Brown adipose tissue in the treatment of obesity and diabetes: Are we hot enough?

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ABSTRACT

The identification of functional brown adipose tissue in human adults has intensified interest in exploiting thermogenic energy expenditure for the purpose of weight management. However, food intake and energy expenditure are tightly regulated and it is generally accepted that variation in one component results in compensatory changes in the other. In the context of weight loss, additional biological adaptations occur in an attempt to further limit weight loss. In the present review, we discuss the relationship between increasing energy expenditure and body weight in humans, including the effects of cold exposure. The data raise the possibility that some processes, particularly those involved in thermogenesis, induce less compensatory food intake for a given magnitude of additional energy expenditure, a state we term the 'thermogenic disconnect'. Although cold exposure increases thermogenesis and can putatively be exploited to induce weight loss, there are multiple adaptive responses to cold, of which many actually reduce energy expenditure. In order to optimally exploit either cold itself or agents that mimic cold for thermogenic energy expenditure, these non-thermogenic cold responses must be considered. Finally, the relative contribution of brown adipose tissue vs other thermogenic processes in humans remains to be defined. However, overall the data suggest that activation of cold-induced thermogenic processes are promising targets for interventions to treat obesity and its secondary metabolic complications. (J Diabetes Invest, doi: 10.1111/j.2040-1124.2011.00158.x, 2011)

KEY WORDS: Brown adipose tissue, Energy expenditure, Weight loss

INTRODUCTION

To date, therapeutic interventions aimed at reducing caloric intake have not been successful. Caloric restriction in combination with exercise is effective in the short term, but long-term weight maintenance is poor and weight regain, sometimes rising above pre weight loss levels, is common[1–3]. Modestly effective pharmacological interventions, such as sibutramine and rimonabant, have both been withdrawn from the market owing to side effects. Only orlistat, a gastrointestinal lipase inhibitor, remains available; however, it is of limited efficacy and causes unpleasant side effects. An alternative strategy is to increase energy expenditure (EE) as a means to prevent weight gain and to induce weight loss.

Weight reduction through caloric restrictions results in a series of responses that minimize weight loss. Rosenbaum *et al.*[4,5] demonstrated that weight loss through caloric restriction reduces total daily EE (TDEE) by 25% beyond that predicted by post-weight loss fat-free mass. This is accounted for, in part, by increased efficiency of skeletal muscle metabolism, altered hypothalamic–pituitary–thyroid axis, and lowered leptin levels. In addition, hunger and satiety scores after weight loss continue to favor caloric intake beyond daily needs. These changes are sustained over a follow-up period of 1–2 years. Conversely, caloric excess promotes a largely opposite response to caloric reduction, although the response is short lived[6]. This ability to defend body energy stores as fat was termed the 'adipostat hypotheses'.

Based on the adipostat hypothesis, increasing EE to promote weight loss should be hindered by opposing responses, particularly an increase in food intake. Ideally, any strategy to increase EE should be achieved with only a small, or preferably no, reliance on concomitant caloric restriction, thus improving long-term compliance. More recently, Cannon and Nedergaard[7] reviewed murine models examining the role of cold-induced thermogenesis (CIT) in weight control and concluded that increasing EE, particularly by utilizing brown adipose tissue (BAT), does not result in caloric intake that matches the elevated metabolic rate. In the present review, we will term the concept that, in the absence of caloric restriction, energy loss through thermogenesis is not completely matched by an increase in food intake as the 'thermogenic disconnect'.

Recently, BAT has gained renewed interest as a target for increasing EE as a means to treat obesity[7]. A putative therapeutic role for BAT was first postulated over three decades ago. Rothwell and Stock suggested that resistance to diet-induced obesity (DIO) in rats was a result of increased EE in response to a cafeteria diet[8]. This increase in EE was later shown to be mediated by BAT and termed 'diet-induced thermogenesis'[9]. Rothwell and Stock postulated that rats resistant to DIO were able to

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evoke an apparently wasteful increase in EE as a protective mechanism against an inadvertent increase in food intake.

The discovery that BAT is present in energetically significant amounts in adult humans makes this tissue a potential therapeutic target against obesity[10–12]. In the present review, we provide a brief summary of the relationship between increasing EE and weight loss. Because there are no data regarding specific BAT EE in humans, we will review data regarding the effects of cold exposure, a state known to be the most potent physiological activator of BAT in rodents and humans, on EE and body weight. Finally, using the cold response as a model, we will review the potential issues involved in exploiting CIT.

INCREASING ENERGY EXPENDITURE FOR WEIGHT LOSS

Obesity is a result of chronic caloric imbalance. On a population level, this net imbalance is thought to be small, resulting in gradual but progressive weight gain amounting to approximately 1 kg/year in Western populations[13–15]. A weight gain of 1 kg/year equates to only 105 kJ (approximately one teaspoon of sugar) per day. However as weight (both fat and fat-free mass) is gained, caloric intake would necessarily rise to maintain energy balance. A larger organism requires more energy, due to both an increase in cell numbers and an increase in mechanical work. Bouchard[16] argue that when an individual gains weight from a body mass index (BMI) of 25 to 35 kg/m^2, his/her intake would have to increase by 2.7 MJ/day compared with his/her leaner self to maintain energy balance. That is, a net 105 kJ/day positive balance will result in a gain of 3.5 kg after 3.5 years. After factoring the effects of age (of 3.5 years), TDEE will rise by 105 kJ/day, effectively balancing the excess caloric intake. In order to explain the sustained rise in body weight, caloric intake would have to increase not only to match the added expenditure, but also to maintain the net positive balance. Therefore, the magnitude of caloric restriction required for an obese person to lose weight would be much larger than 105 kJ/day[17].

Conversely, the reasons for increasing EE to promote weight loss go beyond simple thermodynamics. Individuals who have a lower then predicted weight-adjusted resting metabolic rate (RMR) are more likely to become obese[18,19]. Obese individuals who have lost weight also have a lower than predicted TDEE compared with their pre-weight loss state that cannot be explained by the loss of fat-free mass alone. Therefore, increasing EE would be expected to counter these biological effects.

RELATIONSHIP BETWEEN THE MAGNITUDE OF ENERGY EXPENDED AND DEGREE OF WEIGHT LOSS

Energy Expenditure and Weight Loss Through Exercise

Obesity is thought to be driven, in part, by the modern sedentary life style, although this remains a matter for debate[20]. Evidence for a decrease in physical activity was noted in epidemiological studies, cross-sectional studies, and from data demonstrating an increase in sedentary behavior, such as rising car sales and television watching. Therefore, promoting physical activity was postulated as an effective treatment for obesity.

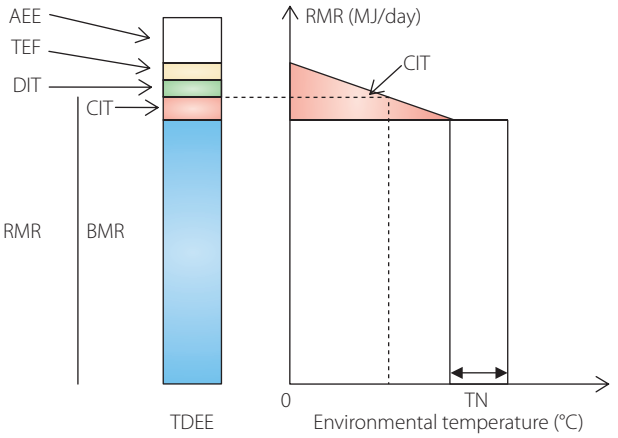

Figure 1 | Components contributing to total daily energy expenditure and the role of cold-induced thermogenesis (CIT). The bar graph on the left depicts the relative contribution of various components to total daily energy expenditure (TDEE), namely activity energy expenditure (AEE), thermal energy of feeding (TEF), diet-induced thermogenesis (DIT), CIT, and basal metabolic rate (BMR). The BMR is measured in a lightly clothed subject at rest in a thermoneutral environment. Below thermoneutrality, CIT is evoked to defend core body temperature. In this situation, the measured energy expenditure at rest is termed the resting metabolic rate (RMR). The graph on the right shows the magnitude of the RMR for a given environmental temperature. At thermoneutral temperatures (TN), RMR = BMR. At cooler temperature, CIT (shaded area) is evoked. The magnitude of CIT is determined by the severity of cold exposure (dotted line).

To determine how much energy is required to induce weight loss through exercise, it is important to understand that measurements are influenced by the method of quantification (free living vs laboratory measurements). In addition, energy balance in the short term is highly variable, but remains remarkably well balanced, particularly in weight-stable lean individuals, over the mid to long term[21,22]. This is of significant importance when extrapolating short-term energy balance data to long-term weight loss. The use of doubly labeled water to measure average daily EE (ADEE) coupled with calorimetry to asses resting metabolic rate (RMR) is generally accepted as the gold standard for determining free living activity EE (AEE). In this method, thermal energy of feeding and diet-induced thermogenesis are assumed to account for 10% of ADEE; hence, ADEE = 10% of ADEE + RMR + AEE (Figure 1).

In the context of weight loss, there appears to be a critical threshold above which increases in AEE are not matched by an increase in caloric intake[23]. Using Physical Activity Level (PAL; whereby PAL = ADEE/RMR) as a marker of AEE, short- to mid-term measurements from the general population and among soldiers in training indicate that the average sustainable PAL score falls between 1.4 and 2.5[24]. At a PAL score above 2.4, significant weight loss occurs. Although food intake

increases with increased PAL, it is thought that consumption and/or absorption of nutrients becomes limiting. This is supported by data from elite athletes, in whom PAL scores >2.4 without weight loss are sustainable only through the consumption of energy-dense supplements[25].

If we take an obese sedentary individual (PAL 1.4) with an RMR of 7.5 MJ/day, this individual will have an estimated ADEE of 10.5 MJ/day. To achieve sustained weight loss through exercise, an increase of ADEE to 18.1 MJ/day (achieving a PAL score of 2.4), equivalent to an increase of 7.6 MJ/day, is required. Four hours of moderate physical activity at four MET (where a MET, or metabolic equivalent, is the ratio of work metabolic rate to resting metabolic rate; by definition, one MET is equivalent to 1 kcal/kg/h or 4.185 kJ/kg/h) expends 4.0 MJ of energy. Energy expenditures below this is expected to induce some initial weight loss followed by a new and lower steady state weight.

Not surprisingly, exercise alone has achieved limited success in weight loss studies. Numerous studies comparing the effects of diet and exercise, diet alone, or exercise alone indicate that weight loss induced by moderate exercise alone is modest. A meta-analysis of 28 studies[26] suggested that exercise alone results in weight loss of 3 kg after 30 weeks intervention in men and 1.4 kg after 12 weeks intervention in women. A subsequent meta-analysis of weight loss trials following the publication of the 1998 National Institutes of Health Obesity Report[27], demonstrated that prescribed exercise intervention for a minimum of 1 year results in an average weight loss of approximately 2 kg at 6 months, followed by a gradual rise thereafter[3].

In terms of energy expended, most exercise intervention studies achieve an increase of between 1.2 and 2.8 MJ/day[28]. A 16-month study by Donnelly *et al.*[15] showed that despite a sustained increase of 1.6 MJ/day throughout the study, the net result was a 5.2 kg weight loss that was achieved within the first 9 months of the study. In addition, female subjects did not lose weight despite a sustained increase of 875 kJ/day over 16 months. In that study, ADEE was measured with doubly labeled water and exercise was performed under strict supervision. Although significantly larger than the imbalance (approximately 105 kJ/day) thought to cause weight gain, this increase in EE still falls below the PAL score of 2.4 required to overcome compensatory increases in food intake.

In contrast, exercise has been shown to be effective in weight maintenance and this can be achieved at PAL scores well below 2.4. The precise amount of exercise required is still debatable, but it is expected to be higher than that recommended for promoting cardiovascular health. Energy expenditure of 8.4 MJ/week (1.2–1.3 MJ/day) may be sufficient for the purpose of weight maintenance[29]. Although the magnitude expended is 10-fold >105 kJ/day, the food intake is perfectly matched with EE and so weight remains stable.

In summary, exercise is an effective means of increasing EE. Despite this, the expected weight loss is not observed (even when compliance is maximized). Exercise-induced EE expenditure is fully compensated for by an increase in food intake up to a PAL score of 2.4. However, exercise can aid weight maintenance at a much lower PAL when combined with caloric control.

Energy Expenditure and Weight Loss in Hyperthyroidism

Individuals with hypothyroidism on average gain approximately 15% of their body weight[30,31]. Not surprisingly, it was once thought that obesity may be a result of thyroid hypofunction. However, the majority of obese individuals have normal thyroid function[32,33]. Although there is debate regarding the possibility of subtle thyroid dysfunction in obesity[34–36], it is unlikely that it can account for the obesity epidemic. Individuals with hyperthyroidism lose, on average, 15% of their premorbid weight. In cohorts of untreated hyperthyroid subjects, an increase in RMR of approximately 30–47% was observed compared with weight-matched controls[37,38]. Considering a median delay of 4 months between the onset of symptoms and diagnosis[36], an 80-kg woman with a premorbid RMR of 6.7 MJ/day will expend an additional 1.9–3.1 MJ/day once she becomes hyperthyroid. After 4 months, this equates to 6.4–10 kg of fat mass loss, which is equivalent to weight loss of 8–12.5%.

Additional interest in the thyroid status in obese individuals comes from the observation that EE falls during caloric restriction and weight loss. This fall is greater than that predicted for weight and is accompanied by a fall in tri-iodothyronine (T_3) and a rise in reverse (r) T_3[39–41]. This fall in EE would rapidly balance the caloric restriction, resulting in the stabilization of weight. As such, a number of studies have looked at the effects of supplementing T_3 during caloric restriction[42–45]. Supplementation with T_3 was expected to allow sustained weight loss by preventing the hypometabolic effects of caloric restriction. In one study in which RMR was measured[45], supplementation, following initial caloric restriction, for 3 months with T_3 (20 μg, 8 hourly) resulted in an approximate 35% higher adjusted RMR compared with placebo controls. Given that the reported average subject weight of 100 kg and that control subjects dropped their RMR by 27%, the expected post-weight loss RMR would be 5.8 MJ/day in the control group. A 35% higher RMR (2.0 MJ/day) in the treated group would result in an additional 4.4 kg weight loss, which compares well with the reported 5.5 kg weight loss[45]. Other shorter-term studies using higher doses of T_3 produced proportionally higher amounts of weight loss[42,46].

Hyperthyroidism is associated with an increase in food intake and yet, despite this, weight loss occurs. The weight loss in response to T_3 described above is mostly accounted for by increased RMR, implying that any additional calories consumed are either inefficiently absorbed and/or energy is expended through thermal energy of feeding and AEE. Crucially, and in contrast with what is observed with exercise (typically 1.2–2.8 MJ/day to lose approximately 2 kg after 6 months), increases in EE due to elevated thyroid hormones (1.9–3.1 MJ/day to lose 6.4–10 kg after 4 months) translate to weight loss and are not fully compensated for by food intake.

Despite decades of research, the relative contribution of various mechanisms responsible for thyroid hormone-mediated EE has not been completely worked out. Various processes have been postulated (for a review, see Silva[47]). In general, increased fuel consumption via oxidative metabolism can only occur under states of increased ATP utilization or a reduced bioenergetic efficiency of ATP synthesis[47,48]. In this regard, thyroid hormone is believed to increase ATP utilization via futile cycling of substrates (glycolysis–gluconeogenesis, lipolysis–lipogenesis, protein synthesis–proteolysis) and ions (Na^+–K^+ cycling, skeletal muscle Ca^{2+} cycling), with heat, rather than useful work, the ultimate energetic product. In addition, increased thyroid-mediated sympathetic nervous system (SNS) tone may contribute to higher non-voluntary skeletal muscle activity. In rodents, thyroid hormones have been found to be crucial for the activation of BAT and for cold acclimation[49,50]. Cold-induced SNS activation of BAT increases the expression of deiodinase 2 (DIO2), which raises the local levels of T_3. Together, T_3 and β-adrenergic receptor stimulation promote the expression of uncoupling protein 1 (UCP1), resulting in mitochondrial uncoupling and diminished efficiency of ATP synthesis[49]. Mice deficient in DIO2 are cold intolerant[50]. Whether BAT uncoupling contributes significantly to EE in adult humans is unknown.

In summary, excess thyroid hormone is associated with an increase in EE. The magnitude of increase is similar to that achieved through exercise. Unlike exercise, much of the increase in EE is translatable to weight loss. Thyroid hormone regulates metabolism through a number of possible mechanisms, including an increase in ATP-utilizing processes and, in rodents, an increase in UCP1 expression in BAT. The incomplete compensation in terms of food intake in hyperthyroid states suggests the possibility of a thermogenic disconnect in humans. Why this should be applicable to hyperthyroid states is uncertain. It is possible that: (i) following hyperthyroid weight loss, the orexogenic signal of low leptin levels is dampened by hyperthyroid states; (ii) the EE through involuntary hypermetabolic processes may not be appropriately sensed; and (iii) the induction of low-grade EE, sustained throughout the day, may have a qualitatively different effect on appetite compared with bouts of EE from exercise.

Energy Expenditure and Weight Loss with Chemical Uncouplers

Studies using isolated mitochondria demonstrate that respiration is inhibited when ADP is depleted[51]. Depletion of ADP removes the substrate for ATP synthase and results in a rise of the inner mitochondrial membrane proton gradient to a point where the electron transport chain is inhibited. However, respiration can recommence in the absence of ADP if the mitochondrial membrane is disrupted and the proton gradient released, thus uncoupling oxidative metabolism from ATP synthesis. In BAT, the expression of UCP1 serves as a regulated uncoupler allowing an increase in biochemical heat production without reliance on ADP or build up of surplus ATP. The heat generated contributes to regulation of core body temperature.

In the early 1930s, dinitrophenol (DNP), a chemical uncoupler, was introduced as a means of increasing metabolism for the purpose of weight loss[52–54]. It was found to be highly effective at increasing metabolic rate and resulted predominantly in a loss of fat with a conservation of muscle mass. However, patients exposed to DNP were later found to develop cataracts months after completing treatment[53,55], resulting in the eventual withdrawal of DNP from the market. In one early study, DNP was administered to 170 subjects without caloric restriction. Five subjects did not lose weight, but the remainder lost an average of 7.8 kg over an average period of 88 days. The average dose used in that study was 340 mg/day, resulting in a 33% rise in RMR[56]. If we take an obese male with an RMR of 7.5 MJ/day, a 33% rise in RMR over 88 days (2.5 MJ/day) would result in a predicted weight loss of 5.2 kg.

The relationship between an increase in energy expended and net weight loss due to DNP is surprisingly similar to that in hyperthyroid states. The predicted amount of weight loss underestimates the observed weight loss, despite the absence of caloric restriction. No food intake data are available, but it would appear that intake does not increase sufficiently to compensate and may even fall. Like hyperthyroidism, chemical uncouplers induce a state of sustained low-grade hypermetabolism and use involuntary biochemical processes. The only difference is that the mode of action of DNP is through mitochondrial uncoupling, whereas hyperthyroidism use a number of biochemical processes. However, if BAT and regulated uncoupling are shown to be functionally relevant in humans, this may point to a common mechanism that may result in a state of thermogenic disconnect.

Energy Expenditure and Weight Loss with BAT Activation

There are no data regarding the magnitude of EE inducible in humans through BAT activation. Virtanen *et al.*[11] compared fluorodeoxyglucose uptake in five healthy individuals following 2 h exposure to approximately 18°C compared with no prior cold exposure. Using data from one subject, the authors estimated the supraclavicular BAT depot to have a mass of 63 g. Assuming that glucose accounts for 10% of substrate utilized, with the remainder from fat, the authors estimated that sustained activation of 63 g BAT will metabolize an equivalent of 4.1 kg fat over 1 year. That is, the estimated increase in EE due to BAT activation is approximately 418 kJ/day (equivalent to a 4.5% increase in TDEE for an average male with 9.2 MJ TDEE).

Although it would be wrong to assume that cold-induced increases in EE are equivalent to BAT thermogenesis, it is reasonable to assume that BAT thermogenesis will fall within the range observed in cold exposure studies, given that cold is the most potent physiological activator of BAT. Interestingly, the estimated increase in EE that could be attributed to BAT (as calculated by Virtanen *et al.*[11]) falls within the range of increases observed in calorimetric studies under similar cold stimulation conditions. Increase in TDEE of 2% (22°C vs 16°C for 48 h)[57], 5% (22°C vs 16°C for 84 h)[58], 6% (24°C vs 19°C for 12 h)[59],

7% (28°C vs 22°C for 30 h)[60], 7–11% (22°C vs 15°C for 3 h)[61], and 17.8% (27°C vs 19°C for 1.7 h)[62] have been described. Provided that the estimates of Virtanen *et al.* are accurate, it would appear that cold-induced BAT activation could contribute significantly to adult human thermogenesis.

Whether these increases are sufficient to alter body weight remains uncertain. Compared with the values known to induce weight loss through exercise, hyperthyroidism, or DNP (as described above), mild cold-induced increases in EE would appear insufficient. Conversely, if cold exposure can induce a state of thermogenic disconnect, weight loss can potentially occur with this degree of increased EE. In rodents, prolonged cold exposure leads to an increase in BAT tissue mass and an increase in maximal thermogenic capacity[49]. Whether CIT could be augmented in adult humans following repeated or prolonged cold exposure needs to be examined.

Indigenous populations living in desert environments are exposed to extreme fluctuations in temperature. During the night, temperatures can fall below 5°C. In addition, shelter and clothing are often minimal. As such, it can be considered a natural form of repeated cold exposure in humans. As opposed to data observed in rodents, studies of Aborigines living in the deserts of Australia[63] and Bushmen living in the Kalahari Desert of southern Africa[64] reveal that repeated nocturnal cold exposure does not increase EE; rather, core body temperature and RMR are observed to fall in the cold. Such a response would be of benefit for short-term energy conservation with the expectation that the prevailing thermal stress will be transient. In keeping with this, Leppaluoto *et al.* have published extensively[65–75] on the hormonal changes that occur following repeated cold exposure. They observed that the endocrine (rise in thyroid hormone and adrenergic response) and metabolic (rise in free fatty acids) changes in a standardized cold stimulation tend to fall following repeated cold exposure. Such a response is termed hypothermic cold adaptation. The defended core body temperature is set lower (by approximately 0.5°C) and the core temperature that triggers shivering thermogenesis is lowered by a similar amount.

Despite hypothermic adaptation, measurements of EE following repeated cold exposure are much more encouraging. Bittel[76] exposed individuals to repeated cold water immersion and demonstrated the effects of hypothermic adaptation as described above. However, EE induced by acute cold stimulation did not diminish despite this. Furthermore, in some individuals, an additional 10% increase in EE was demonstrated upon a standardized cold exposure compared with the pre-acclimated state. This augmentation in EE despite cold adaptation is further supported by other studies[77].

The increase in EE is much more consistent following sustained cold exposure. A recent study looked at the effects of cold acclimatization following the winter months and compared this with the summer[61]. Individuals were exposed to a standardized mild cold exposure of 15°C for 3 h. In summer, the increase in RMR was 7% and in winter it was 11.5%, representing a 64% increase in metabolic capacity following winter acclimatization. Data from longer-term cold exposure (>6 months) necessarily comes from colder environments at latitudes closer to the poles[78,79]. Although these data will be less controlled, the consistency of the findings suggests that long-term cold exposure does increase thermogenic capacity in terms of responses to an acute cold stimulation compared with pre-acclimatization. More importantly, the magnitude of the increase appears higher than that inducible through repeated cold exposure. The effect of long-term cold exposure in soldiers stationed at different latitudes was published by Johnson and Kark[78]. The authors collected estimates of food intake and demonstrated a progressive increase in total caloric requirements at higher latitudes and cooler environmental temperatures. Leonard *et al.*[79] published a meta-analysis of data from studies of Indigenous and non-Indigenous population living at high latitudes. Both Indigenous and non-Indigenous populations showed a higher RMR than that predicted for their age, height, weight, gender, and surface area. Non-Indigenous male subjects had a consistently higher RMR of between 10 and 19% compared with that predicted for fat-free mass. This supports the possibility that, in humans, cold-induced EE can be augmented following cold acclimatization.

Whether this increase in EE translates to weight change is of more relevance. To date, no controlled data regarding the effects of cold exposure on weight loss are available. Bergmann's rule states that at lower ambient temperature, a reduction in the surface area to volume ratio will reduce heat loss and be of survival benefit[80]. Numerous studies have shown that this may be applicable to humans because there is a trend for increased body weight in populations living at higher latitudes[81,82]. Other studies have looked at the effects on weight during extended polar expeditions. In general, these studies conclude that food intake[83,84] and EE[85–87] both increase in the cold, with significant fluctuations between the Antarctic/Arctic winter and summer months. More importantly, most individuals gain a small amount of weight over the period of their stay, which ranges from a few weeks to as much as 3 years[88]. This would suggest that the higher EE from cold exposure is not associated with weight loss. However, these studies involve extreme climates and it may be possible that less extreme exposures applied in a regulated manner may have an impact on body weight regulation.

In summary, cold exposure induces an increase in EE. This increase is small relative to amounts known to achieve weight loss under conditions of exercise, DNP treatment, or hyperthyroidism. However, the increase in EE can be augmented with repeated or sustained cold exposure. Although long-term cold exposure is associated with increased EE, it is not associated with lower body weight.

RESPONSE TO COLD IN HUMANS AND POTENTIAL BARRIERS TO EXPLOITING CIT FOR WEIGHT MANAGEMENT

Homeotherms can maintain body temperature at a consistently stable level despite fluctuating environmental temperatures.

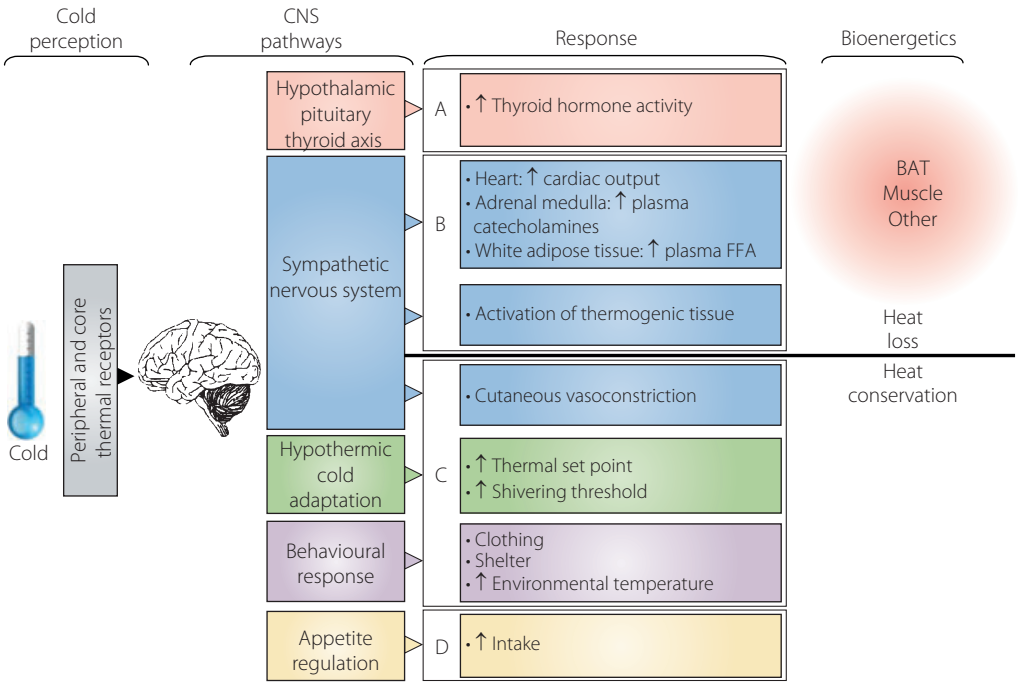

Figure 2 | Physiological responses to cold exposure. From left to right, cold stimuli are sensed by peripheral and central thermal receptors. Within the central nervous system (CNS), neurobehavioral and endocrine responses are activated to defend core body temperature, including the hypothalamic–pituitary–thyroid axis, the sympathetic nervous system, behavioral responses, hypothermic cold adaptation, and appetite regulation. In turn, these produce physiological responses that support thermogenesis (A), activate thermogenic effector tissues (B), conserve heat (C) and increase caloric intake (D). For maximal thermogenic induced weight loss, Processes A and B should be optimal, whereas Processes C and D should be minimized. BAT, brown adipose tissue; FFA, free fatty acids.

Thermoneutrality is defined as a narrow range of temperature whereby additional EE above obligatory thermogenesis is not required for thermal homeostasis (Figure 1). In a lightly clothed human, thermoneutrality lies between 23 and 26°C[89,90]. Temperatures below thermoneutrality will elicit a series of mechanism to defend core body temperature.

In humans, behavioral and physiological processes both contribute to thermal defense. In humans, behavioral responses predominate, such as seeking shelter; increasing clothing levels, and, perhaps most commonly in modern life, increasing our environmental temperature. Physiological responses can be separated into three major forms: (i) hypothermic (including habituation); (ii) insulative (vasoconstriction and piloerection); and (iii) metabolic (facultative thermogenesis or CIT). The latter is the only response that involves purposeful heat production. In addition, CIT can be further divided into non-shivering thermogenesis and shivering thermogenesis. Pertinent to weight management, activation of molecular processes involved in non-shivering thermogenesis represents the most promising (and comfortable) approach (Figure 2).

The relative contribution of each physiological response is dependent on interindividual differences[23,91], the severity of cold exposure[67], the rate of temperature change[92], and prior acclimatization[58]. The neurocircuitry regulating the balance of these three physiological responses is unclear. Much of the work has been done on rodents and is beyond the scope of the present review (for recent reviews, see Whittle *et al.*[93] and Morrison *et al.*[94]). Briefly, environmental temperature is sensed by specific cold receptors (belonging to a class of the transient receptor potential [TRP] family) located in the periphery. Together with central core temperature sensors, thermal signals are integrated within the central nervous system (CNS). Within the CNS, various pathways are thought to modulate food intake, the hypothalamic–pituitary–thyroid axis, and SNS tone to the adrenals, heart, skeletal muscle, cutaneous blood vessels, white, and brown adipose tissue. The resultant rise in core temperature forms a feedback that reduces the stimuli on core thermal receptors. If core or environmental temperatures rise above optimum, heat is sensed by separate warm receptors and their activation effectively terminates the cold response. Any further rise in core temperature will trigger vasodilatation and sweating. Any attempts at exploiting CIT for weight loss will require a specific cold signal to activate thermogenic processes, minimize insulative and hypothermic adaptations, and maximize heat loss (Figure 2).

The molecular mechanism underlying heat production during CIT in humans is not completely understood. In cold-adapted murine models, CIT is largely determined by BAT activity. Little is known about the role of BAT in humans, but the factors required for optimal BAT function in rodents should be applicable to humans. Following cold adaptation in mice, there is

hypertrophy and an increase in blood flow in BAT. Cardiac output is increased, with concomitant cardiac hypertrophy such that up to 60% of output is delivered to the BAT[95]. The SNS tone to the BAT is increased, which regulates the expression of lipoprotein lipase and glucose transporters, thus increasing substrate uptake[49]. Central to the ability of BAT to generate heat is the expression of UCP1. This requires both SNS activity and local increases in thyroid hormone activity. Finally, facilitated SNS tone to white adipose tissue increases the rate of lipolysis. The free fatty acids released are then delivered directly to the BAT or indirectly via hepatic production of triglyceride-rich lipoproteins.

Although the cold-induced changes within the BAT have yet to be documented in humans, many of the changes outside of the BAT have been described (Figure 2). Following acute mild cold exposure in humans, serum catecholamines, thyroid hormone, free fatty acids, and triglycerides are increased[68,72,96,97]. Although heart rate falls, blood pressure is increased, indicating a rise in total peripheral resistance. This is most likely mediated by cutaneous vasoconstriction and is seen as a fall in skin temperature in the extremities. Heart rate variability analysis performed during cold exposure reveals that autonomic tone to the heart is altered with increases in both low-frequency (sympathetic and parasympathetic) and high-frequency (parasympathetic) components[72]. Altogether, optimal activation of BAT/CIT requires a complex series of physiological changes.

In summary, the effects of cold in humans are complex and the physiological processes are regulated by feedback loops centered around the defense of core body temperature. Non-physiological manipulation of thermogenic effector tissues (BAT or otherwise) in isolation may benefit from coordinated changes in the vasculature, hormonal profile, and circulating substrates. Alternatively, physiological activation of thermogenesis through cold exposure will require minimization of non-thermogenic responses and manipulation of core temperature negative feedback.

CONCLUSIONS

From a thermodynamic perspective, increasing EE to induce weight loss is a practical approach. When applied to biological systems, compensatory increases in energy intake often occur. The adipostat hypothesis postulates that fat storage is well defended, especially in the context of negative energy balance.

Exercise is an effective means of increasing EE. Despite this, energy expended during exercise does not result in the predicted amount of weight loss, even when compliance with exercise is maintained. Consistent with the adipostat hypothesis, EE induced by exercise is largely compensated for by an increase in food intake. Conversely, metabolic states, such as DNP treatment and hyperthyroidism, which result in conversion of energy to heat, do not appear to be fully compensated by increases in food intake. Although it is possible that factors other than EE, such as appetite suppression or reduced absorption, may be involved, the current data suggest that it is more than plausible that activating thermogenesis can induce clinically relevant weight loss without caloric restriction, a state of thermogenic disconnect.

The effects of CIT share many common mechanisms with thyroid hormone heat production. In addition, the discovery of functional BAT in adult humans raises the possibility of exploitable mitochondrial uncoupling in humans. Targeting BAT mass and activity as a means of increasing EE has many potential benefits. First, unlike the use of DNP, uncoupling will only occur in one tissue type. Potentially, this would reduce any toxic effect on tissues not normally suited to high rates of uncoupling. Second, BAT contains the necessary vasculature to ensure an adequate supply of nutrients and the dissipation of excess heat. If humans are appropriately cold adapted, nutrient delivery and heat dissipation would be expected to meet BAT activity and thus maximize EE, heat loss, and ultimately weight loss. Finally, BAT activation may have other metabolic benefits independent of weight loss. In murine models, BAT has been shown to contribute significantly to whole-body glucose and lipid clearance. Although this may not directly alter insulin resistance at the molecular level, BAT could contribute to concomitant glycemic control[98–102] and lipid lowering[103,104] in obese individuals.

In the absence of an optimal method of specifically activating BAT in humans, studies using cold exposure without inducing shivering are a practical alternative. As such, a number of areas require further investigation, as outlined below.

1. Can CIT be augmented (through controlled cold exposure) to achieve a level sufficient to induce weight loss?
2. How much does food intake change in response to CIT? (That is, can we quantify the magnitude of the thermogenic disconnect?)
3. In terms of magnitude, what is the relative contribution of BAT-mediated compared with non-BAT-mediated processes to CIT in humans?
4. Can BAT (or other CIT processes) be activated without cold exposure?
5. If weight loss is not possible, will activation of thermogenic processes contribute to weight stabilization?
6. Will thermogenesis contribute to metabolic health independent of weight loss?

Some tools that may be required to investigate the questions listed above include: (i) a practical way of applying cold exposure outside the laboratory; (ii) a method to quantify BAT and non-BAT-mediated thermogenesis; and (iii) a method of distinguishing BAT activity from BAT mass. The answers to these questions and further investigations into the molecular basis of thermogenesis may reveal therapeutic targets that will be translatable into effective obesity and metabolic therapy.

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