 in Myf5-positive brown adipocytes causes a severe loss of brown fat characteristics and induces muscle differentiation in vivo. Kajimura further presented data to indicate that Ehmt1/GLP stabilizes PRDM16 protein and is critically required for BAT development in vivo. Importantly, adipose-specific deletion of Ehmt1/GLP leads to cold intolerance, diet-induced obesity and insulin resistance in mice, further demonstrating its role in the thermogenic function of beige/brite adipocytes (unpublished observations).

In addition to PRDM16, bone morphogenetic proteins (BMPs) and other members of the transforming growth factor-β superfamily of proteins have been shown to play an important role in the cellular fate determination, proliferation and differentiation of brown adipocytes, both during embryogenesis and adulthood, which were discussed by Yu-Hua Tseng from the Joslin Diabetes Center, Harvard Medical School, USA. Certain members of BMP family participate in white adipogenesis, while BMP7 has been shown to be essential for embryonic brown fat development and to induce brown adipogenesis in multipotent progenitor cells (52). Additionally, BMP7 overexpression in mature brown adipocytes is associated with enhanced fatty acid uptake and oxidation via an up-regulation of CD36- and CPT1-dependent pathways (53). Similarly, other members of the transforming growth factor-β/ BMP superfamily such as growth differentiation factor 3, myostatin and BMP8B were shown to regulate browning of WAT depots and BAT thermogenesis in rodents (54–56), pointing towards an important role of BMP signalling in the development and thermogenic function of brown adipocytes. Activation of BMP signalling cascade requires binding of a ligand to two receptor types, type 1 and type 2 receptors (BMPR1 and BMPR2), which are serine threonine kinases that form a hetero-oligomeric complex that relays the signal to the downstream targets. Three type 1 receptors, activin-type receptor-like kinase 2, activin-type receptor-like kinase 3 (also known as BMPR1A) and activin-type receptor-like kinase 6 (also known as BMPR1B), as well as three type-2 receptors, BMPR2, activin-type-2A receptor and activin-type-2B receptor, are known to bind BMPs. Among these, BMPR1A has been shown to promote adipogenesis in mesenchymal stem cells, and genetic variations in its expression have been associated with human obesity. In order to study the role of BMPR1A in the development and thermogenic function of
brown adipocytes, Tseng’s group created a mouse model that had specific deletion of BMPR1A from the constitutive or classical brown adipocytes. Despite normal muscle development, these mice exhibit severe defect in embryonic BAT development that persisted as reduced classical BAT mass throughout their post-natal life. Interestingly, a reduction in the classical BAT was associated with compensatory browning of WAT depots of these mice that occurred in association with an enhanced SNS drive to the WAT depots (32). As the consequence of compensatory browning, the knockout mice were able to maintain normal body temperature even after prolonged cold exposure and were resistant to high-fat diet-induced obesity (32). Surgical denervation of classical BAT also induces loss/reduction of classic BAT mass and total compensation of its thermogenic activity by inducing browning of WAT depots (57), which would support this notion. Although the relevance of this compensatory phenomenon for human BAT development and thermogenic function remains to be determined, studying its molecular mechanisms may allow us to find therapeutic approaches that can induce the emergence of beige/brite adipocytes in human WAT depots.

**Molecular distinction between classical brown and beige/brite adipocytes**

Despite the functional (to become thermogenic upon stimulation) similarity among classical brown and beige/brite adipocytes, it is clear that beige/brite adipocytes maintain anatomical and developmental distinction in rodents. In addition, recent studies have indicated that beige/brite adipocytes exhibit a distinct molecular profile or gene signatures upon cyclic adenosine monophosphate stimulation (29), cold exposure (31) and peroxisome proliferator-activated receptor-γ agonist treatment (58–59), signatures that are not shared by either white or classical brown adipocytes. Kajimura presented data highlighting that murine beige/brite adipocytes expressed a panel of beige/brite selective genes upon rosiglitazone treatment that were not expressed by the classical brown adipocytes. The expression of beige/brite selective genes was further shown to correlate significantly with PRDM16 gene expression, an established marker of brown adipocytes (58). Additionally, Kajimura’s group identified Cbp/p300-interacting trans-activator, with Glu/Asp-rich carboxy-terminal domain, 1 (CITDEA1) as a beige selective marker whose protein expression could be seen in UCP1-positive beige/brite murine adipocytes (58).

Using these gene markers, Kajimura’s group further reported that human BAT samples isolated from multiple anatomic locations including cervical supraclavicular, posterior mediastinal, retroperitoneal, intra-abdominal and mesenteric depots of a paediatric cohort exhibited a beige-selective gene expression profile. Immunohistological presence of CITDEA1 was also reported in these samples suggesting that human BAT is beige/brite in nature (58). Similarly, using gene markers identified in their own study, Wu *et al.* (29) indicated that human BAT samples isolated from the supraclavicular depot obtained from two independent cohorts exhibited beige-specific gene expression profile. However, Tseng’s group recently observed that human neck fat exhibited a gene expression profile that resembled that of rodent classical BAT (60). Additionally, classical brown adipocyte-specific gene expression profile was reported in the interscapular fat depot of human infants in a separate study (61), corroborating the presence of classical brown adipocytes in humans. A further recent study reported an overlap of gene signatures for both classical brown and beige/brite adipocytes in the supraclavicular depot of adult humans (62). Taken together, all these studies have clearly ignited a debate on the nature of human brown fat and further raise the question of whether the presence and activity of classical brown adipocytes vs. beige/brite adipocytes is affected both by the site studied (63) and by the age, gender, ethnicity and metabolic status of humans. Moreover, demonstrations of the presence of both types of brown adipocytes in humans further highlight the need to understand the origin, regulation and activation of each of these cell types in humans.

**Central circuitries involved in the regulation of brown adipocyte thermogenesis**

Owing to its heat-producing capacity upon activation, BAT thermogenesis is believed to have evolved for the thermal protection of mammals from environmental stressors such as cold temperatures. Cold-induced thermogenesis (thermoregulatory thermogenesis) therefore represents the phenomenon that outlines the activation of BAT thermogenesis upon cold exposure in order to maintain optimum core body temperature (3). Regulation of optimum core body temperature (cold-induced thermogenesis) is ensured by brain circuitries that integrate environmental cues (i.e. cold exposure) with the body’s homeostatic mechanisms and communicate with BAT depots via the SNS. Hence, the system executes the ultimate homeostatic control on BAT thermogenesis (64). An overview of the central regulation of BAT thermogenesis with a focus on neuronal circuitries involved in cold-induced thermogenesis and sympathetic innervation of BAT was provided by Timothy Bartness from Georgia State University, USA and Shaun Morrison from Oregon Health & Science University, USA. That the SNS plays a critical role in the regulation of cold-induced thermogenesis was established by denervation studies, where upon denervation, no changes in blood flow, metabolic activity and BAT thermogenesis were observed upon cold exposure (65–67). Using Siberian hamsters as animal models and retrograde
transneuronal tract tracer techniques with the pseudorabies virus, Timothy Bartness and his team demonstrated that nuclei involved in the regulation of the SNS outflow to BAT (i.e. to interscapular BAT, being the most commonly studied classical BAT depot) are distributed in most brain areas including forebrain, midbrain and brainstem such as the preoptic area, zona incerta, suprachiasmatic nucleus, retrochiasmatic area, dorsomedial hypothalamus, paraventricular hypothalamus, lateral hypothalamus and arcuate hypothalamus, nucleus of the solitary tract, raphe pallidus and intermediolateral column (68). On the contrary, interscapular BAT does not exhibit significant parasympathetic innervations (69). Bartness’s group has further reported that majority of neurons found in the brain regions involved in the regulation of sympathetic outflow to BAT express the melanocortin 4 receptor (MC4R), indicating that the melanocortin system plays a major role in the control of BAT thermogenesis (70). In support of this, central injections of the MC4R agonist melanotan II in the third ventricle increase norepinephrine turnover rate in BAT (71), and UCP1 gene expression in BAT (72). Additionally, site-specific stimulation of MC4R in the sub-zona incerta (73) and the paraventricular hypothalamus (67) using single nanoinjection of a specific MC4R agonist was associated with an increase in BAT temperature, which was abolished upon treatment with an MC4R antagonist. Williams et al. (72) further reported that melanocortin circuitry within caudal brainstem is capable of regulating BAT thermogenesis independent of other brain centers, an observation that can form the basis of future pharmaceutical approaches for activating BAT. In addition to the sympathetic innervations, immunohistochemical presence of calcitonin gene-related peptide, an established marker for sensory nerves, has been reported in the nerves innervating BAT blood vessels and BAT parenchyma (74–76). Using anterograde tract tracers, Bartness’s group further reported that the central pathways involved in the sensory innervation of BAT overlap with the brain areas previously recognized to control sympathetic innervation of BAT, pointing towards the possible existence of sympathetic-BAT-sensory neuronal circuits that work together to ensure proper thermogenic outcomes in rodents (77). Capsaicin-induced BAT sensory denervation was shown to inhibit an increase in BAT temperature upon acute cold exposure, supporting this notion (77,78). Although the functional relevance of having sympathetic-BAT-sensory feedback loops remains uncertain at this time, it can be proposed that BAT possesses sensory neurons to provide thermal feedback to the central nervous system or to regulate lipolysis in brown adipocytes, as shown previously for white adipocytes (69,79).

Morrison continued the discussion on the central control of BAT thermogenesis by providing an overview of the neuronal circuits involved in cold-evoked BAT thermogenesis. He described that upon cold exposure, thermal receptors present in the skin and brain activate neural circuits responsible for inducing thermoregulatory effectors, which include heat conservation mechanisms such as cutaneous vasoconstriction, as well as thermogenic mechanisms such as shivering, cardiac and BAT thermogenesis (80). Indeed, BAT thermogenesis is the principal effector of cold-induced thermogenesis in acclimated animals and its activation is appropriately regulated by the central nervous system. In the cold-evoked, feedforward reflex pathway, temperature information is proposed to be sensed by the thermal receptors in the skin, such as the transient receptor potential family of cation channels member 8 that transmits this information to the dorsal horn of the spinal cord via primary somatosensory fibres. These fibres then reach the thermoregulatory integration site in the hypothalamus (median preoptic area) via the lateral parabrachial neurons (81,82). Activation of the median preoptic area leads to the disinhibition of tonic inhibitory neurons that project to caudal brain regions including the dorsomedial hypothalamus and the rostral raphe pallidus nuclei, whose excitation then stimulates the sympathetic preganglionic neurons of the intermediolateral column of the spinal cord that further connect with BAT via sympathetic postganglionic fibres (81,82). A critical role for rostral raphe pallidus neurons in regulating sympathetic activity to BAT was demonstrated by Morrison’s lab, when activation of rostral raphe pallidus neurons was shown to stimulate (83,84), and inhibition of these neurons reversed, the skin cooling-mediated increase in BAT sympathetic nerve activity (83,85). Glutamatergic and serotonergic neurons in the rostral raphe pallidus were shown to primarily regulate the excitability of BAT sympathetic preganglionic neurons (86–88). Additionally, Morrison’s group has demonstrated that BAT sympathoexcitatory neurons in the dorsomedial hypothalamus are under a gamma-aminobutyric acid-ergic tonic inhibition from the median preoptic area (89). In contrast to the stimulatory inputs, nucleus of the solitary tract regions, ventrolateral medullary regions and paraventricular hypothalamic regions were identified as inhibitory regulators of BAT sympathetic activation (90). Although the exact neuroanatomical and neurochemical signatures of the involved pathways remain to be ascertained, physiological factors including hypoxia, hypoglycaemia and glucoprivation were shown to reduce BAT sympathetic activation via activating neurons in these regions (91,92). Lastly, Morrison discussed a role for orexin neurons in the perifornical area of the lateral hypothalamus in the regulation of BAT activation (93,94). Anatomical observations from viral tract tracing studies indicate that orexin neurons of the perifornical area of the lateral hypothalamus are synaptically coupled to BAT and also project to rostral raphe pallidus (95,96), and both injections of orexin into rostral raphe pallidus, and glutamatergic activation
of orexinergic neurons by nanoinjections of N-methyl d-aspartate into the perifornical area of the lateral hypothalamus, produced a long-lasting activation of BAT-sympathetic activation and BAT thermogenesis (96). This supports a role for orexin neurons in the regulation of BAT thermogenesis. However, a basal level of BAT sympathetic activation, usually generated by a slight cooling of core body temperature, was critical for the orexin-mediated increase in BAT SNS activation (96), pointing towards a role for orexin in the rostral raphe pallidus to change the gain of response of BAT sympathetic premotor neurons of this region to their activating excitatory inputs (80).

Neuropeptides involved in the control of brown adipocyte thermogenesis

The role of orexin in the control of BAT thermogenesis and its peripheral actions were further discussed by Devanjan Sikder from the Sanford Burnham Medical Research Institute, Florida, USA. Orexin deficiency is associated with disordered sleep patterns of narcolepsy and obesity in both humans and mice (97). In order to understand the underlying mechanisms, Sikder and his team studied orexin-null mice and observed that despite a reduction in food intake, these mice exhibit weight gain on high-fat diet, owing to enhanced metabolic efficiency that results from inadequate BAT-mediated diet-induced thermogenesis (98). Closer inspection of interscapular BAT from orexin-null mice demonstrated a reduction in triglyceride accumulation that occurred due to poor differentiation of its brown adipocytes. Following development of BAT in these mice, Sikder’s group observed that orexin is required for the proper development and differentiation of BAT during pre- and post-natal life since BAT dysfunction could be rescued in the offspring when dams were injected with orexin during pregnancy. The group further demonstrated that orexin controlled brown fat differentiation via p38 MAP kinase and SMAD signalling (98). Sikder also presented data pointing towards the therapeutic potential of orexin, where systemic administration of orexin in diet-induced obese mice was associated with reduction in body weight and improved metabolic status in association with an increase in their BAT thermogenesis. Orexin therapy increased BAT mitochondrial content, thermogenic gene expression and core body temperature in these mice, while heart rate and blood pressure were not affected. Additionally, he pointed towards a role for orexin in browning of white fat mediated via orexin receptor 2 (unpublished observations). These observations may lay the basis for future clinical trials testing the role of orexin as an anti-obesity and anti-diabetic agent in humans.

Brian Oldfield from Monash University, Australia, followed up the discussion on the involvement of other hypothalamic peptides in the control of BAT thermogenesis, and the role of BAT function in a range of physiological, pathophysiological and therapeutic situations, using rodent models. An inappropriate activation of BAT, even at thermoneutrality, in association with the hypermetabolic status of mice with cancer cachexia, was observed by his team. This was proposed to occur as a result of inflammatory signalling to BAT (99). Similarly, activation of BAT thermogenesis was observed in the rodent models of adjustable gastric band surgery that may underlie the shift in energy balance usually observed in these mice after surgery (unpublished observations). In addition, Oldfield and his team have studied the role of a variety of neuropeptides known to contribute to the regulation of energy balance including orexin, melanocortin-concentrating hormone, endocannabinoids and melanocortins in the activation of BAT thermogenesis. Monotherapies with both orexin- and melanocortin-concentrating hormone receptor antagonists were shown to activate BAT thermogenesis. Even more substantial activation of BAT thermogenesis was observed after combined therapy of orexin- and melanocortin-concentrating hormone receptor antagonists (100). Correspondingly, the antipsychotic drug olanzapine induced a weight gain that was associated with lower UCP1 expression and a reduction in interscapular BAT temperature in rats upon chronic treatment (101). These effects were associated with enhanced Fos protein levels in a number of spinally projecting neurons with discrete hypothalamic and brainstem sites, including the perifornical area of the lateral hypothalamus where some neurons co-stained positively for orexin A, indicating a role for the orexin system in olanzapine-induced weight gain (101). Similarly, the cannabinoid 1-receptor antagonist rimonabant-induced weight loss was shown to associate with an increase in BAT temperature and UCP1 expression, which was blunted upon BAT denervation, indicating that central cannabinoid mechanisms participate in rimonabant-induced weight loss via the sympathetic control of BAT thermogenesis. Recently, glucagon-like peptide 1 signalling was also reported to activate BAT thermogenesis via central mechanism involving the sympathetic activation of BAT. However, this pathway was not critical for cold-evoked activation of BAT thermogenesis (102).

An additional pathway for activating BAT thermogenesis and more specifically for inducing ‘browning’ of WAT depots was presented by Mathieu John During from Ohio State University, USA. During’s group demonstrated that mice living in an enriched environment, which consisted of physically and socially more complex housing, were leaner than their control counterparts on regular chow and were also protected from diet-induced obesity and its related metabolic abnormalities (103). This protection was attributed to enhanced energy expenditure in these mice that was not related to an increase in their physical activity,
but to the emergence of brown-like adipocytes in the retroperitoneal WAT depot. During’s group further demonstrated that enriched environment-induced browning effects were adaptive in nature. Additionally, enriched environment was shown to induce an increase in both norepinephrine levels and β- and β1-adrenergic receptors in the retroperitoneal WAT depots, indicating that an enriched environment specifically enhanced the SNS responsiveness of WAT depots (103). During’s group had previously identified tumour-regressing effects of an enriched environment in mice. These effects were mediated by brain-derived neurotrophic factor (BDNF) via activation of the hypothalamic-sympathoneural adipocyte axis (104). Additionally, hypothalamic BDNF has been shown to contribute to the regulation of energy balance (105,106). Thus, During’s group investigated a plausible role for BDNF in the enriched environment-induced browning effects. Indeed, hypothalamic overexpression of BDNF was able to replicate the browning effects of an enriched environment, whereas hypothalamus-specific gene silencing of BDNF abolished the browning effects of an enriched environment, indicating that BDNF is involved in the enriched environment-induced browning phenomenon. Although the question of whether BDNF signalling is leading to the browning effects of an enriched environment remains to be ascertained, During’s team observed that long-term exposure to an enriched environment was associated with an up-regulation of thyroid-releasing hormone in the paraventricular hypothalamus of mice. These effects were mediated by brain-derived neurotrophic factor (BDNF) via activation of the hypothalamic-sympathoneural adipocyte axis (104). Additionally, hypothalamic BDNF has been shown to contribute to the regulation of energy balance (105,106). Thus, During’s group investigated a plausible role for BDNF in the enriched environment-induced browning effects. Indeed, hypothalamic overexpression of BDNF was able to replicate the browning effects of an enriched environment, whereas hypothalamus-specific gene silencing of BDNF abolished the browning effects of an enriched environment, indicating that BDNF is involved in the enriched environment-induced browning phenomenon. Although the question of whether BDNF signalling is leading to the browning effects of an enriched environment remains to be ascertained, During’s team observed that long-term exposure to an enriched environment was associated with an up-regulation of thyroid-releasing hormone in the paraventricular hypothalamus of mice. Thus, thyroid-releasing hormone-mediated up-regulation of the sympathetic drive to WAT could participate in the BDNF-mediated browning of WAT depots (103).

**Involvement of brown adipocytes in diet-induced thermogenesis**

In addition to cold-induced thermogenesis, BAT thermogenesis has been proposed to participate in (or fully mediate) diet-induced thermogenesis, a phenomenon that implies induction of extra energy expenditure under conditions of energy abundance, probably in order to maintain body weight. The relation of diet-induced thermogenesis to BAT was introduced in a seminal paper by Rothwell and Stock (107), but its presence and significance has been much debated, and diet-induced thermogenesis has on several occasions been suggested to be only a myth (108,109). However, evidence supporting its presence in animal models was presented and discussed by Jan Nedergaard from the Wenner-Gren Institute, Stockholm University, Sweden. Nedergaard first clarified the distinction between the obligatory and the facultative components of diet-induced thermogenesis, i.e. between the part of this thermogenesis that represents the energy costs of metabolizing the food, and the part that may be induced for metaboloregulatory purposes. The issue under discussion however was only the latter, the facultative part of diet-induced thermogenesis. Nedergaard emphasized that this part is difficult to approach; one formulation being that its existence can really only be established in its absence, i.e. if diet-induced thermogenesis is eliminated, animals would become obese without increasing their food intake. One way to examine possible development of diet-induced thermogenesis is to expose rodents to high-fat feeding or cafeteria-style diets in order to recruit and enhance BAT activity, which is further associated with an increased response to injected norepinephrine (107,110). That UCP1 is absolutely necessary for these diet-induced adaptive adrenergic thermogenic effects was demonstrated by studies conducted on UCP1-ablated mice. In such mice, the obesogenic diets no longer recruit an enhanced response to norepinephrine (110). However, injecting norepinephrine is not a very physiological way to activate diet-induced thermogenesis, and Nedergaard, therefore, further presented a series of studies that demonstrated an acute activation of thermogenesis during meal time – showing that this activation was meal dependent and could be recruited with prolonged time on an obesogenic diet. Additionally, meal-induced thermogenesis was much reduced and no recruitment was observed when these studies were performed in UCP1-ablated mice, pointing towards the involvement of BAT activity in this phenomenon. Furthermore, such UCP1-ablated mice became obese on chow and high-fat diets – but only when kept at thermoneutrality (110). Nedergaard pointed out that diet-induced thermogenesis is, therefore, one of several physiological phenomena where the outcome is different based on whether animals are kept at normal animal housing temperatures (that in reality is a cold stress for the animals) or when they are kept at thermoneutrality (111); thermoneutrality being a condition that is much more like the conditions under which humans normally live. 

Nedergaard next discussed the question of how diet-induced thermogenesis is induced; whether the induction is based on the total amount of calories ingested, on the nature of the food (e.g. lipid vs. carbohydrate) or on the result of overeating, i.e. on obesity. Nedergaard pointed out that several conditions that lead to overeating are not per se associated with induction of diet-induced thermogenesis, and that a switch in food constituents does not necessarily lead to a chronic increase in BAT activity, leaving obesity as a likely candidate for inducing diet-induced thermogenesis. This may seem contradictory at first but Nedergaard pointed out that even in humans, there is evidence that increased obesity can be associated with increased UCP1 gene expression (112,113). Indeed, recruitment of diet-induced thermogenesis by increased obesity is exactly what would be expected of a homeostatic system trying to stabilize body weight. Nedergaard further discussed...
leptin as a candidate factor that could trigger the signalling underlying such phenomenon since leptin is generally accepted to promote thermogenic reactions in the body and is also known to activate BAT (114). There are only a few studies examining the source(s) of leptin-induced thermogenesis, but Nedergaard pointed to convincing studies implying that leptin-induced thermogenesis is totally located to BAT (115). Furthermore, there are classical studies demonstrating that the development of obesity in leptin-less rodents can be partly attributed to their inability of inducing diet-induced thermogenesis – despite their ability to demonstrate a normal cold acclimation-recruited non-shivering thermogenesis (116). Nedergaard, therefore, concluded that it is likely that a pathway exists that leads from the onset of obesity to an activation of diet-induced thermogenesis – and if this pathway does not work as intended, obesity will ensue.

Now, when it is accepted that humans possess BAT, these pathways should also be relevant for human obesity, and it is likely that an impairment in such pathways leads to the development of obesity in humans. A possibility can be further sketched in which human BAT activity is balanced between inhibitory effects of glucocorticoids and promoting effects of sex hormones. Therefore, in younger humans, BAT should be very active, whereas during late middle age, the lowering of sex hormone activity would mean that the inhibitory effects of glucocorticoids would become dominating, and BAT involution would occur (117). This may be one of the reasons for the increasing prevalence of obesity with age, with its morbidity consequences. To keep up a good activity of BAT may therefore counteract the development of obesity with increasing years.

**Presence and metabolic activity of brown adipocytes in humans**

The recent interest in BAT thermogenesis is fuelled by the observations that adult humans possess BAT (4). Both retrospective and prospective studies utilizing detection of 18-fluoro-deoxyglucose (18F-FDG) uptake using positron emission tomography/computed tomography (PET/CT) scanning as a measure of BAT presence have proved instrumental in demonstrating the presence, as well as activity, of BAT in humans of most age groups (4–9). BAT has been identified at supraclavicular, cervical, paraspinal, para-aortic and perirenal regions in humans. Additionally, large cohort studies analyzed in a retrospective manner have allowed us to identify physiological factors that contribute to the prevalence of BAT in humans (9,118). Denis Richard from the Quebec Heart and Lung Research Institute, Canada, provided an overview of factors affecting the presence and activity of BAT in humans. A variety of factors including outdoor temperature, seasonal variation, age, gender, body adiposity and presence of diabetes have been identified to affect the detection of BAT in humans in multiple studies (6,9,118,119). While colder outdoor temperature and winter season are associated with enhanced detection of BAT, older age, male gender, increased body weight and fat mass as well as presence of diabetes is associated with lower detection of BAT in humans (64). In addition, there is some evidence to indicate that thyroid status (120) and enhanced SNS activity resulting in significantly increased circulating norepinephrine levels as seen in case of phaeochromocytomas associate with enhanced detection of BAT in humans (121).

Richard’s team has further demonstrated that human BAT is metabolically active and contributes to significant energy expenditure upon activation in humans (122). In a recent study by the group, an 80% increase in the basal metabolic rate of healthy participants was observed upon a 3-h cold exposure, part of which could be attributed to the activation of BAT thermogenesis. An increase in radio-density of BAT was also observed, indicating that the intracellular triglycerides stored within brown adipocytes served as the major fuel for enhanced metabolic activity of BAT in these subjects (122). Continued BAT stimulation may also enhance the utilization of circulating non-esterified fatty acids (derived from WAT lipolysis) or of fatty acids from circulating lipoproteins (123), released due to lipoprotein lipase activity (124), which may lead to their clearance from the circulation in humans. Uptake of circulating triglyceride-rich lipoproteins by BAT upon cold exposure has been reported in mice, which would support this notion (125). Similarly, Richard’s team reported positive associations between expression of UCP1 (and other genes involved in thermogenesis) and circulating triglyceride and HDL cholesterol levels in the epicardial adipose tissue of humans (126). This study points towards a direct or indirect functional association between the presence of brown adipocytes and circulating lipids in humans.

Masayuki Saito from Tenshi College, Japan, further extended the discussion on human BAT. He presented data from dedicated studies using slight cold exposure prior to measuring 18F-FDG uptake in healthy volunteers to indicate that presence of BAT in humans is affected by age, seasonal variation, body mass index and body fat mass. Confirming the observations of retrospective studies, older age, summer months and higher body mass index were found to associate with lower detection of BAT in healthy humans (127). Additionally, Saito’s team observed that, despite similar body mass index, body fat mass as well and basal energy expenditure at thermoneutrality (27°C), BAT-positive subjects (those with 18F-FDG uptake in supraclavicular and paraspinal regions), upon cold exposure exhibited significantly higher energy expenditure compared with BAT-negative subjects (with no appreciable 18F-FDG uptake) (128). In addition, the cold-induced drop in supraclavicular skin temperature was observed.
to be lower in BAT-positive subjects compared with BAT-negative subjects. A positive correlation between energy expenditure and 18F-FDG uptake activity was also observed in BAT-positive subjects, indicating that activation of BAT contributes to the observed cold-induced thermogenesis in these subjects (128). Using a separate cohort, the group further reported that the incidence of cold-activated BAT prevalence was much higher in younger individuals, such that more than 50% subjects were BAT-positive in twenties, but less than 10% subjects in their fifties and sixties were BAT positive (129). Moreover, adiposity-related parameters such as the body mass index, total body fat mass and abdominal fat mass did not change with age in BAT-positive subjects, whereas these parameters increased with age in BAT-negative subjects, pointing towards a protective effect of BAT presence in age-associated development of obesity in humans (129). Besides cold-induced thermogenesis, Saito’s group also investigated diet-induced thermogenesis in dedicated studies and reported that some dietary agents such as capsinoids can increase energy expenditure in BAT-positive subjects. However, no change in skin temperature was observed in these subjects (130). Similar to capsinoids, Aframomum melegueta (grains of paradise), a species of the ginger family, extract also enhanced energy expenditure in BAT-positive subjects (131). These observations would point towards the plausible involvement of human BAT in enhancing energy expenditure by these dietary agents; however, this is yet to be demonstrated.

Therapeutic utility of brown adipocytes for treating obesity

Eric Ravussin from the Pennington Biomedical Research Center, Baton Rouge, USA, addressed the question of the therapeutic utility of BAT thermogenesis for human obesity. Although the presence of BAT in humans through most ages at this time is now generally accepted, the actual prevalence of BAT in humans of different age groups has not been optimally determined so far. Currently, measuring 18F-FDG uptake using PET/CT scanning is the most commonly used ‘non-invasive’ method for determining the presence of activated BAT in humans. Various studies using this method have reported the BAT prevalence in adult humans to vary from 2% to 100% (5,9,132,133). This variation can be explained based on the nature of the study being retrospective (normally yielding BAT prevalence much below 10 % as reviewed by Nedergaard and Cannon (132)) or prospective (yielding much higher prevalence), subject characteristics or their cold exposure conditions. Ravussin pointed out that PET/CT scanning methods are limited in their capacity to reflect on the true prevalence of BAT in humans. While PET/CT methods cannot fully discriminate between the presence and activity of BAT, the reproducibility of the data obtained using these methods remains somewhat low (only 13–15%) (134,135) but this is likely mainly due to variations in examination room conditions, etc., between the repeated observations. Additionally, inclusion of healthy subjects and their repetitive measurements in such studies is hampered by high doses of radiation exposure that is generally required for PET/CT scanning. Thus, it is important to explore alternative approaches that can possibly overcome the limitations of PET/CT methods.

Some of these alternatives include iterative decomposition with echo asymmetry and least squares estimation magnetic resonance imaging, infrared thermography and utilization of negative Hounsfield units during CT scanning. Infrared thermography may also be relevant for assessing the amount of BAT in adults, children and babies. Using better techniques in future studies may allow us to study human BAT more closely, estimate its presence and activity, and further understand its plasticity under various physiological and pathophysiological conditions (136).

In terms of the therapeutic utility of BAT thermogenesis in humans, Ravussin pointed out that BAT mass and BAT activity represent two separate parameters in the equation of BAT-mediated increase in energy expenditure in humans. While central mechanisms aimed at the recruitment of BAT represent one avenue to increase BAT mass, promoting the differentiation of adipocyte precursors within adipose tissue depots into brown adipocytes represent another approach to achieve greater BAT mass. However, considering that prospective studies have pointed towards near 100% prevalence of BAT in (younger) humans, it might be important to understand the factors that interfere with the activity of already existing BAT in humans.

Even if there is no doubt that BAT can be activated by behavioural or pharmacological means, it is doubtful that BAT activation will be a viable strategy for weight loss. Based on the recent studies reporting enhanced energy expenditure in humans upon stimulation of BAT thermogenesis (122,137), it is conceivable that activation of BAT may rather be developed as a strategy for maintenance of weight loss achieved by other therapies. Indeed, BAT activation would counteract the ‘metabolic adaptation’ occurring in response to weight loss.

In terms of its magnitude, Ravussin highlighted the estimations made by Rothwell and Stock (107) in their seminal study where 50 g of active BAT was projected to burn additional 125 kcal d−1 in humans. Similarly, more recent studies have estimated that 63 g of active BAT could burn an additional 126 kcal d−1 (5) or 31–329 mL of BAT could contribute to an additional expenditure of 8–100 kcal d−1 in humans (122). A constant long-term activation of BAT thermogenesis may thus help in
sustaining weight loss achieved by pharmacological and/or lifestyle interventions.

Considering that energy balance is a tightly regulated phenomenon, it is likely that BAT-mediated increase in energy expenditure may activate metabolic adaptations or counter-regulatory mechanisms in the body, which could oppose weight loss, in a similar fashion to what is seen with caloric restriction- or exercise-induced weight loss programmes (136). Ravussin’s group has previously reported an unexplained drop in the resting metabolic rate of the participants of a weight loss programme (The Biggest Loser), despite the restoration of fat-free mass to a large extent (138). Although the metabolic adaptations underlying the reduction in resting metabolic rate upon weight loss remain largely unknown, data indicates that the reduction in body fat stores results in lower circulating leptin levels. Lower circulating leptin levels may cause a reduction in the sympathetic drive and in thyroid activity in the body, which would slow body metabolism and may lead to body-weight regain (136). Whether BAT-mediated weight loss would promote similar metabolic adaptations and counter-regulatory mechanisms – including an increase in food intake remain to be determined. Thus, although BAT thermogenesis is an attractive candidate, we are still in the preliminary stages of utilizing this tissue as a therapy for obesity. It would be important to practice ‘caution in extrapolating indirect histological and thermographic evidence to a major role for BAT in human energy metabolism’ as urged by Rothwell and Stock (139) and reminded to us by Ravussin.

**Conclusions and outstanding questions**

With the renaissance of interest in BAT as a thermogenic tissue in adult humans, these are exciting times for obesity researchers to examine and develop the potential of this tissue as an anti-obesity target in humans. Rapid increase in the number of publications involving BAT since 2007 can vouch for the current interest in understanding this depot. In addition to the demonstrations of BAT presence and its metabolic activity in humans, we have made significant progress in our understanding of the developmental origins and nature of brown adipocytes. Significant progress has also been made in our understanding of the brain regions and neuronal circuitries that are involved in the regulation of BAT thermogenesis. Additionally, a variety of neuropeptides and peripherally derived circulating factors (such as irisin, fibroblast growth factor 21 and natriuretic peptides) that can serve to enhance BAT thermogenesis in rodents and plausibly in humans have now been identified (140–142). The 11th Stock Conference was, therefore, held very timely with a focus on BAT. In addition to the discussions of our progress to date in each domain, conference participants in the final discussion also recognized a variety of important research questions (given below) that would enhance our current understanding of BAT, and may further allow us to realize the goal of utilizing its thermogenic potential as an anti-obesity tissue in humans.

1. What are the specific molecular players that participate in the development and differentiation of beige/brite adipocytes?
2. Why do various WAT depots differ in their browning capacity? What are the molecular players that support browning in one WAT depot relative to other?
3. How do sex hormones and glucocorticoids participate in the browning of WAT depots and activity of classical BAT?
4. Do the central regulation of beige/brite vs. classical brown adipocytes differ?
5. Are there separate neural pathways regulating the browning of WAT relative to activation of classical BAT depots?
6. Does the physiological relevance of classical brown adipocytes and beige/brite adipocytes differ? If yes, can compensatory mechanisms be targeted to support the development of one type of brown adipocytes relative to the other in humans?
7. Can cold exposure be utilized as a means to activate human BAT?
8. If diet-induced thermogenesis exists in humans, what is its magnitude in terms of activating BAT thermogenesis?
9. Do omega-3 fatty acids serve to activate or regulate UCP1 activity? If yes, can dietary intake of omega-3 fatty acids allow us to active human BAT?
10. Do peripheral factors such as fibroblast growth factor 21, irisin and natriuretic peptides interact with the SNS to execute their regulation of BAT thermogenesis? Can these factors be safely used clinically to activate brown adipocytes in humans?
11. From the perspective of integrative physiology, do various metabolically active organs including liver, heart, skeletal muscle and brain participate in the regulation of BAT thermogenesis in a collaborative or antagonizing manner?

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