Breaking BAT: can browning create a better white?

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Abstract

Obesity and its comorbidities are a growing problem worldwide. In consequence, several new strategies have been proposed to promote weight loss and improve insulin sensitivity. Recently, it has been demonstrated that certain populations of white adipocytes can be 'browned', i.e., recruited to a more brown-like adipocyte, capable of thermogenesis through increased expression of uncoupling protein 1. The list of browning agents that induce these so-called beige adipocytes is growing constantly. However, the underlying mechanisms are often poorly understood, with the possibility that some of these agents cause browning as a secondary effect. Moreover, it remains unclear whether beige adipocytes can contribute sufficiently to affect whole-body energy expenditure in a functionally significant manner. This review presents an overview of the different molecular pathways leading to the induction of beige fat, including direct stimulation and indirect actions on the CNS or the immune system. We discuss the available evidence on the capacity of beige adipocytes to influence whole-body energy expenditure in rodents, and lastly outline the potential problems of translating browning capacity into the potential treatment of human metabolic diseases.

Introduction

Obesity and its comorbidities are a growing worldwide health problem (Lobstein *et al.* 2015). Consequently, a lot of money and effort have been invested into understanding the problem, namely the overflow of lipid storage capacity in fat and inappropriate lipid deposition in other organs, as well as finding a potential therapeutic target to remove the excess fat. Recent studies have put forward preliminary evidence that the solution to the problem might lie in the fat itself, which could be turned to burning fuel instead of storing. This review summarizes the current knowledge on thermogenic adipose tissue and discusses its possible use in elevating energy expenditure to counteract obesity and metabolic diseases such as type 2 diabetes.

Three kinds of fat

To date, three major types of adipose tissue have been identified. White adipose tissue (WAT) is characterized by large white adipocytes with few mitochondria, and can be found in mice predominantly around the gonads (gonadal WAT, in male mice also called epididymal WAT) and subcutaneously close to the hind limbs (inguinal WAT) (Bartelt & Heeren 2014). In humans, the major sites of WAT are under the skin (subcutaneous WAT) and associated with the digestive tract (visceral WAT), but it can also be found around internal organs such as the heart (epicardial WAT), kidney (perirenal WAT), lung (pulmonary WAT), and arteries (periadventitial WAT). WAT is generally considered as insulation against cold and as a safe storage space for fatty acids esterified into

triglycerides. When its storage capacity is exceeded, fatty acids can no longer be safely cleared from the systemic circulation and accumulate in other organs such as the liver and the muscle. This concept called lipotoxicity can subsequently lead to insulin resistance and result in type 2 diabetes and/or cardiovascular disease (Huang-Doran *et al.* 2010, Speakman & O'Rahilly 2012).

Brown adipose tissue (BAT) is characterized by smaller lipid droplets and a high number of mitochondria. In rodents, BAT can be found in the interscapular region, located between the shoulders, and to a lesser extent as perirenal and axillary BAT (Sidossis & Kajimura 2015). In humans, BAT is found in the neck region of infants with a volume of around 3.5 ml (van Marken Lichtenbelt *et al.* 2009, Virtanen *et al.* 2009, Devlin 2015) – presumably to warm the blood supply of the brain (Sidossis & Kajimura 2015, Symonds *et al.* 2015), supraclavicular area and perirenal area. Interestingly, although BAT and WAT both have the capacity to store lipids, they are derived from different progenitors. BAT is derived from precursor cells, which share the same lineage as skeletal muscle cells and are positive for Myf5. In contrast to skeletal muscle cells, BAT progenitors initiate the expression of PRDM16 and BMP7 during development, which drives them to become mature brown adipocytes (Seale *et al.* 2008, Tseng *et al.* 2008, Rajakumari *et al.* 2013).

The most important feature of brown fat is the capability to oxidize substrates to produce heat for facultative thermogenesis, which is required to maintain body temperature under conditions below thermoneutrality, and for arousal from torpor (Cannon & Nedergaard 2004). At the molecular level this process depends on uncoupling protein 1 (UCP1), which shortcuts the proton gradient in mitochondria to produce heat instead of ATP. Several other molecules have been identified to be integral in BAT thermogenesis, the most important being cell death-inducing DFFA-like effector A, peroxisome proliferator-activated receptor gamma coactivator 1, and receptor-interacting protein 140 (Uldry *et al.* 2006, Emont *et al.* 2015).

Recently, a third type of adipocyte was identified in rodents and termed beige or brite fat (brown in white; Wu *et al.* 2012). It is characterized by the expression of UCP1, but localized in classical WAT depots. Although beige adipocytes share some features of brown adipocytes such as similar levels of UCP1 in the mitochondria (Shabalina *et al.* 2013), their expression profile is distinctly different and even varies substantially depending on fat depot and origin. The lineage of these beige fat cells is not well understood. Evidence suggests that these cells can either

arise directly from Myh11+ smooth muscle precursor cells (Long *et al.* 2014), from preadipocytes derived of mesodermal stem cells or through transdifferentiation from white adipocytes by a process called 'browning' (Asano *et al.* 2014, Carey *et al.* 2014, Sanchez-Gurmaches & Guertin 2014, Sidossis & Kajimura 2015, Symonds *et al.* 2015), although the term appears to be interchangeable in the literature regardless of origin.
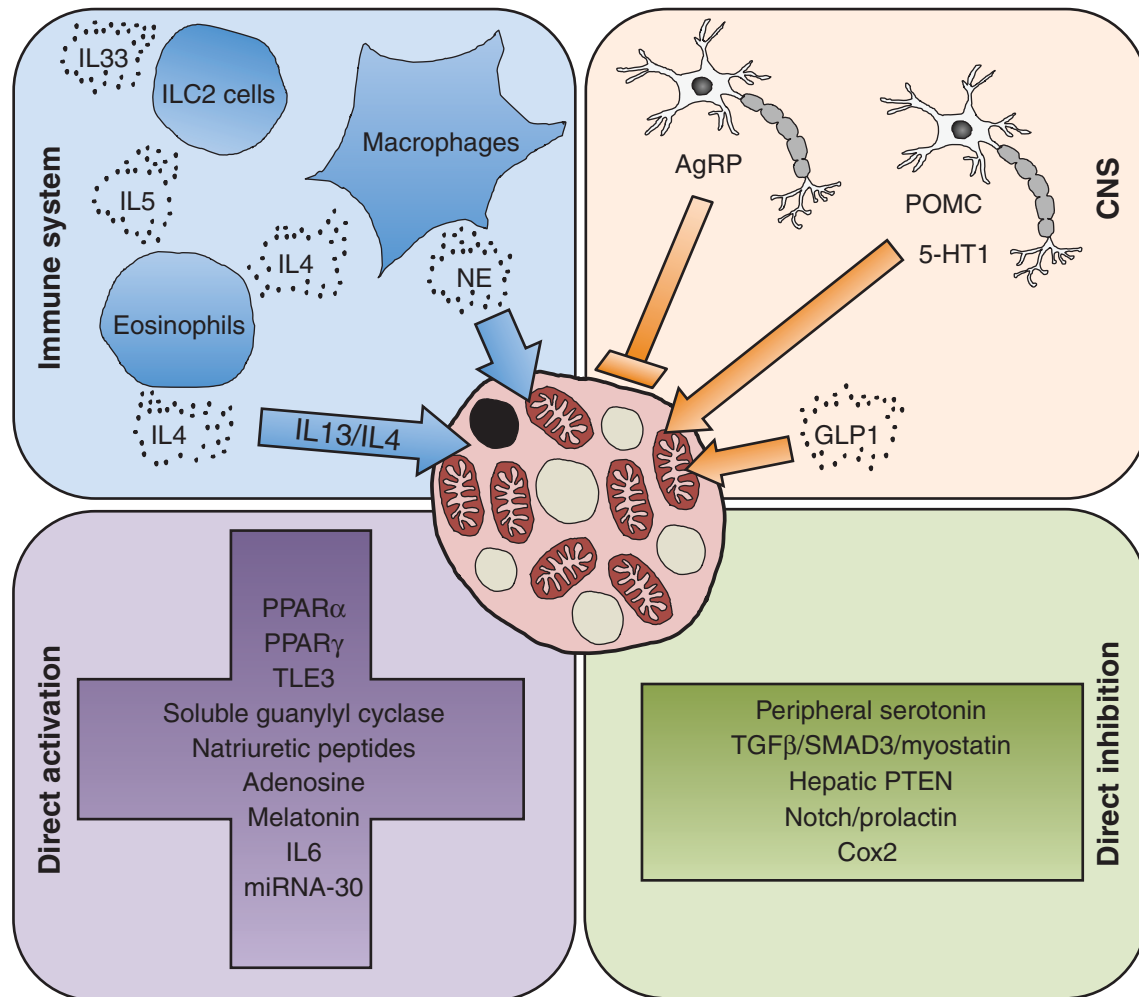
The relative contributions of these pathways most likely depend on the specific depot of WAT hosting the UCP1-positive adipocytes, while perigonadal WAT does not readily produce beige adipocytes and also lacks a response to norepinephrine in terms of oxygen consumption, inguinal WAT shows significant browning (Okamatsu-Ogura *et al.* 2013). In humans, browning has also been observed (Wu *et al.* 2012, Jespersen *et al.* 2013), for instance in WAT associated with organs such as the epicardial depot (Sacks *et al.* 2013). To date, a plethora of browning agents has been identified (Fig. 1), and the list is likely to expand further. Therefore, it seems conceivable that several distinct pathways to beige adipocyte generation exist, and that they might be species- and WAT depot-specific.

Ways of browning

Browning of WAT can be achieved by several different means, including CNS activation modulating sympathetic output to WAT, the recruitment and activation of immune cells in WAT, or by direct action on white adipocytes or beige precursor cells (Fig. 1).

With regard to the CNS, the focus has been on neurons in the hypothalamus, which constitutes the master regulator of the autonomic nervous system. This includes pro-opiomelanocortin and agouti-related peptide producing neurons, well known for appetite regulation, which promote or inhibit browning respectively (Ruan *et al.* 2014, Dodd *et al.* 2015). Furthermore, hypothalamic GLP1 was found to stimulate browning (Beiroa *et al.* 2014, Lopez *et al.* 2015) and to increase triglyceride-derived fatty acid uptake in subcutaneous WAT (Kooijman *et al.* 2015). Likewise, 5-hydroxytryptamine neurons are also important to maintain beige UCP1 mRNA levels (McGlashon *et al.* 2015). These examples, which are likely only the tip of the iceberg, clearly demonstrate the existence of central mechanisms that modulate browning, most likely by adjusting the sympathetic tone to WAT.

The immune system has only recently been implicated in browning. Initially, it was observed that macrophages in WAT can undergo alternative activation and

Figure 1

Different ways leading to browning of white adipose tissue, including immune system, CNS, direct activation, or direct inhibition. AgRP, agouti-related peptide; Cox, cyclooxygenase; GLP1, glucagon-like peptide 1; IL, interleukin; ILC2 cells, type 2 innate lymphoid cells; miRNA, micro ribonucleic acid; POMC, proopiomelanocortin; PPAR, peroxisome prolif-erator-activated receptors; PTEN, phosphatase and tensin homolog; TGF, transforming growth factor; TLE3, transducin-like enhancer of split 3; 5-HT1, 5-hydroxytryptamine receptor 1.

begin to release catecholamines (Qiu *et al.* 2014), which triggers browning via pathways similar to sympathetic stimulation. Interestingly, cold-induced browning of WAT also appears to be dependent on the immune system, namely eosinophils and the interleukin 4 (IL4)/IL13 signaling pathway (Qiu *et al.* 2014). A more recent study shed more light on the underlying complex immuno-logical pathways: when ILC2 immune cells are triggered by IL33, they produce IL5, which in turn activates eosinophils to release IL4. This subsequently activates macrophages to produce norepinephrine, which starts browning events in WAT. However, IL4 and IL13 seem to also have direct browning properties (Brestoff *et al.* 2015, Flach & Diefenbach 2015, Lee *et al.* 2015).

Most studies have identified mechanisms that directly cause browning of WAT, for instance, by activation of PPARα (Rachid *et al.* 2015) or PPARγ (Petrovic *et al.* 2010), through FGF21 (Coskun *et al.* 2008), IL6 (Petruzzelli *et al.* 2014), natriuretic peptides (Bordicchia *et al.* 2012), adenosine through A2A receptors (Gnad *et al.* 2014), melatonin (Jimenez-Aranda *et al.* 2013), TLE3 (Villanueva *et al.* 2013), soluble guanylyl cyclase (Hoffmann *et al.* 2015), or even micro RNA such as miRNA-30 (Hu *et al.* 2015). Unfortunately, the precise mechanisms are often unclear, and some of the identified candidates might well act through indirect mechanisms such as increasing sympathetic tone systemically, which leads to thermogenesis through β-adrenergic activation

(Bachman *et al.* 2002), or by altering body temperature through the promotion of heat loss (Warner *et al.* 2013). Moreover, at least for FGF21, it has been shown that some of the beneficial metabolic effects are in fact not dependent on UCP1 (Samms *et al.* 2015, Veniant *et al.* 2015).

In addition to these direct positive stimuli, a large number of pathways have been identified that seem to actively suppress browning. Consequently, the inhibition of these pathways in turn leads to the appearance of beige adipocytes. This includes peripheral serotonin levels (Crane *et al.* 2015), the TGFβ/SMAD3/myostatin cascade (Shan *et al.* 2013, Singh *et al.* 2014), hepatic PTEN (Peyrou *et al.* 2015), Notch or prolactin receptor signaling (Auffret *et al.* 2012, Bi *et al.* 2014), as well as Cox2 (Madsen *et al.* 2010).

Do beige adipocytes contribute to whole body energy expenditure in rodents?

Studies *in vitro* have shown that beige adipocytes have almost the same thermogenic potential as brown adipocytes (Shabalina *et al.* 2013). However, in rodents it is estimated that *in vivo* beige fat can only reach about 20% of the UCP1-dependent oxygen consumption per gram of tissue compared to canonical BAT, and the total mitochondrial mass of inguinal beige fat reaches at best 30% of the levels found in BAT (Shabalina *et al.* 2013). Likewise, the β3-adrenergic-induced increase in glucose metabolism in pooled subcutaneous WAT of Zucker diabetic fatty rats was over 40 times less than in BAT (Liu *et al.* 1998). Whether recruited beige adipocytes can contribute significantly to whole-body energy expenditure *in vivo* therefore remains controversial, particularly since convincing direct measurements of its thermogenic contribution are currently lacking. There are three major reasons for this gap in knowledge: the complex relationship between BAT and browning of WAT, which clearly interfere with each other (Pan *et al.* 2014), the use of cold exposure and pharmacological agents such as β3-adrenergic agonists, which act on both brown and beige fat in parallel, and the use of UCP1 mRNA as a readout of thermogenic activity (Nedergaard & Cannon 2013).

The reporting of UCP1 mRNA expression as verification of significant browning and thermogenesis is a setback when determining whether browning of white fat is of functional relevance (Keipert & Jastroch 2014, Jastroch & Andersson 2015). As UCP1 mRNA expression is already relatively high in BAT at ambient temperature, further activation of BAT by cold exposure or pharmacological agent will only cause a modest increase in mRNA levels, which usually translates into a measurable change in tissue oxygen consumption and thermogenesis. In contrast, WAT depots such as inguinal or epididymal exhibit a very low and often very heterogeneous levels of baseline UCP1 mRNA expression (Wu *et al.* 2014), and any increase presented as fold elevation from virtually nothing can be a misrepresentation towards functional change (Nedergaard & Cannon 2013). Consequently, absolute threshold cycle (CT) values should be reported (Keipert & Jastroch 2014). In addition, increases in UCP1 mRNA expression do not necessarily translate to measurable UCP1 protein expression in subcutaneous and gonadal WAT, which becomes especially difficult in animals kept at thermoneutrality or in obese animals. Moreover, this is additionally confounded by the fact that many commercially available UCP1 antibodies detect proteins other than the target, often very close to the correct band weight, requiring optimal separation conditions and the need for a BAT lysate control (Veniant *et al.* 2015). Different blot exposure times between BAT and subcutaneous WAT are almost always needed, but usually not reported, giving the illusion that the presence of UCP1 is at a similar level when shown in the same figure. Consequently, when assessing browning of WAT, it is recommended to provide mRNA and protein data in direct comparison with BAT samples.

More importantly, alternative and additional readouts for thermogenesis should be provided to gain further insights into the biological relevance of the browning process at the tissue level. These alternatives could include tissue-specific substrate uptake studies using a variety of tracers, thereby determining functional significance by separating out the substrate uptake in each tissue *in vivo* without 'disrupting the system' (Bartelt *et al.* 2011, Khedoe *et al.* 2015, Kooijman *et al.* 2015). Using this approach, subcutaneous WAT has been shown to increase triglyceride uptake under cold exposure, although compared to BAT the effect was minimal (Bartelt *et al.* 2011). Moreover, blood flow to BAT and WAT depots can be an important indicator of metabolic function. The use of microspheres (Foster & Frydman 1978, Rothwell & Stock 1981) and more recently microbubbles (Baron *et al.* 2012) to measure blood flow, and therefore an approximation of oxygen consumption, can prove useful for measuring functional significance of individual tissues. Furthermore, more direct measurements of thermogenesis, for instance by recording tissue temperature (in relation to body and BAT temperature) through implantable transmitters or direct infrared thermography of fat pads, could shed light on the individual contributions to whole body thermogenesis.

However, here it needs to be taken into consideration that browning might be highly heterogeneous for the different WAT depots.

An alternative approach to discriminating BAT from beige fat thermogenesis could be the functional removal of interscapular brown fat via either chemical sympathectomy or physical denervation. However, this is not ideal as a means to study beige contribution, due to the ability of the other BAT depots to fully compensate (Rothwell & Stock 1989). Moreover, in genetic models of BAT ablation it is difficult to separate true beige fat thermogenesis from developmental compensation, despite showing that browning of white fat is increased (Schulz et al. 2013).

As an opposite approach, transplantation of fat depots into recipient mice has proved successful in improving some metabolic parameters with BAT (Stanford et al. 2013, Liu et al. 2015), and to a lesser extent subcutaneous WAT into the visceral cavity (Tran et al. 2008). Also matrix assisted stem cell-derived beige fat transplantations generated some interesting effects (Tharp et al. 2015). Unfortunately, these approaches create very artificial situations, with yet unknown effects exerted by the donor tissue, including endocrine mechanisms, alterations of body temperature or other physiological parameters that are sensed by the brain and compensated for through changes in sympathetic tone, or even immune or stress responses. Consequently, detailed further studies potentially involving conditional beige vs BAT specific knockouts are urgently needed to delineate the respective individual contributions. This approach, however, requires detailed knowledge on brown and beige specific genes that can be used to drive tissue-specific manipulations.

Taken together, there is currently sparse evidence that beige fat contributes significantly to whole-body energy expenditure and thermogenesis under physiological conditions in rodents. Only under certain conditions, where for instance specific BAT depots are incapacitated or beige fat has been implanted, have significant actions been observed. Due to the issues previously indicated, this remains to be further validated.

Role of thermogenic fat for weight loss in humans

Owing to its capability to dissipate stored energy in the form of heat, there has been a renewed interest to use thermogenic fat for the treatment of obesity with the rediscovery of brown fat in the supraclavicular region of adult humans (van Marken Lichtenbelt et al. 2009, Virtanen et al. 2009). This brown fat depot seems to be highly variable, depending on gender, age, and ambient temperature (Cypess et al. 2015, Wang et al. 2015a). Moreover, it is still unclear whether this thermogenic fat is in fact brown, beige, or even a mixture of both (Wu et al. 2012, Jespersen et al. 2013), and whether the adipocytes are derived from $Myf5^+$ precursors like true BAT or whether they are transdifferentiated white adipocytes. What is certain, however, is that these depots express UCP1 and have true thermogenic capacity, which can increase their uptake of circulating glucose and free fatty acids. They can also be activated by cold and β3-adrenergic stimulation as well as by weight loss (Boon et al. 2015).

The most crucial point is whether the capacity for energy expenditure in these depots is high enough to shift energy expenditure compared to other means like exercise or food restriction (Fig. 2) and bring about tangible therapeutic benefits. First estimates from simple upscaling of rodent data suggested that a fully activated adult human BAT would burn about 300 kcal/day, which would be a substantial contribution to the daily energy expenditure of a human. However, when factoring in tissue specific alterations in energy expenditure for species and size such as the Kleiber's law, the estimate would be around 50 kcal/day for fully activated BAT (Devlin 2015). The first studies using real life conditions rather than algorithms now estimate that human BAT activated by mild cold exposure would contribute expenditure of 15–25 kcal/day (Muzik et al. 2013).

These findings are rather discouraging in the context of obesity treatment as an overweight person would require about 15 years of fully active BAT to melt off 20 kg of fat. This becomes even less feasible when considering that BAT is more difficult if not impossible to recruit in obese people (van Marken Lichtenbelt et al. 2009, Virtanen et al. 2009), highly variable between individuals (Cypess et al. 2009, Lee et al. 2013, Xue et al. 2015), and that any increase in energy expenditure is often met by increased compensatory food intake. More importantly, it is not sufficient to activate the BAT on a single occasion, as the additional thermogenesis will cease as soon as the individual returns to the usual thermoneutral environment, around 22 °C for clothed humans. This was clearly demonstrated in a recent study, where BAT was activated by cold exposure (15–16 °C) for 6 h daily for 10 days, but resting metabolic rate was not elevated when the patients returned to thermoneutrality (van der Lans et al. 2013).

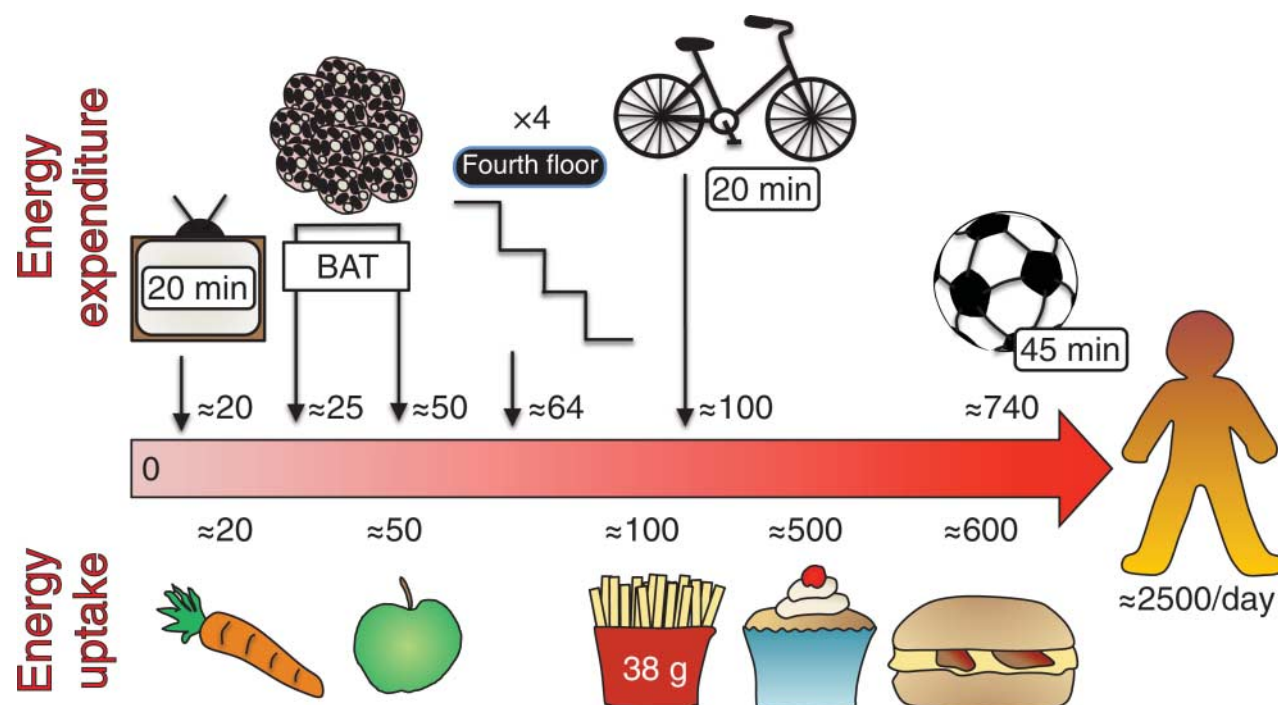It can certainly be argued that if a conversion of additional human WAT depots to beige fat were possible,

Figure 2
Estimated energy expenditure generated by fully activated human BAT (Muzik *et al.* 2013, Devlin 2015) in relation to other every day life events that concern energy uptake or expenditure in kcal (for a 75 kg person (Bassett *et al.* 1997, Gao *et al.* 2012)).

resulting in more thermogenic fat than in the supraclavicular region alone, this could substantially contribute to energy expenditure, especially in obese patients. Unfortunately, so far no significant beige adipocyte populations have been observed in the traditional WAT depots: UCP1+ cells were only observed in perirenal, visceral and subcutaneous fat of non obese children, but not at all in obese children or adults (Rockstroh *et al.* 2015). Likewise, mild cold exposure for 10 days did not induce browning in abdominal WAT (van der Lans *et al.* 2013). So far evidence of browning in humans has been observed only in extraordinary circumstances such as cachexia (Petruzzelli *et al.* 2014) or severe adrenergic stress through burns (Sidossis *et al.* 2015).

Therefore, it is currently uncertain how anatomical regions other than the supraclavicular depots can successfully be converted to thermogenic fat and how they can contribute significantly to overall energy expenditure.

Pharmacological activation of human thermogenesis

Emphasis on the activation of human thermogenesis to increase energy expenditure as a therapeutic intervention for obesity and related metabolic disorders such as type 2

diabetes is pervasive. While it might be questionable whether this approach could really be effective for weight loss, given the potentially low daily energy expenditure as previously mentioned and possible compensatory hyperphagia, the real benefit may lie in reducing circulating glucose and fatty acids, therefore increasing insulin sensitivity. Recent studies have shown that by increasing the volume of BAT through cold exposure, glucose clearance also improves (van der Lans *et al.* 2013, Chondronikola *et al.* 2014). However, this requires constant activation and substrate turnover of the BAT, as its storage capacity is limited. Most importantly, unwanted side effects must be considered, including increased sweating and hyperthermia, hunger and even cardiovascular problems, as UCP1 seems to have a negative effect on arterial plague formation (Dong *et al.* 2013). Consequently, well-controlled human studies are urgently required, if possible using several alternative strategies to assess BAT function, as the commonly used [18]FDG–PET–CT technique measuring glucose uptake does not account for fatty acid oxidation, and the mechanism of preferential uptake between substrates is poorly understood (Townsend & Tseng 2012).

The most obvious mechanisms to initiate thermogenesis in humans would be cold exposure or direct

β3-adrenergic agonists. While recent studies have shown some promise (Cypess *et al.* 2009), β3-adrenergic agonists have historically proven less effective in patients compared to rodents (Arch 2008), probably because the β3-adrenergic signaling pathway is generally less sensitive in humans (Lafontan & Berlan 1993), and cardiovascular side effects might be an issue. Consequently, many of the proposed activation strategies in rodents might ultimately fall short if they depend on β3-adrenergic signaling. Conversely, cold exposure works impressively well, increasing energy expenditure and thermogenesis as well as the capacity for glucose clearance (van der Lans *et al.* 2013). However, this effect seems to require constant cold activation, as a return to thermoneutrality normalizes resting energy expenditure (Yoneshiro *et al.* 2011, van der Lans *et al.* 2013). Since constant cold exposure is somewhat uncomfortable for humans, pharmacological mechanisms that allow for permanent induction of thermogenesis could be favourable to keep the tissue in a constantly activated state. This would then circumvent any adaptational measures such as a reduced sympathetic tone in response to elevated body temperature, since it has been shown that browning can be reversed upon cold acclimation (Jankovic *et al.* 2015) and beige adipocytes can undergo 'whitening' again if not constantly activated (Rosenwald & Wolfrum 2014).

Problems in translating current rodent data

There has been no shortage in recent publications demonstrating the induction of thermogenic fat in rodents. However, the precise mechanism is often unclear, and simply tipping the sensitive regulation of body temperature to hypothermia can already stimulate thermogenesis (Nedergaard & Cannon 2014). This could include peripheral heat loss, changes in insulative capacity due to alterations in subcutaneous fat, skin or fur, or even behavioral changes that alter nesting. Additionally, many practical factors in experimental design affect the accurate measurement of thermogenesis in rodents, such as housing conditions, ambient temperature, and metabolic measurement (Virtue & Vidal-Puig 2013, Xiao *et al.* 2015). Fortunately, studies are increasingly being conducted at both room temperature and thermoneutrality to discern true action of potential browning agents from other factors such as thermoregulatory compensation (Warner *et al.* 2013). Moreover, there are even distinct differences between different strains (Guerra *et al.* 1998, Li *et al.* 2014), age (Xue *et al.* 2007, Lasar *et al.* 2013) and adiposity (Kooijman *et al.* 2015), which affect the ability to induce beige fat in mice. Finally, several studies use doses that are at a high pharmacological level (Commins *et al.* 1999, Fisher *et al.* 2012, Wang *et al.* 2015b), which are likely to be unrealistic for use in humans.

The lack of the precise mechanism for the induction of thermogenesis will certainly hamper the translation of rodent data to the design of human studies, as a number of species differences are known between mice and humans that affect specific thermogenic systems. In addition to the aforementioned changes in β3-adrenergic signaling, there are also differences for instance in the thermogenic response to food, the so-called diet-induced thermogenesis, between species. This phenomenon depends on BAT in rodents (Rothwell & Stock 1981, Feldmann *et al.* 2009), but is still somewhat enigmatic in humans and may differ for different substrates (Schlogl *et al.* 2013). Moreover, adult human thermogenic fat does not share the same gene expression profile as either rodent BAT or beige fat, with current data suggesting that it could even be a mixture of both types, with the deepest neck areas possessing the most brown fat characteristics and displaying the highest expression of UCP1 (Park *et al.* 2015).

The future of beige adipocytes

Despite an overwhelming number of comprehensive studies on thermogenic fat in humans and browning of white fat in rodents, several issues still remain enigmatic: What exactly is the thermogenic fat in adult humans? Where does it come from? Which mechanisms activate and deactivate it? How does it compare to BAT or beige fat in rodents? Moreover, it remains uncertain what the role for beige fat is in rodents, especially as there is accumulating evidence that the thermogenic contribution might be insignificant. Could it be that beige fat constitutes some kind of thermogenic appendix, given that UCP1 evolved before a specialized tissue for thermogenesis such as BAT existed (Nedergaard & Cannon 1990, Oelkrug *et al.* 2015)? While white fat expressing UCP1 might certainly be an advantage in the absence of functional BAT (Schulz *et al.* 2013) and sufficient fuel would also be available from surrounding WAT depots, it still would require some time to recruit this tissue for thermogenesis given the relatively low capacity of available mitochondria. Alternatively, one could also speculate that beige fat might have a different physiological function than thermogenesis. This is supported by the fact that it can be induced by exercise (Bostrom *et al.* 2012, Stanford *et al.* 2013, Rao *et al.* 2014),

which is a condition where thermogenesis is certainly not needed and might even be counterproductive, or by thyrotoxicosis, a condition where body temperature is already elevated by the increased basal metabolic rate (Obregon 2014).

Finally, the big questions: which of the rodent mechanisms are also applicable to the human condition, and which mechanisms really unlock the true metabolic potential of thermogenesis without severe side effects? Here a vast number of agents capable of browning by several different means under very specific circumstances must be considered. Unfortunately for many of these agents, the underlying mechanisms are unclear and might turn out to be a consequence of reducing body temperature or activating the immune system. Furthermore, alternative strategies aiming at activating brown (not beige) fat in rodents through for instance hypothalamic AMPK activation (Lopez *et al*. 2010) or reducing body temperature by increasing heat loss (Warner & Mittag 2014), might provide more successful strategies for metabolic improvement. However, additional more comprehensive studies aiming to address these issues are therefore urgently needed to facilitate the translation of rodent thermogenesis for human metabolic health.

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