Strategies for targeting BAT thermogenesis

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Abstract

Modern imaging techniques have confirmed the presence of thermogenically active brown adipose tissue (BAT) in adult humans, leading to suggestions that it could be stimulated to treat obesity and its associated morbidities. The mechanisms regulating thermogenesis in BAT are better understood than ever before, with new hypotheses for increasing the amount of brown fat or its activity being put forward on a weekly basis. The challenge now is to identify safe ways to manipulate specific aspects of the physiological regulation of thermogenesis, in a manner that will be bioenergetically effective. This review outlines the nature of these regulatory mechanisms both terms of their cellular specificity and likely effectiveness given the physiological paradigms in which thermogenesis is activated. Similarly, their potential for being targeted by new or existing drugs is discussed, drawing on the known mechanisms of action of various pharmacological agents and some likely limitations that should be considered.

Introduction

By no means is brown adipose tissue (BAT) a novel discovery; the "hibernating gland" was first described in rodents by the Swiss naturalist Conrad Gessner as early as 1551[1]. In more recent times throughout the 20th Century it has been the focus of multiple waves of scientific study, each of which waned as BAT's relevance to adult humans was called into question, largely due to limitations in our ability to measure its presence or activity. The recent resurgence in interest stems from data provided by modern PET-CT imaging studies firmly demonstrating that healthy adults do indeed possess significant depots of BAT [2-5]. Moreover, amounts of detectable, active BAT are inversely correlated with age, BMI, fat mass and insulin sensitivity [6]. At the same time the increased availability of genetically modified murine models has shone more

light than ever before on the endogenous mechanisms that exist to regulate BAT function [7]. Attention has now returned to how we might exploit this tissue more effectively and whether any of these regulatory systems lend themselves to pharmacological or therapeutic intervention. It is posited that increasing the prevalence and activity of BAT might be an effective strategy to treat conditions such as obesity, diabetes and cardiovascular disease, via increased energy expenditure, the removal of toxic lipid species and a direct reduction in the demand for insulin production. This review will focus on the latest knowledge surrounding BAT regulation and highlight potential approaches to target thermogenesis safely and effectively. It will also comment on situations where dysregulation of BAT may contribute to certain metabolic diseases.

The master regulators of BAT, lest we forget

While the views towards BAT's prevalence and relevance have changed dramatically over recent decades one fact has remained widely undisputed. BAT is under the direct regulation of the sympathetic nervous system, the tone of which to BAT is increased by exposure to a cold environment or in response to increased caloric intake. Subsequent activation of various isoforms of the β-adrenergic receptors by noradrenaline to induce both the thermogenic activity of existing brown adipocytes and the recruitment of new cells to BAT depots is therefore essential for heat production [8]. Similarly, intact thyroid hormone signalling is crucial for thermogenesis in BAT, signified by two key observations. Circulating thyroid hormone and BAT expression levels of the thyroid hormone activating enzyme deiodinase II (DioII) are strongly increased in situations of increased thermogenesis and genetic ablation of *DioII* in BAT renders animals incapable of adaptive thermogenesis [9]. Therefore when

considering strategies to increase BAT thermogenesis we should be mindful of the necessity to coincidently increase or at least maintain tonic levels of BAT activation by these endogenous regulatory mechanisms. Failure to do so will likely negate the efficacy of approaches targeting more specific aspects of brown adipocyte biology.

Increasing the amount of BAT

To understand how to increase the amount of BAT, we must first learn where it comes from. In mouse models, cell lineage tracing has shown fairly conclusively that in development brown adipocytes arise from an origin distinctly different to that of white adipocytes. Brown adipocytes arise from a cell lineage expressing the myogenic gene *Myf5,* which are also able to differentiate into skeletal muscle cells [10]. In BAT the induction of key transcription factors leads to repression of myogenic gene expression and facilitates brown fat determination. In the last 5 years a number of these factors, PR domain contain 16 (PRDM16), Sirtuin 1 (SIRT1) and the signals that can induce them, such as bone morphogenetic protein 7 (BMP7) have begun to be identified [10-12]. Despite their different origins, all adipose tissues are dependant on certain fundamental transcriptional regulators to drive preadipocyte cells towards mature adipocytes, namely peroxisome proliferator-activated receptor-gamma (PPARγ) and CCAAT/enhancer-binding proteins (C/EBPs) [13]. Expression of just C/EBPβ, the C/EBP more highly expressed in BAT, and PRDM16 in either human or mouse skin fibroblasts is sufficient to drive these precursors to differentiate into cells displaying the key characteristics of brown adipocytes (expression of uncoupling protein 1, lipid accumulation, responsiveness to cAMP). What is more, when transplanted back into mice these genetically modified fibroblasts formed an ectopic

fat pad that appeared and behaved similarly to BAT, taking up labelled glucose to give a positive PET-CT signal [14].

Obviously there are important differences between mice and men, a crucial one being our perception of our environmental temperature. In the aforementioned study by Kajimura *et al*. the modified BAT-recipient mice were housed at room temperature (20 °C – 23 °C), a sub-thermoneutral temperature for animals of their size. Thermogenic demand would have thus been reasonably high such that they were driven to activate their additional pool of new BAT. The same temperature requires no additional heat productions for humans to maintain their core temperature and therefore very little sympathetic activation of thermogenesis. Given the importance of adrenergic tone to BAT it is likely that a similar study in humans would have simply seen the extra thermogenic capacity of the transplanted cells largely or even completely unutilised. That said, the study does demonstrate that population of modified adipocytes, under the right environmental setting can engraft and acquire adequate vascularisation and innervation to become metabolically active to a significant degree. Also, whilst GM-cell transplants are currently some way off use in humans they do provide a potential future approach to avoid deleterious off-target effects, as to date very little is known about the effects of factors such as PRDM16 in non-adipose tissues.

Increasing the number of "beige" adipocytes

When mice are exposed to colder environmental temperatures and adaptive thermogenesis is activated, increased numbers of "brown-like" or "beige" adipocytes appear in traditional white adipose tissue depots. These cells are unique in the sense

that they express all of the thermogenic machinery but not Myf5, indicating they arise from a different developmental origin to canonical BAT [15]. The same effect is seen in animals treated with agonists of PPARγ [16] and interest in these cells has grown for a two key reasons. First, strains of mice that have a higher propensity for "browning" of their white fat show a greater degree of protection from diet-induced obesity when treated with β3-adrenergic receptor agonists [17] (this is the main receptor isoform regulating thermogenic activity in BAT); and second, it has recently been suggested that the gene expression profile of adult human BAT bears greater resemblance to that of beige adipocytes than murine brown adipocytes found in traditional interscapular depots [18]. This finding is hugely encouraging, as traditional BAT depots decline rapidly in childhood and into adolescence, leaving only small residual repositories of thermogenically active tissue by post-pubertal ages [19]. In adult mice beige adipocytes require active induction and recruitment under specific environmental conditions, therefore these human observations at very least suggest that such "browning" mechanisms might remain intact and effective at maintaining a population of thermogenic cells well into later life.

When seeking to increase the pool of beige adipocytes in white adipose tissue (WAT) we may only have to look as far as skeletal muscle. It had been observed that exercise was sufficient to increase the expression of thermogenic genes in WAT whilst at the same time protecting mice from the deleterious effects of a high fat diet [20]. Subsequently an exercise-induced myokine, namely irisin, has been characterised with the ability to induce a thermogenic program of gene expression in white adipose tissue, increase energy expenditure and reduce rates of weight gain in mice [21]. At present this finding should encourage people of the multitude of benefits resulting

144 from increased exercise. However, before irisin is hailed as “magic switch” for
145 turning white fat brown, a couple of important points must be addressed. At present
146 the irisin receptor remains elusive and the effects of irisin in other crucial organs have
147 not been examined. A molecule that integrates increased muscle and thermogenic
148 function may also be expected to act on the heart to meet the extra requirements of
149 oxygen provision. In obese individuals already carrying an increased cardiovascular
150 burden, such effects may not be well tolerated.
151
152 Other avenues for increasing beige adipocyte content do exist. MicroRNA-133
153 (miRNA-133) is highly expressed in skeletal muscle and is heavily downregulated in
154 brown and white adipose depots in response to cold-exposure in mice. Further studies
155 show that miRNA-133 directly targets PRDM16 to repress its browning effects in
156 adipose tissue and that inhibition of miRNA in BAT or WAT precursor cells allows
157 increased expression of thermogenic genes and increased oxidative metabolism [22].
158 Given that pharmacological strategies that suppress individual miRNAs have already
159 been tested with some success in humans to treat hepatitis [23], this strategy may also
160 show promise for BAT-centric, anti-obesity therapy. As always there is air for caution.
161 miRNA-133 has recently been identified as an important tumour-suppressor [24], while
162 its downregulation associates with cardiac hypertrophy and infarction [25, 26], perhaps
163 making global pharmacological repression of its expression less than favourable.
164
165 **Increasing BAT activity**
166 As alluded to earlier in this review, it is likely of limited benefit to provide an
167 additional pool of thermogenic cells to an individual unless the physiological setting
168 is such that they will receive at least a minimal level of stimulation from the

169 sympathetic nervous system. Similarly, the efficacy of any BAT-expanding approach
170 is likely to be enhanced by parallel strategies to enhance adrenergic stimulation. To
171 this end bioactive food ingredients such as methylxantines (caffeine or theophylline),
172 ephedrine, polyphenols (catechins, resveratrol, querectin, kaempferol) and certain
173 fatty acids or drugs such as sibutramine, which increase or maintain sympathetic
174 nervous system activation, are effective at increasing energy expenditure and
175 lowering body weight [27-29]. However, the fact that the effects of sibutramine and other
176 sympathomimetics cannot be targeted to a specific tissue has rendered them unsafe for
177 use in humans [30].
178
179 Increasingly researchers are attempting to define more specific physiological
180 mechanisms that regulate the response of BAT to endogenous levels of sympathetic
181 stimulation, with some success. An increasing number of cytokines and hormones
182 have been shown to have positive effects on the thermogenic activity of BAT and
183 perhaps most interestingly, none of them appear to act via the adrenergic receptors
184 directly but instead through more specific aspects of brown adipocyte biology.
185
186 FGF (fibroblast growth factor) 21 is secreted predominantly by the liver, where levels
187 rise during fasting, feeding a ketogenic diet or after amino acid deprivation; but also
188 by other tissues such as WAT, BAT, skeletal muscle and pancreatic β-cells following
189 different metabolic signals [31, 32]. FGF21 signals to BAT to increase expression of
190 thermogenic genes and enhance its heat-producing capacity and regulates the
191 browning of white adipose tissue though PGC1α signalling [33-35]. In line with this,
192 treatment of obese mice with FGF21 is sufficient to reduce bodyweight by 20%,
193 almost entirely due to increased energy expenditure [36]. Paradoxically, circulating

194 levels of FGF21 increase in obesity in humans. However further investigations reveal
195 that the same is true in mice and that treatment of obese mice with additional FGF21
196 brings about markedly reduced activation of FGF21 targets, such as ERK1/2. In light
197 of this, obesity has been suggested to constitute a state of FGF21 resistance, which
198 may go some way to contribute to obesity-associated metabolic complications and
199 perpetuate a state of positive energy balance [37].
200
201 Although the intracellular pathways activated by FGF21 in BAT are unclear its
202 membrane interaction is well characterised. FGF21 signals through traditional FGF
203 receptors conjugated to the membrane protein βKlotho [38], which may offer
204 opportunities to target the thermogenic properties of FGF21 more selectively of other
205 growth factor-activated pathways.
206
207 We have recently characterised bone morphogenetic protein 8b (BMP8B) as another
208 thermogenically regulated protein, enriched in mature brown adipocytes in line with
209 their level of thermogenic activity [39]. BMP8B is a secretory peptide that acts in an
210 autocrine/paracrine manner to increase the thermogenic capacity of BAT by
211 enhancing the intracellular response to adrenergic stimulation. Mice that lack BMP8B
212 have lower core body temperatures, reduced thermogenic capacity and are
213 significantly more susceptible to diet-induced obesity due to a reduction in their
214 metabolic rate. Any ability to modulate adrenergic sensitivity in a tissue specific
215 manner is likely to be of great potential benefit for the treatment of a range of
216 conditions in addition to obesity, such as hypertension and various forms of fibrotic
217 liver disease. Whilst the precise mechanisms of BMP8b remains unclear, its distinct
218 expression profile of and the high degree of cellular specificity in the BMP receptor

219 system, recently reviewed by Sieber et al [40], make it a promising future target for
220 activating BAT independently of other sympathetically regulated organs.
221
222 Of perhaps even greater interest is the fact that a significant portion of the
223 thermogenic effect of BMP8B can likely be attributed to its central role. ICV
224 treatment with BMP8b results in an immediate increase in sympathetic tone to BAT
225 and this effect is dependant on the level of AMP-activated protein kinase (AMPK) in
226 the ventromedial nucleus of the hypothalamus, a well defined regulatory node for
227 thermogenic activation [41]. Whilst the global role of AMPK in cellular bioenergetics
228 would appear to make it a difficult target when aiming to selectively activate BAT,
229 these findings to highlight that there are discrete nuclei within the central circuits
230 regulating BAT that might one day be amenable to therapeutic manipulation. The fact
231 that BMP8B may be an endogenous activator of such nuclei makes dissecting its
232 mechanism of action all the more important. Already it has been shown that blockade
233 of an individual BMP receptor, Activin Receptor IIB, induces thermogenesis and
234 brown adipogenesis [42].
235
236 **Targeting central regulation of BAT**
237 Our understanding of the central control of thermogenesis has been expanded vastly
238 in the last decade. This is thanks largely to detailed electrophysiological investigations
239 of specific neuronal population by Morrison and Madden, which are the focus of
240 recent review [43]. In summary, classical cold-activated thermogenesis is brought about
241 when cold sensitive temperature receptors in the skin signal to the pre-optic area of
242 the hypothalamus (POA). Activation of these GABA-ergic neurons disinhibits
243 neurons of the dorsomedial hypothalamus (DMH), which are themselves suppressed

by signals from warm-sensitive temperature receptors. Subsequently projections from the DMH activate sympathetic pre-motor neurons in the rostral raphe pallidus of the hindbrain (rRPA), which signal to BAT to increase thermogenesis. Certain nutritionally-derived molecules are known to act as agonists for various isoforms of these temperature receptors, offering a means to activate the endogenous machinery at the most upstream point.

Capsaicin, the compound found in hot chilli, acts a ligand for the transient receptor potential cation channel subfamily V member 1 (TRPV1). This receptor is highly expressed in sensory neurons and is stimulated by intense heat [44]. Seemingly paradoxically, treatment of rats with capsaicin is known to actually increase activation of thermogenesis [45] and more recently capsaicin has been shown to cause similar effects in humans [46]. Whilst this raises the possibility of nutritionally-mediated increases to energy expenditure to treat obesity the studies performed to date have only been conducted in the acute setting. The rat studies indicate some level of protection from diet induced obesity but it should be noted that animals of this size already have good levels of thermogenic activation, and indeed the capsaicin had no effects in BAT-negative individuals.

Menthol is also able to stimulate thermogenesis through a separate temperature receptor, TRP subfamily M member 8 (TRPM8). TRPM8 is also expressed in the sensory and central nervous system, as well as on brown adipocytes themselves [47]. Menthol treatment of mice is also effective at protecting them from diet-induced obesity through increased thermogenesis, but a portion of this effect can be attributed to direct effects on BAT itself. TRPM8 treatment of brown adipocytes increases

expression of UCP1 via a mechanism not hugely dissimilar to BMP8B, whereby the phosphorylation status of key intracellular kinases, in this case PKA, is also increased. It should still be considered however that activation of traditional cold-induced thermogenesis is known to have a potent positive effect on food intake [8], potentially limiting its long terms effects on energy balance in humans.

Additional central effectors of BAT thermogenesis and the link with nutrition

In addition to cold, increasing caloric intake via western or high fat diets also increases heat production in BAT, via a process termed diet-induced thermogenesis [48]. As yet the central mechanisms driving this phenomenon is unknown, but there is an ever increasing number of neuroactive molecules that could be involved in feeding-related changes to sympathetic outflow to BAT. Such mechanisms are of interest for two reasons. First, in situation where food intake is the signal for activating thermogenesis, it is unlikely further food intake would be stimulated as is the case in cold-stimulated thermogenesis. Therefore mimicking these signals may be more efficacious for weight loss. Second understanding the interaction between thermogenesis and feeding may open the door for inhibiting compensatory food intake when pharmacologically mimicking cold-stimulated thermogenesis.

Appetite-regulating molecules released by the digestive system (such as ghrelin and calcitonin gene-related peptide) have documented effects on BAT activity [49-51]. Equally, changes in nutrient availability might be directly sensed by the brain. Specific hypothalamic neurons including orexin/hypocretin neurons are sensitive to nutrients (including glucose, amino acids, and fatty acids), and glucose and lipid species can act centrally to activate thermogenesis [52, 53].

294 The inclusion of the hypocretin system and also the dopaminergic and serotonergic
295 systems [54] in the list of central BAT-regulatory mechanisms suggests that there may
296 also be links between thermogenesis and arousal status, reward and stress. Further
297 evidence for a link between more complex behavioural traits and thermogenesis may
298 be inferred from the observation that in sufferers of anorexia, treatment to induce
299 weight gain is often hindered by inappropriately high levels of feeding-induced
300 thermogenesis [55].
301
302 **Antipsychotics, body weight and BAT**
303 It is well documented that certain atypical antipsychotics lead to increased weight
304 gain in both humans and rodents [56]. Closer examination of the known mechanisms of
305 action of these drugs can shed light on a potential link to altered thermogenesis in
306 BAT. Schizophrenia is largely characterised as a state of dopamine receptor
307 supersensitivity and indeed the efficacy of different antipsychotics correlates with
308 their ability to bind and antagonise dopamine D_2 receptors [57]. Olanzapine for example
309 is a D_2 antagonist which causes weight gain but importantly it is a far more potent
310 inhibitor of serotonergic signaling through its antagonism of various 5-HT receptor
311 isoforms [58]. Recently it has been shown that chemical inhibition of these same
312 receptor subtypes has a dramatic effect of body temperature, significantly reducing it
313 by blocking nervous stimulation of BAT [59].
314
315 In this sense one might envisage that selective serotonin reuptake inhibitors (SSRIs)
316 could help to induce the opposite effects. Indeed early studies in humans did indicate
317 weight loss in patients taking SSRIs for relatively short periods, but this was
318 apparently driven largely by reduced food intake [60]. Sibutramine on the other hand,

which is also a noradrenaline reuptake inhibitor, does have a positive effect on energy expenditure and BAT activity in humans [61]. Taken together these finds suggest that serotonin acts further downstream in thermogenic signalling in coordination with norepinephrine and that at a hypothalamic level its modulation has more potent effects on appetite. It also highlights the inherent complexity of the multiple levels of interaction between energy expenditure and intake.

Increasing the contribution of muscle to thermogenesis

Like BAT, skeletal muscle is well innervated and responds positively to noradrenaline levels through β3- and β2-adrenergic receptors. In skeletal muscle, heat production occurs through shivering and non-shivering mechanisms. As shivering is a mechanical muscle response and cannot be sustained for long time the main target processes to sustain prolonged thermogenesis are: mitochondrial uncoupling (as in BAT), futile calcium cycling, and fatty acid/triglyceride cycling that has been shown to be increased following mild-cold exposure [62, 63].

Whilst the functional capacity for myocytes to generate heat through uncoupling is still a matter of debate, an adipocyte progenitor population in human skeletal muscle tissue has been described with a high potential for inducing UCP-1 expression. It has been suggested that these cells explain the small amount of UCP1 mRNA often detected in adult human skeletal muscle and that under the relevant conditions they could contribute significantly to energy expenditure. In this instance, part of the heat generated by uncoupling in skeletal muscle may be due to this quiescent brown cell population [64], which may be amenable to enhancement by the same strategies designed to target BAT directly.

Futile calcium cycling has also been suggested as a potential mechanism to increase skeletal muscle heat production. Here, calcium (Ca^{2+}) released into the cytoplasm is recovered to the sarcoplasmic reticulum by SERCA proteins (sarco/endoplasmic reticulum Ca^{2+}-ATPase), which use ATP. In some cases, as in human malignant hyperthermia, there is an uncontrolled leakage of Ca^{2+} that stimulates calcium cycling without muscle contraction, producing heat [65]. In line with this cold-exposure induces expression and activity of SERCA, and in UCP1 deficient mice leptin-induced thermogenesis increases *Serca-2a* expression to maintain core body temperature [66, 67].

More recently, Sarcolipin has been implicated in skeletal muscle thermogenesis by its role controlling calcium cycling through a direct interaction with SERCA. Sarcolipin deficient mice are more sensitive to cold-exposure, and to a high fat diet (having increased adiposity and glucose intolerant). In terms of adaptive thermogenesis the Sarcolipin-SERCA interaction might be exploited to enhance nonshivering heat production in animals with reduced (humans) or non-functional BAT (pigs) activity [68, 69]. Although more studies need to be done to evaluate the impact of Sarcolipin-SERCA modulation in human skeletal muscle and heart, this mechanism may open new targets to increase thermogenesis in skeletal muscle by modulating Ca^{2+} cycling.

Summary

There are a constantly expanding number of regulatory nodes and pathways that integrate BAT function with global physiological changes to ensure its tight regulation. This is to be expected, as the capacity for BAT to "waste" energy is huge. As we learn more about the endogenous molecules and neuronal systems that control

thermogenesis, we are beginning to identify key points that might be amenable to manipulation and thus allow us to disconnect BAT from the tight leash on which it is kept. It may also be true that metabolic changes that occur in certain disease states are disproportionately inhibiting thermogenesis. In either situation identifying the molecular pathways that bring about changes to BAT function is likely to offer therapeutic opportunities. There are already suggestions that increasing levels of key endogenous molecules, such as irisin and FGF21, might be metabolically beneficial in obese states and there is no shortage of receptors to hunt for that likely bring about the thermogenic effects of some of the other molecules described in this review.

Key to the medicinal utilisation of BAT thermogenesis, as with any intervention, will be specificity and efficacy. Thankfully it seems that the unique qualities of brown adipocytes bring with them some unique regulatory systems, which may help address the former of the problems. More difficult will be overcoming the complex central regulatory mechanisms that sense heat production and modulate sympathetic nervous stimulation of BAT accordingly. Still it seems that even in the heterogeneity of neural networks there may lie key nuclei that integrate information on temperature and energy availability that might be specifically targeted to alter their level of control over BAT activation. What is almost certain, is that approaches mindful of the multiple levels of regulation of thermogenesis in BAT are likely to be the most effective.

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