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Title: Human Brown Adipose Tissue as a Target for Obesity Management; Beyond Cold Induced Thermogenesis

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Running Title: BAT adaptive thermogenesis in humans

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Abstract

Elevating energy expenditure via adaptive thermogenesis in brown adipose tissue (BAT) is a potential strategy to reverse obesity. Much early enthusiasm for this approach, based on rodent studies, was tempered by the belief that BAT was relatively inconsequential in healthy adult humans. Interest was reinvigorated a decade ago when a series of studies re-identified BAT, primarily in upper thoracic regions, in adults. Despite the ensuing explosion of pre-clinical investigations and identification of an extensive list of potential target molecules for BAT recruitment, our understanding of human BAT physiology remains limited, particularly regarding interventions which might hold therapeutic promise. Cold-induced BAT thermogenesis (CIT) has been well studied, though is not readily translatable as an anti-obesity approach, whereas little is known regarding the role of BAT in human diet-induced thermogenesis (DIT). Furthermore, human studies dedicated to translating known pharmacological mechanisms of adipose browning from animal models are sparse. Several lines of recent evidence suggest that molecular regulation and physiology of human BAT differs to that of laboratory rodents, which form the majority of our knowledge base. This review will summarise knowledge on CIT and expand upon the current understanding and evidence gaps related to human adaptive thermogenesis via mechanisms other than cold.

Keywords: brown adipose tissue, BAT, adaptive thermogenesis, cold-induced thermogenesis, CIT, diet-induced thermogenesis, DIT, obesity, facultative thermogenesis, postprandial thermogenesis

Abbreviations: AR, adrenergic receptor; BAT, brown adipose tissue; BeAT, beige adipose tissue; cBAT, classic brown adipose tissue; CIT, cold induced thermogenesis; CNS, central nervous system; DIT, diet-induced thermogenesis; FDG, ^{18}F-fluorodeoxyglucose; PET/CT, positron emission tomography/computerised tomography; PPARγ, peroxisome proliferator-activator receptor γ; SNP, single nucleotide polymorphism; SNS, sympathetic nervous system; T3, triiodothyronine; T4, thyroxine; TAG, triacylglycerol; TRP, transient receptor potential; TRPV1, transient receptor potential cation channel subfamily V member 1; TSH,

thyroid stimulating hormone; TZD, thiazolidinedione; UCP-1, uncoupling protein-1; WAT, white adipose tissue.

Introduction

Obesity is associated with several comorbidities such as type 2 diabetes, certain cancers and several forms of cardiovascular disease. In recent years, the incidence and prevalence of obesity and its associated diseases has been increasing [1, 2], creating unsustainable pressure on healthcare systems and budgets globally [2]. Lifestyle interventions such as physical activity and diet, as well as current and past pharmacological approaches, have had limited success in sustaining weight loss [3]. On this background, it is necessary to explore new approaches which support enduring weight loss across the lifespan.

Brown adipose tissue (BAT) is currently an attractive therapeutic target to manage obesity and is a highly fertile field of preclinical and clinical investigation. Current research is predicated on BAT's key function of heat production, which, in a physiological context, is invoked primarily in response to acute cold exposure. During this process, the increase in BAT cell energy expenditure is mainly released as heat into the surrounding blood stream where it can be systemically distributed [4]. Importantly, chronic stimulation in this manner causes BAT adaptation, resulting in enhanced capacity for heat production and thus energy expenditure. Conceptually, this ability to increase energy expenditure via 'adaptive thermogenesis' has been proposed as a potential therapeutic approach for the management of obesity and its complications. At present, there are no therapeutics that specifically target increased energy expenditure via BAT. However, this is a field of intense investigation, particularly in rodents and more recently in humans.

Interest in the study of BAT has exploded in the past 5-10 years, largely in response to *in vivo* demonstrations of functional BAT in human adults [5-9]. Preclinical research has resulted in development of well-characterised, immortalised human BAT cell lines [10, 11] and revealed many possible therapeutic targets ranging from genes that regulate BAT development, proliferation and function, as well as small molecules and hormones that could increase BAT recruitment and thermogenesis. These factors have been recently reviewed extensively [12, 13] and will not be the focus of the present review.

While our understanding of human BAT has expanded during recent years, it has become increasingly clear that there are differences in BAT function between species, and that there is a relative dearth of knowledge in humans. This review will outline the current understanding of cold-induced thermogenesis (CIT) in human BAT, then expand on other mechanisms of BAT thermogenesis; primarily diet-induced thermogenesis (DIT) as well as mimicking CIT via pharmacological means. We will also discuss species differences which are becoming increasingly evident and must be fully understood in the context of future therapeutic development.

BAT function and contribution to whole body energy metabolism

Adipocytes are currently classified into three broad sub-types: white adipose tissue (WAT), classic brown adipose tissue (cBAT) and brite, or beige adipose tissue (BeAT) [14, 15]. The molecular and functional characteristics of each broad subtype have been reviewed recently [16]. These tissues are primarily distinguished by differential expression of uncoupling protein-1 (UCP-1), which has a key role in heat generation (higher for cBAT). When UCP-1 is inactive (eg. during 'coupled' respiration), the proton gradient generated is linked to the ATP synthase enzyme complex, resulting in ATP resynthesis. However, when UCP-1 is present and activated, it initiates a proton leak whereby the energy stored in the proton-motive force is released as heat instead of ATP production [4].

From an energy regulatory standpoint, white adipocytes function to store lipids predominately in the form of triacylglycerol (TAG) in a single large droplet, but have low mitochondrial density, UCP-1 content and oxidative capacity. cBAT adipocytes, in contrast, are more flexible, whereby when adapted to thermoneutral conditions will have a relatively low oxidative capacity. When a prolonged cold stimulus is applied, classic brown adipocytes increase turnover of intracellular and extracellular-derived lipids through dramatically increased oxidative capacity and UCP-1 content [17]. Subsequently, cold-adapted cBAT can increase whole body energy expenditure 2-4 fold above resting levels in cold exposed mice [4]. Although both cBAT and WAT are classified as adipose tissues, in addition to this

obvious functional divergence, they possess distinct developmental lineage and molecular characteristics [18, 19].

The concept of transitions between brown- and white-ness for each tissue is not new, particularly in reference to 'browning'. This refers to the acquisition of functional brown adipose characteristics in any adipose tissue [17], and could include processes of proliferation, differentiation and/or recruitment. Most commonly, however, the term is used in reference to appearance of brown-like adipocytes in WAT, therefore alternatively termed 'beiging' [20]. The browning process in WAT generally results in an increase in UCP-1 protein expression, which may be accompanied by BAT hyperplasia and/or hypertrophy. More contemporary animal work demonstrates that browned white adipocytes have generally arisen from the distinct cell type now most commonly termed beige adipocytes. Depending on the prevailing physiological conditions, these cells demonstrate variable degrees of brown or white characteristics [21, 22]. In rodents, the increase in energy expenditure associated with near maximal browning of cBAT and BeAT is functionally relevant for whole body energy metabolism and weight control [23-25, 15]. Notably, while recent evidence demonstrates browning of adipocytes in WAT depots in mice results in thermogenic cells capable of uncoupled respiration [25, 15], cBAT most likely remains the primary [15], and potentially only [26] physiologically relevant contributor to whole body cold and adrenergic agonist-stimulated thermogenesis. In addition to cold-stimulated heat production, in mice, DIT is also significant [27]. This is likely cBAT rather than BeAT dependent, as high energy diets in mice result in expansion and browning of cBAT, but whitening of beige adipose [28]. Recent evidence from mice on the context-dependant nature of cold-induced browning of cBAT and BeAT suggests similar further attention is required in both laboratory animals and humans [17].

Studies based on morphology and whole-body physiology from the 1960's suggested that BAT is present and functional in human infants [29, 30]. This evidence was not based on molecular characterisation of adipose tissues, but since infants have poorly developed capacity to shiver and contain distinct BAT depots, it was inferred that BAT contributed to the near doubling of energy expenditure observed at ~25 °C compared to ~35 °C [29]. Recent

evidence identifying *bone fide* BAT in infants suggests this is likely mediated by cBAT which is present in human infants, but less so in adults [31]. Cold-exposed adult BAT contributes relatively less to whole body energy expenditure. In non-shivering, mild-cold exposed adults, 10-20% increases in energy expenditure have been reported [7, 8]. While more severe cold exposure further increases thermogenesis [32], it is difficult to tease out the relative contribution of BAT (**Table 1**). This is further complicated by variation in cooling protocols used by different laboratories. Rodent studies demonstrate that increased skin surface area results in greater sympathetic nervous system (SNS) outflow to BAT [33]. Thus, it is likely that experimental methods involving whole body cooling [32, 34] will maximise the SNS signal and therefore the thermogenic activity of BAT. Best estimates suggest BAT would account for approximately 1-7% of the increase to whole body energy expenditure in humans [35-38].

The relatively small contribution to whole body energy expenditure attributed to BAT in humans has provoked suggestion that it is virtually irrelevant to whole body energy turnover and therefore obesity management [39]. However this relevant concern cannot yet be fully justified based on our current understanding of human BAT. It should be evident that modern research techniques have not studied human BAT under conditions of extreme thermogenic adaptive pressure, such as in rodents, where it has been pushed to its limits of oxidative capacity (eg, the equivalent of weeks-months, continuous exposure of mice to <4 °C) [36]. Further, the capacity to clear systemic glucose and lipids is highly relevant to maintenance of metabolic health in the absence of significantly increased energy expenditure. Recent mouse [40] and human [41, 42] studies support the notion that the key therapeutic role for BAT could be in reducing risk associated with hyperglycemia, hyperlipidemia and ectopic lipid deposition.

BAT and the species divide

Our understanding of BAT is predominately based on the study of laboratory animals, which identify it as an organ responsible for heat production in response to cold exposure via SNS

activation [4]. In mice, adipose tissue exists in distinct regions with major depots being generally representative of one of the three sub-types of adipocyte [43]. While not in complete contrast, humans differ such that an almost continuous subcutaneous layer of WAT is present throughout the majority of the body. Other human WAT depots such as visceral and mesenteric adipose express brown markers [44, 45]. However, the regions which contribute meaningfully to human adult cBAT and BeAT are likely to be those observable in cold-exposed individuals via ^{18}F-fluorodeoxyglucose (FDG) positron emission tomography/computerised tomography (PET/CT) and include: deep upper thoracic/supraclavicular, axillary, cervical, paravertebral, mediastinal and renal [46-48]. Other relevant depots may exist (eg. pericardial adipocytes) but have yet to be well characterised.

The prominent BAT depots evident via PET imaging in humans appear as a mixture of small discrete islands (~10g; eg. paravertebral and mediastinal) or larger, often interconnected networks (upper thoracic) that may be over 100g in some individuals. Interestingly, mice contain analogous networks in the deep cervical region extending into the axillae which are cBAT. Based on several studies involving molecular characterisation of human supraclavicular and/or cervical adipose and mouse brown and beige adipose tissues, the majority of adipocytes in these depots in humans are a mixture of BeAT and cBAT [49, 50]. While most adipocytes are currently presumed to be BeAT [51, 14], with a predominance of cBAT found only in the deepest cervical regions [49], the relative proportions in each region remain subject to further investigation [17]. Critically, no relevant quantity of interscapular cBAT has been identified in adults.

Responsiveness of human BAT to canonical activation signals mirrors that of mice. Acute cold exposure increases BAT activity [6, 41, 52, 32] and regular daily cold exposure results in adaptive thermogenesis [53, 54, 34, 55] in a timeframe similar to mice (~2-4 weeks) [4]. It is unknown whether the relative degree of adaptation in human BAT in response to chronic cold differs from mice. After a period of adaptation in humans, an equivalent degree of mild cold exposure increases BAT activity, measured via FDG-PET/CT by approximately ~25-

60% more than prior to adaptation (**Table 2**). This has been associated with a doubling of BAT total oxidative metabolism [34]. The well-established several-fold increase in energy expenditure in mice after cold adaptation has been attributed solely to BAT [4], and represents a greater overall contribution to whole body energy expenditure than in humans. While the maximal rate of oxygen consumption per unit of tissue volume in humans is unlikely to approach that of mice, we hypothesise that increases beyond those observed to date in humans are likely possible given the extremes of human BAT have not, and probably will not, be tested in a research setting.

All adrenergic receptors (AR) (α_1, α_2, β_1, β_2, and β_3) are expressed in adipose tissue. These receptors respond to catecholamine signals from the central nervous system (CNS) and the circulation and mediate the majority of the key adipocyte functions related to regulation of energy substrate storage and expenditure [4, 56]. All β-ARs are required for maximal induction of thermogenesis in mouse cBAT [57]. Moreover, while the β_3-AR is expressed in tissues such as skeletal, smooth and cardiac muscle and WAT at low levels, it is now well established that high levels of β_3-AR expression are almost exclusive to cBAT and BeAT, and are primarily responsible for thermogenesis [57, 4, 56]. Interestingly, the β_3-AR is also expressed highly and is the predominate β-AR in the human detrusor urinae muscle, a smooth muscle which controls contraction and relaxation of the bladder and is now the target of approved therapy for overactive bladder [58, 59]. Although AR agonists increase BAT thermogenesis in both mice and humans [27, 36, 60], only limited evidence is available regarding β_3-AR agonist signalling in humans. A lack of efficacy of β_3-AR agonists in humans for obesity management during the 1990s served to foster the notion that humans contained no functional BAT. The failure of early ligands was primarily attributed to differences between the rodent and human β_3-AR, resulting in poor binding kinetics [61], low bioavailability and/or that human BAT/WAT may contain low levels of β_3-AR [62, 57, 4]. Second-generation β_3 agonists were also unsuccessful, supposedly due to low potency for human β_3-AR and poor oral bioavailability [63-65]. More recently, and unlike prior drugs in this class, the β_3-AR agonist mirabegron has been shown to have higher *in vitro* binding

affinity to human β_3-AR and possesses satisfactory bioavailability [66]. A single dose of this drug in humans increases FDG uptake in supraclavicular BAT [60]. It is, however, yet to be determined whether mirabegron-stimulated FDG uptake in BAT is linked to classic uncoupled thermogenesis, as distinct from receptor-stimulated glucose disposal, storage and/or coupled oxidation. Lastly, based on comparison of separate human and mouse studies, the absolute increase in BAT activity and general thermogenesis induced by β_3- and pan-β-AR agonists is lower in humans compared to mice.

Beyond differences in the volume, anatomical distribution and oxidative capacity of cBAT and BeAT between species, is accumulating evidence of disparate functional and adaptive responses, particularly regarding pharmacological mechanisms of activation and adaptation. Three distinct pharmacological approaches have now been reported to elicit divergent responses between species during both *in vivo* and *in vitro* experiments. All-*trans* retinoic acid increases browning of mouse adipocytes in culture, but impairs browning and differentiation of cultured human BAT cells [67]. Chronic treatment with the pan-β-AR agonist ephedrine increases BAT adaptive thermogenesis in mice [68]. In contrast, we demonstrated that chronic ephedrine treatment downregulated BAT activity in humans [69]. Ramage *et al.* [70] also demonstrated contrasting cross-species effects, whereby acute prednisolone treatment decreased and increased thermogenic responses in mice and humans, respectively.

Disparate BAT responses also occur with respect to exercise. Endurance exercise training increases BeAT in mice, putatively via secretion of skeletal muscle contraction induced secretory factors such as irisin and meteorin-like protein 1 [71, 72]. Conversely, while no human studies have definitively determined whether endurance training results in browning of adipose tissues, current evidence suggests the reverse. In a cross-sectional study, young highly endurance trained males had lower cold-stimulated BAT activity compared to a matched sedentary group [73] and in a separate intervention study 2 weeks of training decreased insulin-stimulated FDG uptake in previously untrained healthy men [74]. Short-

term intense endurance training (10 days), also exerted no browning effect on human subcutaneous WAT in previously untrained young healthy males [75]. Skeletal muscle secretory factors (myokines) are theorised to drive adipose tissue browning as an evolutionarily conserved mechanism to complement shivering thermogenesis [71, 76] and this mechanism may be operative in humans [76]. Nevertheless, *in vivo* evidence thus far from trained mice does not translate to humans.

To date, no pharmacological approaches have been proven to promote BAT adaptive thermogenesis in humans. Given the aforementioned species disparities in physiological and pharmacological activation of BAT, careful study is required to establish human relevance prior to pursuing potential therapeutic avenues.

The imperative to characterise human BAT

Vella *et al.* [77] recently highlighted concern regarding a reduction in support for clinical mechanistic research from shrinking research budgets in the USA, and that this is of particular concern for diabetes research. The recent surge in attention for BAT as a therapeutic target for metabolic disease, combined with the issues described above, should highlight that small, tightly controlled, rigorous clinical studies are integral in the therapeutic development pipeline. This does not detract from the need for continued pre-clinical studies, but highlights the necessity for studies in clinically relevant animal models performed in parallel with human investigations. To overcome the species divide, other fields such as immunology, regenerative medicine, and infectious diseases have created humanised mice using targeted mutations and engraftment of human haematopoietic stem cells or peripheral-blood mononuclear cells (PBMCs) [78]. Likewise, while not a new concept in BAT research [79, 4], there is a necessity that research animals should be studied under physiologically relevant, thermally neutral conditions [80, 47, 17]. Comfortable ambient room (and animal research facility) temperature (eg. ~20-22 °C) for humans is a cold stress for mice; temperatures of ~30-33 °C are necessary for thermoneutrality in mice. Indeed, this should be an important consideration for any animal research involving energy metabolism.

Obesity and human BAT function; cause or consequence?

It was hypothesised nearly 40 years ago that obesity may be secondary to reduced energy expenditure arising from dysfunctional BAT [81]. In support of this hypothesis, mouse strains with highly inducible BeAT UCP-1 content (eg, A/J and 129) are resistant to obesity in comparison to C57BL/6J mice, which are relatively obesity prone and have less inducible UCP-1 [82, 23, 24]. In humans, numerous studies indicate that BAT activation in obese individuals is impaired in response to a variety of stimuli including cold exposure [6, 7, 41, 83], the sympathomimetic, ephedrine [84] and insulin [85]. Further, two separate studies have reported increased BAT activity in obese individuals who lost weight after a conventional lifestyle modification program [85] and morbidly obese individuals via laproscopic adjustable banding [86]. It is unknown, however, whether BAT dysfunction precedes obesity in humans and may contribute to causation, or whether dysfunction parallels and/or follows the onset of obesity.

Cause? Numerous mouse models with genetically defective BAT function are prone to obesity [87], arguing for a causative role of BAT dysfunction with regard to obesity. In humans a single nucleotide polymorphism (SNP) in the *UCP1* gene promoter (A-to-G, rs1800592) was identified over 15 years ago and has been studied in many populations. While some early reports indicated reduced expression and association with obesity, altogether the evidence is conflicting and ultimately insignificant (for a comprehensive meta-analysis see [88]). Of course, numerous genes beyond *UCP1* are required for optimal BAT function. Therefore, the recent connection between impaired BAT thermogenesis and a SNP in the *FTO* gene (T-to-C, rs1421085), the locus containing variants which exhibit the strongest associations of any gene with obesity, is compelling [89]. The resulting phenotype involves modulation of the expression of key genes (*IRX3* and *IRX5*) during early BAT differentiation. This evidence suggests that BAT can have a causative role in obesity, though corroborating evidence is required.

Consequence? While the mechanism linking SNS function and body weight maintenance in human obesity remains incompletely understood, there is evidence that the SNS dysfunction following obesity may precipitate BAT dysfunction. Increased sympathetic nerve activity to certain organs has been reported in obese individuals, which in most instances downregulates AR expression and sensitivity [90]. Though it has been hypothesised that BAT β_3-ARs do not downregulate during chronic stimulation in mice [91], it is possible that similar to other tissues and ARs, chronic AR signalling may trigger a counter-regulatory mechanism resulting in adrenergic desensitization in humans [92]. In support of this mechanism, we demonstrated that treatment of lean, healthy humans with ephedrine resulted in a reduction in FDG uptake on PET imaging in BAT [69]. This observation supports the contention that BAT dysfunction can also be secondary to development of obesity.

Whether cause or consequence, the well-established existence of BAT dysfunction in the context of human obesity is relevant as a factor perpetuating weight gain. Understanding the mechanisms responsible and developing strategies to recruit BAT therefore remain important for research in this field.

BAT adaptive thermogenesis

Adaptive cold-induced thermogenesis. Upon cold exposure, unacclimatised animals initially increase thermogenesis primarily by shivering, where skeletal muscle contracts rapidly to produce heat as a metabolic by-product [4]. BAT will also contribute to CIT, depending on the level of adaptive pre-conditioning. The cluster of acute heat-producing processes is called facultative thermogenesis [4, 35]. Persistent shivering upon prolonged cold exposure is maladaptive as it will result in short- (loss of coordination) and long-term (tissue wasting) impairment in muscle contractile function, without periods of recovery. Cold adaptation involves adaptive thermogenesis, or adaptive CIT, whereby BAT oxidative machinery is induced to support a higher level of sustained heat production in the absence of shivering. The resulting energy expenditure during a future, similar, cold insult will thus be greater than

prior to adaptation. In mice, the additional portion of energy expenditure above pre-adaptation level can be fully attributed to BAT recruitment [4].

Demonstration of adaptive CIT via PET/CT imaging was a critical step supporting investigation of the therapeutic potential for BAT in humans. Longitudinal evidence indicated seasonal adaptation [6, 93], while several laboratories subsequently presented data from intervention trials in close succession (**Table 2**). These studies showed that daily periods of cold (10-19°C, 2-10 hr/d) exposure for 10-42 days in young healthy individuals resulted in adaptive CIT [53, 54, 34, 55, 94, 95]. One group demonstrated increased cold-stimulated whole body energy expenditure and body fat loss [54], but the others did not [53, 54, 34, 55, 94, 95] (**Table 2**). Interestingly, a key difference in the study reporting fat loss [54], is that participants were pre-selected from a larger cohort for the long-term cold exposure intervention based on low or no cold-stimulated BAT activity prior to the intervention. As a result, these individuals starting from a lower baseline may have been more sensitive to the intervention. In two separate studies Hanssen *et al.* [94, 96] also reported adaptive CIT in individuals with type 2 diabetes and an obese but otherwise healthy group. All studies demonstrated increases in acute cold-stimulated BAT activity after cold adaptation, primarily measured via FDG-PET/CT imaging. Of note, Blondin *et al.* [34] also reported a doubling of BAT total oxidative metabolism using PET/CT with ^{11}C-acetate, a tracer which more accurately reflects total BAT activity compared with FDG.

Limitations of cold exposure to translation. Based on the discoveries described above, support has emerged for chronic cold exposure through reducing ambient home and workplace temperatures as an environmental solution to reduce body weight and improve health in humans [6, 53, 94, 97]. Chronic cold exposure, like many cardiovascular and metabolic stressors (eg. exercise, caloric restriction, heat stress), can provide benefits to metabolic health [94, 42, 98]. It is highly effective for the induction of adaptive thermogenesis in mice and protects from high energy diet-induced obesity [91]. In contrast, when cold exposure is intermittent, and therefore not severe enough to approach the upper

limits of energy expenditure, mice increase body mass and fat content due to the well described hyperphagic response to chronic cold exposure [99, 100]. Of the seven intervention studies cited above and in **Table 2** which examined human BAT adaptive CIT, only one reported a decrease in body fat mass [54] and none reported a loss of total body mass. These interventions are all of relatively short duration, therefore definitive conclusions cannot yet be made regarding the utility of these protocols for human weight loss. Nevertheless, most humans would not tolerate either or both of severe and prolonged cold environments and do not have the capacity of mice for increasing adaptive thermogenesis. In turn, humans would likely experience hyperphagia, which renders this method ineffective as well as being impractical and unrealistic [84, 100]. These data demonstrate the requirement for pharmacological agents targeting BAT for weight loss or maintenance to have direct and specific actions on BAT to increase activity in the absence of central sympathetic and orexigenic signals.

Diet-induced adaptive thermogenesis: a brief history. The concept of adaptive DIT was born out of an observation over 100 years ago by Neumann *et al.* [101], termed luxusconsumption, where the weight gained after prolonged overeating would be less than that predicted by the quantity of excess energy intake. DIT is used interchangeably to describe certain components of both the acute increases in energy expenditure in response to a meal as well as the longer term adaptive increase in energy expenditure (**Figure 1**). The increase in energy expenditure in response to a single meal, often termed postprandial thermogenesis, is composed of an obligatory component linked to digestion and nutrient handling as well as a potential additional component, facultative DIT (similar to facultative CIT), the portion in which BAT may contribute to additional energy expenditure beyond digestion and nutrient handling [35].

Adaptive DIT was first attributed to BAT in rats in pioneering experiments by Rothwell and Stock [102]. The finding was controversial then [103, 104] and has remained so based on critics suggesting the excess energy expenditure could be accounted for by biochemical processes other than simply adaptive expenditure and heat loss [103, 24]. Nevertheless, adaptive DIT after prolonged overfeeding has been demonstrated experimentally in mice [27] and humans [105]. By using high fat diet-fed UCP-1 knockout mice housed at

thermoneutrality, Feldmann *et al.* [27] showed a UCP-1-depenent mechanism exists whereby overfed knockout mice gained more body fat and mass than wild-type mice in addition to reduced norepinephrine-induced energy expenditure.

Determination of the presence and degree of adaptive DIT energy expenditure in humans is difficult mainly due to inherent inter- and intra- (day-to-day) individual variability in energy expenditure, sensitivity of measurement instruments (usually indirect calorimetry) [103] and experimental factors such as the duration and nature of the diet, duration of weight stability and any form of physical pre-conditioning [106]. Yet, in well controlled long-term over- and under- feeding studies, adaptive energy expenditure has been demonstrated by Liebel *et al.* [105]. In these experiments, pre-determined quantities of weight gain and loss, and subsequent weight stabilisation, resulted in increases and decreases in energy expenditure that were larger and smaller, respectively, than values predicted based on the participant's initial lean body mass. While not a universal finding [107], collectively studies in humans have reported changes in adaptive DIT after overfeeding [108, 109, 105, 110] and underfeeding [111, 105].

Diet induced thermogenesis: evidence of a role for BAT in facultative diet-induced thermogenesis. While promising evidence of increased facultative DIT linked to BAT has been reported in humans, these data require substantiation [112, 113, 42]. Limitations of previous studies include BAT activity assessment using FDG-PET/CT during a mixed meal, which likely incorporates a proportionately undefined combination of insulin-stimulated storage and thermogenic BAT glucose uptake (**Table 1**) [112]. Other studies use relatively indirect measures of BAT activity such as supraclavicular fossa skin temperature [42]. Less robust are reports of association between facultative DIT and BAT-positive status based on prior cold-exposure [113]. Evidence gaps thus include the proportion of facultative DIT attributable to BAT in humans and more specifically the effect of specific macronutrients (carbohydrate, fat, protein; **Table 1**).

Diet induced thermogenesis: evidence of a role for BAT in adaptive diet-induced thermogenesis. Energy expenditure can be crudely broken down into the following components: sleeping energy expenditure, resting energy expenditure, postprandial or diet-induced thermogenesis (both obligatory and facultative) and non-resting energy expenditure (both incidental and purposeful activity/exercise) (summarised in **Figure 1**). The first study dedicated to determination of a contribution of BAT to adaptive DIT was published recently by Peterson *et al*. [114]. In their study involving 8 weeks overfeeding by 40% of weight maintenance energy intake in healthy males, sleeping energy expenditure was increased by 4.7% above that expected based on the amount of weight gained. This would be supportive of the hypothesis proposed in **Figure 1A**, except that BAT activity, measured by infrared thermography, was not different in response to the intervention. This study, while laying important groundwork for the field, has not definitively determined a lack of BAT contribution to adaptive DIT. BAT was not measured via the current gold-standard technique of PET/CT imaging, and only a small portion of skin surface (the feet) were placed in ice-water. The size and region of skin surface exposed to cooling is the primary driver of the BAT response, therefore Peterson *et al*. may not have optimised their likelihood of identifying an effect of the intervention [33, 115]. Given that obese individuals do not have higher energy expenditure than those who are lean when corrected for lean body mass, it is possible that tolerance to DIT develops over time, where BAT functional capacity regresses. Further, since human BAT activity is impaired in obese individuals and has only been measured at static time-points in well-established obesity, it is conceivable that, long-term, BAT function diminishes rather than increases during chronic overfeeding (**Figure 1**). Currently no studies have investigated the time-course for adaptive DIT in humans; this may not be linear with respect to time or volume of overfeeding, therefore in this regard, both the whole body and BAT adaptive DIT responses require further study.

A contribution for adaptive DIT to non-resting energy expenditure has been reported if activity is light (incidental) [108, 105, 110], but not strenuous (purposeful) exercise [109]. Similar to other components of energy expenditure, it is unknown whether the increase in light activity-associated thermogenesis involves BAT. Two studies have investigated whether

there are links between facultative and adaptive CIT and DIT. That is, whether BAT conditioning as a result of chronic cold exposure can increase facultative DIT, and *vice-versa*. In a 4-week cold-acclimated healthy human cohort whole-body CIT increased, but this could not be attributed to BAT, and there was no change in facultative DIT in response to overfeeding for 24 hours compared to before the 4 week intervention [116]. In contrast, Lee *et al*. [55] measured facultative DIT (pre *vs* post meal energy expenditure) after 1 month cold acclimation and saw no change in adaptive CIT but demonstrated an increase in adaptive DIT along with increased BAT activity in response to acute cold exposure. The chronic cold exposure protocol as well as the method for measurement of DIT differed substantially between these two studies, providing a likely explanation for the disparate findings and rationalising the need for standardising measurement of these variables. It is also contentious whether CIT and DIT are regulated by the same CNS-derived sympathetic mechanism/s [117-119, 24, 120, 112, 55]. Future studies should examine whether and to what degree both of chronic dietary changes and cold exposure protocols can induce each of cold- and diet-induced adaptive energy expenditure (**Table 1**).

Pharmacological approaches to BAT recruitment and activation in humans

Identification of novel pharmacological agents targeting cellular pathways for proliferation, differentiation, recruitment and activation of BAT is a major focus of this field. A key consideration is the necessity to not only drive induction of BAT browning via proliferation, differentiation and recruitment, but also the need for persistent thermogenic activation via BAT specific β-adrenergic signalling pathways (**Figure 2**). To this end, multiple pharmacological approaches have been studied in humans in recent years, predominantly using PET/CT imaging techniques.

Adrenergic receptor agonists. Because AR signalling in BAT activates various metabolic, including thermogenic, processes and has been comprehensively studied in animal and cell models, pharmacological targeting of this pathway was the first major focus of attention. Studies predating the publications in 2009 [5-9] by 20-30 years provided *ex vivo* biochemical evidence for the presence of functional, recruitable BAT, albeit not in the depots identified as

most abundant in subsequent work [121, 122]. During this early period, evidence for *in vivo* BAT function was indirect, inconclusive and despite robust methodology, ultimately misleading [102, 123, 124]. Subsequent to 2009, two laboratories reported that treatment with either the pan-β AR agonist isoprenaline [125] or ephedrine (1 mg/kg) [52], did not increase BAT metabolic activity, measured via FDG-PET/CT. However, our subsequent work demonstrated that these negative findings reflected low dosage and that, in fact, ephedrine can activate BAT activity at a dose of 2.5 mg/kg [84]. The magnitude of the BAT response to ephedrine, however, was less than for mild cold exposure. We observed a ~2-fold increase in response to ephedrine, whereas mild cold increases BAT activity by >5-fold [8, 52]. Further, the impairment of BAT activation in obese participants identified previously in response to cold remained apparent with sympathomimetic activation [7, 83]. An underlying caveat to these studies is that in all cases, BAT activity was measured via FDG-PET/CT. Therefore it remains to be determined whether any measured activity is thermogenic, or conversely, whether low doses of sympathomimetics increase uncoupled thermogenesis through oxidation of non-glucose substrates.

It was clear, and expected, in the three studies of acute sympathomimetic administration that pan-β-AR agonists would result in significant and dose dependent increases in blood pressure [52, 125, 69]. Cypess *et al.* [60] later repeated a similar study with the β_3-AR specific agonist mirabegron (200mg). BAT activity, again measured via FDG-PET/CT, increased substantially and to a degree approaching that of cold exposure. Spill-over to the β_1-AR is expected with mirabegron at *in vivo* doses above 50 mg [60], however cardiovascular responses were minimal with increases in systolic blood pressure of ~10 mmHg versus the ~45 mmHg elevation which occurs with the ephedrine dose required to elicit BAT activation [84]. Whether mirabegron or other newer generation β_3-AR agonists can induce adaptive thermogenic BAT activity and weight/fat loss remains to be determined (**Table 1**).

Transient receptor potential (TRP) channel agonists. Capsaicin, capsinoids and catechins are food compounds which have been studied extensively in animal models as well as a

number of human studies, specifically in relation to their role in BAT thermogenesis. These studies have been thoroughly reviewed [126]. Of particular note, these compounds increase energy expenditure and have been indirectly linked to increased facultative [127] and adaptive [54] BAT thermogenesis. The proposed mechanism suggests that these compounds activate the transient receptor potential cation channel subfamily V member 1 (TRPV1) located in the upper digestive tract, leading to increased sympathetic-mediated thermogenic activity, including to BAT [126]. Due to this mechanism of action these compounds are indirect activators of BAT thermogenesis because rather than directly acting on adipocytes, they depend on activation of the sympathetic nervous system (**Figure 2**). Moreover, BAT activity in humans has not been directly measured in response to ingestion of these compounds, therefore further human studies are required to characterise the specificity, mechanism and magnitude of BAT activation and whether TRP channel agonists have efficacy for BAT activation in obesity (**Table 1**).

Thiazolidinediones (TZD). The TZD drug family are reportedly the only non-adrenergic stimulatory molecules that have been conclusively shown to promote cell-autonomous browning [47]. While these drugs do not activate thermogenesis (**Figure 2**), in many cell-based and animal models, they induce browning through activation of peroxisome proliferator-activator receptor γ (PPARγ) [128-134]. It is of great interest to directly examine whether prolonged treatment with these drugs can induce browning in humans *in vivo*. However, as yet, this has not been investigated (**Table 1**) [47].

Thyroid hormones. The role of the thyroid hormones triiodothyronine (T3) and thyroxine (T4) in regulating mammalian energy metabolism is well recognised [135]. Due to broad tissue distribution of the nuclear thyroid hormone receptor, these hormones regulate various functions, including the metabolic rate of many cell types including brown adipocytes [136]. Although these hormones directly promote thermogenesis in adipocytes they do not activate the classic adrenergic thermogenic pathway (**Figure 2**).

Patients with hypo- and hyper-thyroidism provide an avenue for the study of thyroid hormones in regulating human BAT activity. There is mixed evidence regarding the effect of thyroid hormones on BAT activity and certain factors indicate the effect may be context dependant. Studies of hyperthyroidism (Grave's disease), compared with matched euthyroid individuals [137] or treatment of hypothyroidism with T4 [138, 139] suggest thyroid hormones increase BAT activity. However, this is not supported by another study of patients with Grave's disease where BAT activity was absent prior to initiation of treatment with methimazole, but was present in one patient post-treatment [140]. Disparate results between the two studies of patients with Grave's disease [137, 140] may be the result of methodological considerations; both studies measured BAT activity in the thermoneutral state. While this infers BAT activity in the basal state and therefore the prevailing thyroid hormone concentrations as the activating stimulus, a physiological activating stimulus such as cold exposure may have better defined whether or not T3 and T4 induced an adaptive response. Moreover, only limited evidence to control for seasonal preconditioning and laboratory conditions were provided and one study did not include a euthyroid control group [140]. Notably, one of these studies [137] employed quantitative FDG-PET/CT as well as ^{15}O-water to assess tissue perfusion, therefore allowing better resolution of thyroid hormone-stimulated BAT activity and support for the contention that thyroid hormones increase BAT activity in hyperthyroid patients. An inconclusive role for thyroid hormones in regulation of BAT is supported by Gavrila *et al*. [141] in patients with hypothyroidism. Nevertheless, we contend that this and other evidence based on mixed patient groups undergoing significant medical treatment interventions cannot clearly define the role of thyroid hormones in regulation of BAT in healthy individuals.

An interesting thyroid hormone-related hypothesis proposes that thyroid stimulating hormone (TSH) may positively regulate BAT thermogenesis directly. Evidence for this is based only on a case study of a severely hypothyroid child [142], but would support the contention by Zhang *et al*. [140] that normalisation of elevated T3 and T4, and concomitant increase in TSH with treatment for Grave's disease increases BAT activity.

It should be apparent that all evidence discussed above on the influence of thyroid hormones on human BAT is based on studying patients with thyroid-related disease. Because of the resulting systemic dysregulation of metabolism caused by these diseases, caution is required regarding definitive conclusions of the effect of thyroid hormones on human BAT. Further human studies are clearly required to understand the potential role of BAT thermogenesis in the weight gain and loss associated with hypo- and hyper-thyroidism, respectively. Regarding the potential of thyroid hormones as pharmacological agents for BAT recruitment and activation, due to their broad tissue targeting, this avenue of investigation is unlikely to be productive.

Glucocorticoids. Excess glucocorticoids have long been linked with obesity and are known to regulate a broad range of processes in adipocytes. Glucocorticoids, primarily dexamethasone, are used broadly for *in vitro* differentiation of various adipocyte cell lines due to their role in proliferation and differentiation [143]. Of note, while important in this role in primary human white and brown adipocyte cultures, they inhibit adrenergic activation of BAT thermogenesis [144]. These mechanisms predict that the actions of glucocorticoids on BAT *in vivo* would be complex and potentially different between recruitment and thermogenesis. Ramage *et al*. [70] showed that 3 doses of prednisolone over 24 hours prior to cold-exposure augmented cold-stimulated BAT activity in humans. However, chronic glucocorticoid use in humans supressed BAT activity. The mechanisms responsible have not been fully established but are presumed to be an increase in UCP-1 expression acutely, but reduction in adrenergic signalling with prolonged treatment.

Similar to thyroid hormones, due to the key role of glucocorticoids in many cell types, targeting these pathways to specifically increase BAT activity is not currently viable. Nevertheless, understanding the role of BAT activity in the context of glucocortoid pathologies (eg Cushing's syndrome) may prove helpful for future patient management strategies.

Conclusion

Whether BAT can make a meaningful contribution to the prevention and management of weight gain and associated metabolic disease remains to be determined. It may be relevant as a therapeutic target for weight reduction, or alternatively, in metabolic disease treatment in the absence of weight loss as a glucose and lipid sink. It is probable, however, that despite induction of adaptive BAT thermogenesis, chronic cold exposure will not promote weight loss due to activation of hyperphagic and WAT growth pathways. This finding points to future strategies which directly and specifically activate BAT thermogenic and browning processes. Future development must consider disparities in BAT distribution, function and signalling pathways between experimental animals and humans which underline the need to pursue clinically relevant animal models. Furthermore, preclinical and mechanistic human trials should progress in parallel, informing each other in a bidirectional manner.

Conflict of interest statement

The authors declare that there is no conflict of interest.

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Evidence gap	Current evidence base and its limitations	Strategies to traverse the evidence gap
What is the absolute contribution of BAT to whole body energy expenditure?	While most recent evidence has been derived from PET/CT techniques which are mainly semi-quantitative (ie, FDG), some laboratories have employed PET techniques which have described absolute energy expenditure and transport of substrates other than FDG (eg, ^{15}O-labelled water and gas, ^{11}C acetate and fatty acids). However, use of these tracers is technically difficult, restricted to few laboratories and involves exposure to ionising radiation, limiting the potential to comprehensively understand a broad range of physiological and pharmacological interventions.	Develop and apply novel *in vivo* techniques (eg. arterio-venous difference, tracer-based substrate turnover or related techniques which are not yet possible in relevant BAT tissues) which directly measure substrate or oxygen consumption and/or heat production in major human BAT depots, or imaging techniques (eg, MRI, infrared thermography) not dependent on ionising radiation. Validate PET and other techniques against each other in recognised BAT depots similar to those conducted in other tissues [145].
Does BAT contribute to facultative and adaptive DIT?	Available evidence is unclear or unavailable regarding whether BAT is involved in DIT, particularly after a period of adaptive preconditioning via regular long-term cold exposure or a hyperenergetic diet.	Studies employing standardised methodology which delineate thermogenic *vs* insulin- or glucose mass action- stimulated uptake are required to determine whether BAT contributes to DIT.
Do the mechanisms contributing to adaptive BAT thermogenesis (eg. cold or diet) overlap with respect to their corresponding acute stimulatory signal (eg. does facultative CIT increase BAT's response to adaptive DIT, and *vice-versa*)?	Currently, only limited and conflicting data are available regarding whether an adaptive stimulus, such as cold exposure, results in increased BAT function after an acute meal and *vice-versa*.	Standardised protocols and methodology are key to uncovering whether a clear role exists for BAT under different adaptive and acute thermogenic stimuli. Ideally, techniques enabling measurement of neural control of BAT in humans are required.
Does variation in dietary macronutrient (carbohydrate, fat and protein) composition contribute to either acute or adaptive DIT?	Limited evidence is available regarding whether altering macronutrient composition (relative proportion or absolute amount) impacts DIT, and this is yet to be explored in BAT.	If it is determined that BAT is involved in DIT, further studies should expand upon whether diet composition plays a role in regulating these responses.

Can chronic administration of the new generation β_3-AR agonists adaptively recruit BAT?	One study has reported that a new generation β_3-AR can potently activate BAT after a single dose. However, dose-response data are unavailable along with any evidence of a response to chronic treatment.	Dose response studies related to BAT activation and off-target effects (eg. cardiovascular activation via $\beta_1/_2$-AR binding), as well as chronic adaptive responses are warranted.
Do any natural or food-based products increase BAT activity and recruitment?	A strong body of evidence demonstrates that capsinoid compounds found in chili peppers and related foods are linked with increased BAT activity both acutely and chronically. This occurs via an indirect (gut-brain-SNS-BAT) mechanism. However, BAT activity has not been directly measured in response to consuming these products.	BAT activity needs to be directly measured in response to treatment with capsinoid extracts. The search should continue for analogous nutrients which may have similar properties. Given promising evidence based on these compounds, drug development targeting this putative mechanism may be justified.
Does chronic TZD treatment recruit BAT *in vivo* in humans?	There is a large evidence base for the browning/recruitment effect of TZDs (primarily rosiglitazone) on WAT and BAT. Despite this, there are no studies that have directly examined if this effect can be replicated in humans *in vivo*.	Studies should be conducted to determine whether the browning effect of TZDs observed in preclinical models exists *in vivo* in humans.
What is the effect of thyroid hormones on human BAT function?	Diseases associated with dysregulated TSH, T3 and T4 suggest thyroid hormones probably increase BAT thermogenic capacity, however these are based on studies of patients with thyroid disease.	Determine the role of BAT in dysregulated energy metabolism associated with hyper- and hypo-thyroidism.
Can the novel molecules identified to recruit BAT in animal/preclinical experiments do so in humans?	Basic/fundamental research rarely involves direct translation to examination of a mechanism, intervention or drug trial in humans. Emerging evidence regarding disparate observations between humans and experimental animals suggests this should be a priority.	Human proof-of-concept studies are required to determine the value of BAT targets identified in preclinical studies.

Table 1. Evidence gaps and research directions for understanding human BAT physiology which are important next steps in therapeutic development. AR, adrenergic receptor; BAT, brown adipose tissue; CIT, cold-induced thermogenesis; DIT, diet-induced thermogenesis; FDG, fluorodeoxyglucose, MRI, magnetic resonance imaging; PET/CT, Positron Emission Tomography/Computerised Tomography; PPT, postprandial thermogenesis; SNS, sympathetic nervous system; T3, triiodothyronine; T4, thyroxine; TSH, thyroid stimulating hormone; TZD, thiazolidinedione; WAT, white adipose tissue.

Study	Participant characteristics (cohorts in which BAT activity measured)	Chronic cold exposure protocol	Change in body mass and body fat mass	#Adaptive change in acute cold-stimulated BAT Activity	#Adaptive change in thermoneutral energy expenditure	#Adaptive change in acute cold-stimulated whole body energy expenditure
Blondin *et al.* [34]	M (6), 23 yrs, 24.5 kg/m^2 no control group	4 wks, 2 hr/d, 5 d/wk, 10°C whole body water perfused suit	NR	45% (a)	-7.1%, NS	-7.4%, NS
Lee *et al.* [55]	M (5), 21 yrs, 22.0 kg/m^2 no control group	1 month, sleeping >10 hr/night at 19°C, light clothing and bed covering only	BM 0.5%, NS BF -1.4%, NS	42% (a) 19% (b)	-4%, NS	-3%, NS
Van der Lans *et al.* [53]	F (9), M (8), 23 yrs, 21.6 kg/m^2 no control group	10 d, 6 hr/d, 15-16°C ambient air temp, light/short clothing	NR	37.3% (a) 16.7% (b) 34.8% (c)	0% NS	3.8%, NS
Yoneshiro *et al.* [54]	M (22), 24.4 yrs, 22.0 kg/m^2 12 cold exposed (8 for BAT analysis), 10 thermoneutral controls	6 wks, 2 hr/d, 17°C ambient air temp, light/short clothing	BM 0% BF -5.2%	58.1% (b)	-2.6%, NS	6.7%
Hanssen *et al.* [94]	M (8) with type 2 diabetes, 59.3 yrs, 29.8 kg/m^2 no control group	10 d, incremental to 6 hr/d, 14-15°C ambient air temp, light/short clothing	BM 0.5%, NS	57% (b)	-3%, NS	3%, NS
Romu *et al.* [95]	F (18), M (10) cold (11 completed) 24.3 yrs, 21.6 kg/m^2 con (12 completed) 26.1 yrs, 22.5 kg/m^2 control group (randomised)	6 wks, participants asked to feel cold for at least 1 hr/d	-0.2%, NS	23.4% (d)	4.3%, NS (p=0.052)	2.5%, NS

Hanssen *et al.* [96]	M (10) overweight-obese, 36.0 yrs, 32.9 kg/m^2 no control group	10 d, incremental to 6 hr/d, 14-15°C ambient air temp, light/short clothing	NR	Pre-intervention BAT-positive: 26% (b); 115% (c) All: 91% (b)	-3.4%, NS (p=0.06)	0%

Table 2: Studies of cold-induced adaptive thermogenesis and BAT activity in humans measured via current best-practice imaging techniques.

M, male (n); F, female (n); BM, body mass; BF, body fat; NR, not reported; NS, not significant; temp, temperature

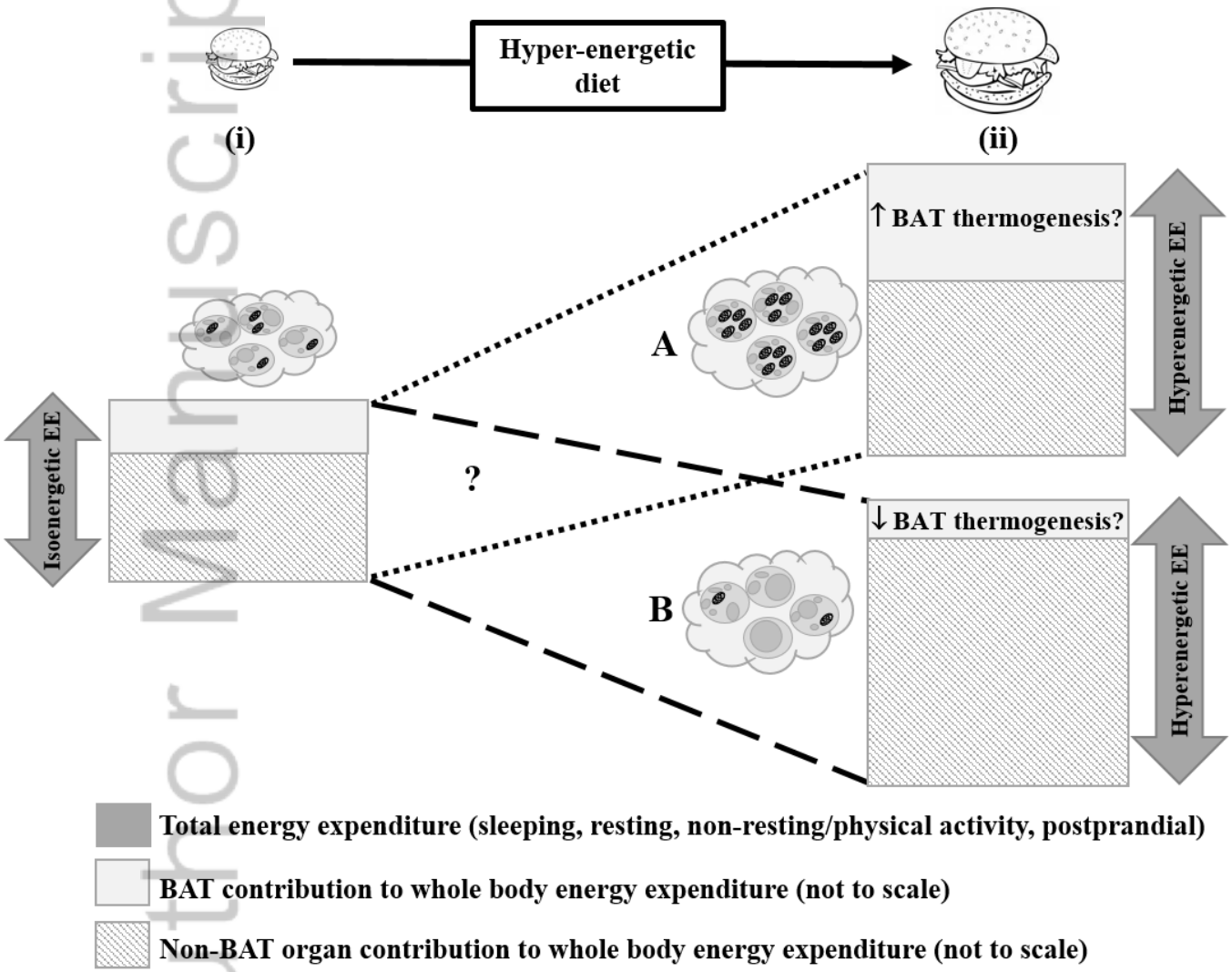
BAT activity: 18F-FDG PET/CT measured as volume of activity (a), SUV_{mean} (b), SUV_{max} (c). MRI-determined BAT volume (d).

Change data calculated as % change based on absolute values for each variable.

[#] Calculated as change from thermoneutral or acute cold-stimulated value before chronic cold interventions to thermoneutral or acute cold-stimulated values after interventions (where relevant, chronic cold-exposed groups only)

Figure 1. Components of energy expenditure (EE) and the (unknown) contribution of brown/beige adipose tissue to whole body EE during diet-induced adaptive thermogenesis. In the transition from an isoenergetic diet that maintains a stable weight (**i**) to a hyperenergetic diet (**ii**), total energy expenditure increases in both mice and humans. In mice this additional energy expenditure has been attributed to brown adipose tissue (BAT). In humans, it is unknown whether BAT adaptive diet-induced thermogenesis (DIT) occurs. Studies in mice and rats predict it should increase in thermogenic capacity (**A**), however studies from obese humans suggest excess body weight is associated with diminished BAT thermogenic capacity (**B**). BAT could be active during any of sleeping, resting, postprandial or non-resting/physical activity energy expenditure, therefore potentially contributing to increases or decreases in adaptive energy expenditure during any of these physiological states. Energy expenditure during the non-sleeping period encompasses normal daily activities associated with wakefulness, environmental (eg. cold) stress, psychological stress, immune activation, eating and non-purposeful and purposeful physical activity. Because of this, and the fact that the total contribution of BAT to whole body energy expenditure in humans is small, direct measurement of BAT activity will be necessary to determine if it contributes to adaptive energy expenditure associated with overeating.

Figure 2. Overview of recruitment and thermogenesis in brown/beige adipocytes. Thermogenic signalling from the sympathetic nervous system (SNS), via β-adrenergic (β-AR) receptors drives and recruitment of mature adipocytes to increase thermogenic capacity. The same SNS/β-AR signalling pathway is responsible for acute activation of thermogenesis in both warm-adapted and cold-adapted brown and brite/beige adipocytes. Uncoupled energy expenditure and heat production from warm-adapted cells is relatively low. Upon chronic thermogenic signalling (eg, repeated or chronic cold exposure), cold-adapted cells become capable of significantly greater heat production. Agents and hormones such as thiazolidindiones (TZDs) and thyroid hormones, which putatively induce production of thermogenic machinery (adrenergic/thermogenic receptors, mitochondrial biogenesis, uncoupling protein 1, enzymes involved in substrate oxidation and substrate handling), may increase the sensitivity of BAT to classic thermogenic signalling, such that it exhibits increased heat production in response to a thermogenic stimulus without prior thermogenic recruitment. Other non-classic pathways may indirectly activate BAT recruitment and thermogenesis via stimulating sympathetic nervous system outflow to BAT (eg, capsinoid compounds).



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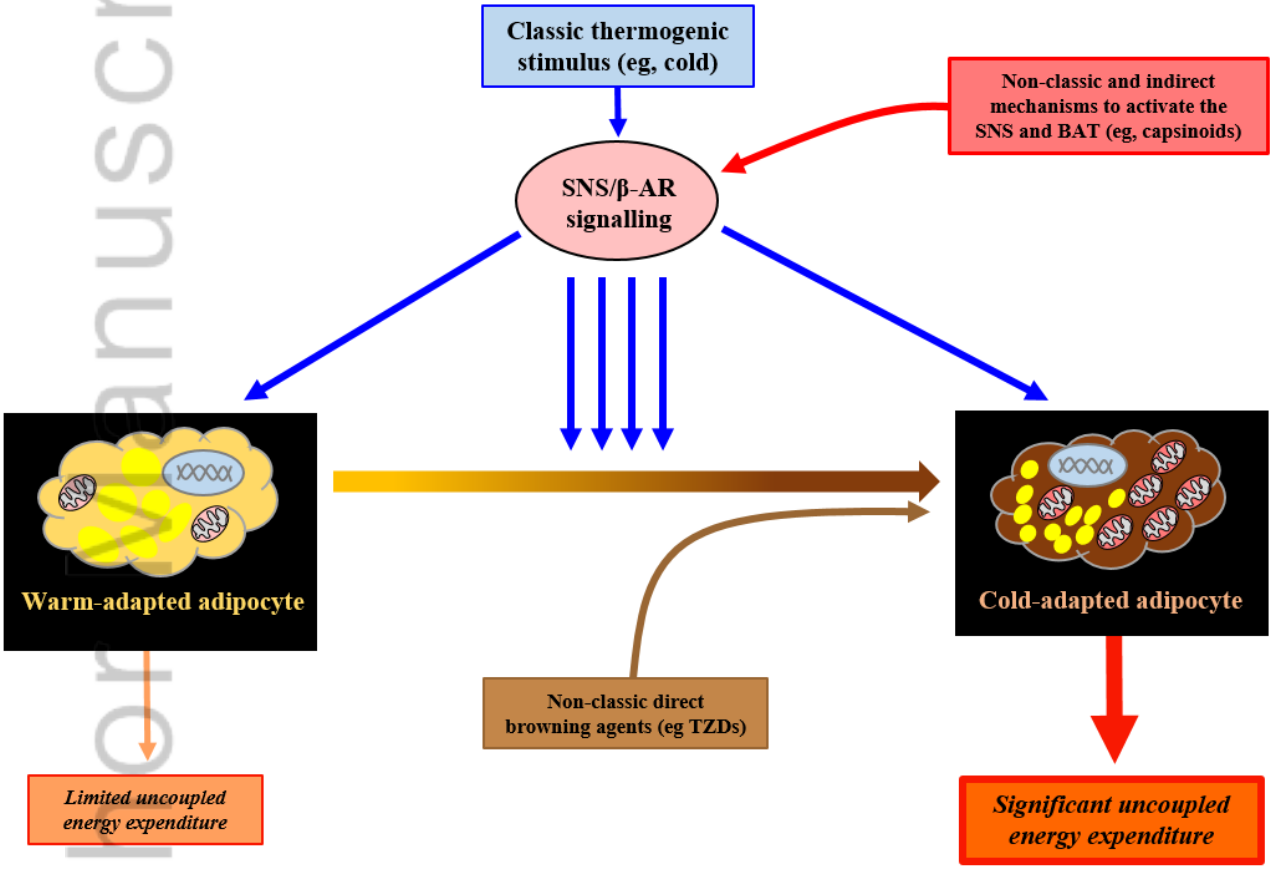

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