Human brown adipose tissue: Classical brown rather than brite/beige?

Barbara Cannon | Jasper M. A. de Jong | Alexander W. Fischer | Jan Nedergaard | Natasa Petrovic

Department of Molecular Biosciences, The Wenner-Gren Institute, Stockholm University, Stockholm, Sweden

Correspondence
Natasa Petrovic, Department of Molecular Biosciences, The Wenner-Gren Institute, Stockholm University, SE-106 91 Stockholm, Sweden.
Email: natasa.petrovic@su.se

Present address
Jasper M. A. de Jong, Department of Comparative Medicine, Yale School of Medicine, New Haven, CT, USA
Alexander W. Fischer, Department of Molecular Metabolism, Harvard School of Public Health and Department of Cell Biology, Harvard Medical School, Boston, MA, USA

Edited by: Jeremy Ward

Funding information
The Swedish Research Council; Novo-Nordisk Foundation; Magnus Bergvall Foundation; Stiftelsen Olle Engkvist Byggmästare

Abstract
Since the presence of brown adipose tissue (BAT) was established in adult humans some 13 years ago, its physiological significance and molecular characteristics have been discussed. In particular, it has been proposed that the mouse adipose tissue depot most closely resembling and molecularly parallel to human BAT is not classical mouse BAT. Instead, so-called brite or beige adipose tissue, which is characteristically observed in the inguinal ‘white’ adipose tissue depot of mice, has been proposed to be the closest mouse equivalent of human BAT. We summarize here the published evidence examining this question. We emphasize the differences in tissue appearance and tissue transcriptomes from ‘standard’ mice [young, chow fed and, in effect semi-cold exposed (20°C)] versus ‘physiologically humanized’ mice [middle-aged, high-fat diet-fed mice living at thermoneutrality (30°C)]. We find that in the physiologically humanized mice, classical BAT displays molecular and cellular characteristics that are more akin to human BAT than are those of brite/beige adipose tissues from either standard or physiologically humanized mice. We suggest, therefore, that mouse BAT is the more relevant tissue for translational studies. This is an invited summary of a presentation given at Physiology 2019 (Aberdeen).

KEYWORDS
beige fat, brown fat, thermoneutrality

1 | CLASSICAL BROWN ADIPOSE TISSUE

In the classical view, brown adipose tissue (BAT) is primarily found in small mammals and is active when the animals are exposed to the cold. The activity, in this respect, is stimulated via centres in the brain that activate the sympathetic nerves innervating the tissue. These nerves release noradrenaline that activates lipolysis in the brown adipocytes and, probably through this, also heat production, via activation of uncoupling protein 1 (UCP1; Cannon & Nedergaard, 2004; Nedergaard & Cannon, 2018). Owing to the activated lipolysis, the BAT in small mammals (e.g. mice) living in what they experience as cold (e.g. 20°C) is rather lipid depleted and consists of small cells with small lipid droplets and dense mitochondria (Figure 1, mouse BAT).

In this symposium report, we first summarize how BAT in humans and in mice is generally described in terms of cellular appearance and gene expression profiles. We then discuss how the physiological conditions (diet, age and ambient temperature) of standard mouse models (chow-fed, young and at 20°C) are different from those of humans and how this might affect the characteristics of brown and brite/beige adipose tissues. This leads us to examine the characteristics of ‘physiologically humanized’ mice (high-fat diet-fed, middle-aged mice, housed at 30°C). We conclude that analysis of such mice shows...
Until 2007, the general view was that, in humans, the presence of active BAT was limited to neonates and that it atrophied and disappeared during late childhood. A few reports of brown fat in adult humans were published. However, these reports concerned rather special physiological conditions (outdoor workers in cold Finnish winters) or pathological conditions (patients suffering from phaeochromocytoma or hibernoma) (reviewed by Lean, 1989). Brown adipose tissue could, therefore, not be assigned any relevance for normal adult human metabolism. However, in 2007, based on a compilation of analyses of positron emission tomography scans obtained earlier from patients undergoing investigations of fluoro-deoxyglucose uptake for the identification of cancer metastases (e.g. Hany et al., 2002), it was established that even adult humans possessed what would seem to be active BAT (Nedergaard, Bengtsson, & Cannon, 2007), at least when defined as adipose depots that showed high glucose uptake when the patients felt cold (Christensen, Clark, & Morton, 2006). Some of these depots had also been verified to express UCP1 (e.g. Lean, James, Jennings, & Trayhurn, 1986). This conclusion was subsequently reinforced through a series of studies in 2009 (Cypess et al., 2009; Saito et al., 2009; van Marken Lichtenbelt et al., 2009; Virtanen et al., 2009; Zingaretti et al., 2009) and now by a large number of further studies. However, when the morphology of the human BAT was examined microscopically, it was observed – initially perhaps disappointing – that it did not resemble BAT as we knew it from small mammals. It did contain UCP1, but the UCP1 was found only in rather small amounts in only some of the cells (in cell clusters), and a large fraction of the tissue presented with large adipocytes containing large fat droplets, perhaps only a single droplet (Zingaretti et al., 2009). Thus, morphologically, the human tissue did not seem to be similar to classical BAT (Figure 1, human BAT).

**New Findings**

- **What is the topic of this review?**
  It has been suggested that human brown adipose tissue (BAT) is more similar to the brite/beige adipose tissue of mice than to classical BAT of mice. The basis of this is discussed in relationship to the physiological conditions of standard experimental mice.

- **What advances does it highlight?**
  We highlight that, provided mouse adipose tissues are examined under physiological conditions closer to those prevalent for most humans, the gene expression profile of mouse classical BAT is more similar to that of human BAT than is the profile of mouse brite/beige adipose tissue. Human BAT is therefore not different in nature from classical mouse BAT.

**FIGURE 1** Initial (but potentially misleading) comparison of tissue appearance between human brown adipose tissue (BAT) versus brown and brite/beige adipose tissues from ‘standard’ mice. The BAT observed in mice living in standard conditions (i.e. young, chow-fed mice, 20°C) has small cells that are multilocular (contain many small lipid droplets [yellow]) and a dense mitochondrial network (black), where large amounts of UCP1 are found (red dots). This is morphologically different from the appearance of human BAT, which mainly has larger cells that are often unilocular (contain one large lipid droplet) and contain few mitochondria; the tissue also contains some clusters of cells that look more similar to BAT in mice and also contain UCP1. Human BAT thus appears to be similar morphologically to the brite/beige adipose tissue (inguinal subcutaneous adipose tissue) observed in standard mice, where some multilocular cells are also found amidst mainly large unilocular cells; some of the multilocular cells in certain mouse strains, e.g. 129Sv, may contain Ucp1 protein, albeit in small amounts.

**2 | BROWN ADIPOSE TISSUE IN HUMANS**

However, there are many different depots of adipose tissue in the mouse: at least some 13 depots can easily be identified anatomically (de Jong, Larsson, Cannon, & Nedergaard, 2015; Zhang et al., 2018). These can, in turn, be divided into three major types of adipose tissues: the BAT depots, the subcutaneous adipose tissue depots (e.g. the inguinal depot) and the visceral depots (e.g. the epididymal and mesenteric depots). This division can be made based on the expression of UCP1 in these tissues: in the brown depots, some UCP1 is always expressed; in the inguinal, some UCP1 mRNA can be observed when the mice are in semi-cold or cold surroundings; and in the visceral depots, there is practically no UCP1 mRNA in any physiological conditions (de Jong et al., 2015). In mice housed in standard or cold conditions, some of these depots, the subcutaneous depots, show a morphology remarkably similar to that of human BAT (e.g. de Jong et al., 2019). Thus, there would appear to be good reason to think that...
human BAT might be more similar to subcutaneous white adipose tissue in the mouse than to classical mouse BAT.

This morphological notion was initially reinforced by molecular observations. Thus, in vitro, it is possible to induce some precursor cells from white adipose tissue depots, even visceral depots, to express Ucp1, by treating cell cultures with rosiglitazone (a PPARγ agonist). In this respect, the adipocytes from the white adipose tissues thus became similar to those from classical BAT. However, when a series of other genes were examined, genes that had earlier been found to be expressed in cell cultures from BAT but not in cell cultures from white adipose tissue (Zic1, Lhx8, Meox2 and Prdm16; Timmons et al., 2007), they were not observed in the Ucp1-expressing white adipocyte cultures. Following recommendations from referees to indicate that these cells were not classical brown adipocytes, the term ‘brite’ (brown-like in white) adipocytes was introduced (Petrovic et al., 2010).

Subsequently, in an elegant molecular study (Wu et al., 2012), it was observed that a series of genes were well expressed in immortalized Ucp1-expressing cell lines originating from subcutaneous depots in the mouse (the inguinal depots), in comparison to their expression in immortalized cell lines derived from classical mouse BAT. Conversely, there were genes that were well expressed in brown immortalized cell lines but less expressed in the Ucp1-expressing white cell lines. Remarkably and unexpectedly, the expression pattern of these genes in human BAT samples was more similar to the expression pattern in the brown immortalized mouse cells than to the pattern in the brown immortalized mouse cells. Thus, it would seem that human BAT more closely resembled the Ucp1-expressing cells from the inguinal depot than the classical mouse BAT. [A similar study arrived at a similar conclusion (Sharp et al., 2012).] It was again suggested that these cells should bear a special name: beige (Wu et al., 2012). Thus, beige adipocytes and brite adipocytes refer to the same cells: Ucp1-expressing cells originating from white adipose tissue depots in the mouse, being different from classical brown adipocytes and proposed to be of a similar nature to human brown adipocytes (Figure 1).

4 | INITIAL ANALYSIS OF ADIPOSE TISSUE IDENTITY ACCORDING TO MARKER GENES

To distinguish between classical BAT and brite/beige adipose tissues, it was thus possible to suggest some genes (the molecular function of which is not relevant for this discussion) as marker genes for each tissue (Sharp et al., 2012; Wu et al., 2012). As examples, Eva1 may be considered a marker gene for brown (its expression is about fivefold higher in brown than in brite/beige adipose tissue collected from mice living in standard animal housing conditions), whereas Tmem26 may be considered a brite/beige marker, because its expression is >10-fold higher in brite/beige than in brown adipose tissue in these conditions (Sharp et al., 2012; Wu et al., 2012; de Jong et al., 2019). Indeed, when we examined the expression pattern of these proposed ‘marker’ genes in human BAT versus brown or inguinal (brite/beige) adipose tissue from standard mice in a principal components analysis, we could confirm that the closest mouse equivalent to human BAT is the inguinal brite/beige tissue rather than the classical BAT (Figure 2).

Thus, both from the morphological observations (Figure 1) and from the marker gene expression analysis (Figure 2) performed with “standard” mice, it would seem that BAT in humans should be considered brite/beige (Figure 1).

5 | WHAT IS A “STANDARD” MOUSE?

From a physiological point of view, a ‘standard mouse’, as used for most experimental research, is a mouse that is in a physiological state that is not very similar to that of adult humans. Firstly, the mice normally used are very young. Metabolic studies are often initiated when mice are 6–8 weeks of age, i.e. mice that have entered puberty and are thus comparable to human teenagers. The mice indeed continue to grow, in total protein content etc., to ≥12 weeks of age. Secondly, the mice are fed a ‘chow’ diet, i.e. a nutritionally well-balanced diet, but one that is dry and not very tasty (at least not to human taste). Most humans in industrialized countries are presently exposed to diets that are tasty, even hedonically so. Thus, we eat not only to obtain calories and nutrients but also because it gives us pleasure. Thirdly, the mice are housed in cages kept at a temperature of ∼20°C. For mice, this is a cold temperature. Mice exhibit their lowest metabolism (their basal metabolic rate) at ∼30°C (their thermoneutral temperature). When they are living at 20°C, they have to almost double their metabolism (food intake etc.) to maintain body temperature (e.g. Fischer, Cannon, & Nedergaard, 2018). For humans, we would have to be constantly (day and night) exposed naked to 10°C to induce an increase in metabolism (Erikson, Krog, Andersen, & Scholander, 1956) and food intake similar to that which the mice experience continuously in standard animal house conditions. Thus, from a translational point of view, ‘standard’ mice might be said to be rather special.

(There are evidently many other factors that distinguish experimental mouse life from normal human life: a low degree of environmental novelty, different degrees of social interaction, different daily rhythm, etc., but for metabolic studies, we would consider those mentioned here to be the most important ones.)

6 | THE PHYSIOLOGICALLY HUMANIZED MOUSE

The main translational target for metabolic studies performed in mice is obviously not human teenagers living naked at 10°C and being fed dry food pellets. Thus, the question may be raised: are young, cold-stressed, chow-fed mice really good models for most adult humans that are middle-aged, living primarily close to thermoneutrality and eating hedonically attractive, rather high-fat and tasty meals?

We would instead suggest that mature (>6 months old) mice fed a tasty high-fat/high-sugar diet (e.g. 45 energy % lipid, plus 35 energy % carbohydrate, of which half is sucrose, the standard 45 % high fat diet
from Research Diets) and living at mouse thermoneutrality (~30 °C) should be better metabolic models for adult humans than are the ‘standard’ mice commonly used in metabolic studies. We will refer to these mice as physiologically humanized.

Exposure to these conditions will evidently affect the general state of the mice. Just as middle-aged humans generally are less lean than teenagers, the physiologically humanized mice are heavier than standard mice, weighing ~50 g, versus 30 g for a standard mouse, with a total lipid content of 20 versus 3 g. However, despite their obesity, these mice are not markedly insulin resistant, at least as estimated from an insulin tolerance test (Abreu-Vieira et al., 2015; de Jong et al., 2019).

7 | DO HUMANS LIVE AT THERMONEUTRALITY?

Most people will agree that humans normally eat food that is different from mouse chow and that most humans are not young teenagers, but a more controversial issue is whether most humans live most of the time at thermoneutrality.

Of course, this question entails several methodological problems. Examinations of (nearly) naked humans imply that human thermoneutrality is rather close to that of mice, i.e. a little lower than 30 °C (Hill, Muhich, & Humphries, 2013). Evidently, this is not an environmental temperature that most humans are exposed to most of the time. However, we are not naked most of the time either, and there is good reason to believe that we generally dress so that we minimize our heat loss in an attempt to maintain thermal comfort. Studies that directly examine this are, however, scarce and would, in any case, not have been performed on larger cohorts of people. Thus, the issue has to be approached in an indirect way.

One indirect way to approach this is to consider the definition of thermoneutrality, i.e. the temperature zone where metabolic rate is lowest. However, metabolic rate, or daily mean energy expenditure (MEE), consists of two components. There is the true basal metabolic rate (BMR) plus what may be referred to as ‘physical activity’ (although this might not be the best term for this factor, because it includes not only physical activity but also components such as diet-induced thermogenesis, but for the present we can ignore this distinction). The so-called ‘physical activity level’ (PAL) for a given individual is the ratio between the mean energy expenditure and the basal metabolic rate: PAL = MEE/BMR (Figure 3).

In humans, it is straightforward to measure the BMR by asking the subject to remain still, lying down, being at thermal comfort (with clothes and blankets) and not recently having eaten and to measure metabolic rate for ~30 min.
Identification of the mouse metabolic state most similar to the human metabolic state. In humans, basal metabolic rate (BMR) can be determined by direct measurements, and the mean energy expenditure (MEE) can be determined through the double-labelled water technique. The double-labelled water technique allows the individuals to be free living, in this respect including living at any temperature they choose and being dressed in any way they choose. The extra energy thus used above the BMR in normal ‘free-living’ conditions may be referred to as the ‘physical activity’ (PA). The ratio between the MEE and the BMR is referred to as the ‘physical activity level’ (PAL) and has been measured to be ∼1.7 for most individuals. In mice, the BMR can be approximated as being the lowest continuous metabolic rate observed in metabolic chambers at thermoneutrality, and the MEE can be obtained from continuous measurements in such chambers. For mice at thermoneutrality (30°C), the ratio between these values (PAL) is 1.7 (Westerterp, 2018). Thus, free-living humans and mice at thermoneutrality have identical PALs. This indicates that these mice are good metabolic models for humans. The correspondence between the human free-living PAL and the mouse PAL does not necessarily demonstrate that humans live essentially at thermoneutrality, but we would consider this an implication. Mice living at the standard animal house temperature have a much higher MEE (nearly double that of the mice at 30°C), owing to a combination of ‘normal’ physical activity and cold-induced thermogenesis (CIT). They may thus be considered less relevant metabolic models for humans. (Principal summary of data from Fischer et al., 2018; Fischer, Cannon, & Nedergaard, 2019; Fischer, Csikasz, von Essen, Cannon, & Nedergaard, 2016; Westerterp, 2018; picture created with BioRender.com)

To obtain the MEE in humans is not as easy. It is possible to confine the subjects for a prolonged time (>24 h) in metabolic chambers, but this probably lowers spontaneous physical activity. A better alternative is to measure metabolism in ‘free-living subjects’, being at whatever temperature they prefer and being clothed however they wish and otherwise doing whatever they want. It is possible to do this by using the ‘double labelled water method’ to measure metabolism. Basal metabolic rate and MEE have been measured in parallel in rather large cohorts of humans (in northern temperate zones and with a Western lifestyle). Although a rather wide spread of values for PAL are obtained from different individuals and different populations, the mean value obtained is ∼1.7 (Westerterp, 2018). This means that during normal life, adult humans expend (only) ∼70% extra energy on ‘activity’, on top of their BMR. It can therefore be argued that a relevant translational mouse model for metabolism should, likewise, expend not more than ∼70% above its BMR for all activities, including heat produced to counteract heat loss in the cold (Figure 3).

The determination of PAL in the mouse also has methodological difficulties. One of these is that it is clearly not possible to ask a mouse to remain still, not to have eaten and to ensure that it is in thermal comfort. What can be done instead is to assume that the mouse in its daily life might be in such conditions spontaneously during certain periods: being inactive and not having eaten recently. Indeed, by using high-time-resolution indirect calorimetry in mice exposed to what are thermoneutral ambient temperatures for mice (∼30°C), it is possible to identify periods of ∼10 min in duration when the energy expenditure of the mice is at its lowest (Fischer et al., 2018). Such periods tend to occur towards the end of the light phase of the mice (Fischer et al., 2018; Keijer, Li, & Speakman, 2019), indicating that they are not random inconsistencies in measurement but represent biological events. It is thus possible to consider the metabolic rate during these periods as a reasonable proxy of the BMR of the mouse. During prolonged measurements of metabolic rates in indirect calorimeters, a value for the MEE at thermoneutrality can also be obtained (note that this value is sometimes incorrectly referred to as the BMR of the mouse, but, exactly as in humans, the total energy utilization is the sum of the BMR plus the extra energy expenditure of physical activity etc.). Thus, for mice maintained at thermoneutrality, a value for PAL can be obtained and, as a mean, this value is 1.7 (Fischer et al., 2018). This indicates that mice living at thermoneutrality have a total metabolic rate 70%
above their BMR; this is exactly the mean metabolism of humans in ‘free-living conditions’. Therefore, mice at thermoneutrality may be considered good metabolic models of humans. Note particularly that at any temperature below thermoneutrality, the metabolic rate (and consequently, the PAL) is higher. Thus, less translationally relevant PAL values are obtained (Figure 3). Given these criteria, housing experimental mice in ‘standard’ conditions might not be optimal for translationally intended metabolic research.

Thus, although these indirect measurements do not demonstrate in themselves that adult humans normally attempt to live in thermoneutral conditions (although we would think that we do), they do demonstrate that mice at thermoneutrality are more physiologically humanized metabolically than mice at any other ambient temperature (Figure 3).

8 | MORPHOLOGICAL APPEARANCE OF BROWN FAT IN PHYSIOLOGICALLY HUMANIZED MICE

Given this understanding of the metabolic characteristics of physiologically humanized mice, it would appear relevant to study these mice to address the question of brown and brite/beige adipose tissue similarities in mice and humans. Visually, the BAT of mice living in physiologically humanized conditions (∼9 months old, fed a high-fat diet for ≥6 months and living at 30°C) contrasts sharply with that of BAT of ‘standard’ mice (only ∼8–10 weeks old, fed chow and living at 20°C). The physiologically humanized mice have a rather whitish-looking ‘brown’ adipose tissue, with large, rather lipid-filled cells, most of which are unilocular. Although UCP1 is present, it is found in only certain cells, and these cells tend to be in clusters of multilocular cells within the tissue (de Jong et al., 2019). In these respects, the mouse classical BAT depots now appear very similar to the BAT observed in humans (Figure 4). A question would be to what extent is this also reflected in the gene expression profile.

9 | DISTINCT GENE EXPRESSION IN ADIPOSE TISSUES OF PHYSIOLOGICALLY HUMANIZED MICE

As would be expected, the gene expression level not only of Ucp1 but also of several other genes normally considered to be part of the thermogenic programme (Pgc1a, Cidea and Elovl3) in adipose tissues is lower in physiologically humanized mice than in standard mice. This is the case in both classical BAT and in the inguinal brite/beige tissue (although the expression of these genes in the inguinal depots is generally at least 10–100 times lower than in classical BAT; de Jong et al., 2019).

Indeed, not only genes directly related to thermogenesis but a very broad array of genes have different expression levels when adipose tissues from mice living in standard or physiologically humanized conditions are compared. Approximately 4500 genes display significantly different expression levels in BAT of standard versus physiologically humanized mice (de Jong et al., 2019). This is not really surprising, because exposure to 20°C is sufficient to induce molecularly almost all the characteristics of recruitment in BAT seen at 4°C, compared with what is seen in mice living at 30°C (Kalivovich, de Jong, Cannon, & Nedergaard, 2017). The differences in gene expression in brite/beige adipose tissue between standard and physiologically humanized mice are somewhat smaller: ∼2000 genes show significantly different expression levels. In total, the differences in gene expression levels between brite/beige and BAT are smaller in physiologically humanized than in standard mice, both for the subset of thermogenesis-related genes and for the entire transcriptomes (de Jong et al., 2019).

10 | A DIRECT COMPARISON BETWEEN HUMAN AND MOUSE ADIPOSE TISSUE TRANSCRIPTOMES

For a general approach to the question of the identity of human BAT versus mouse brite/beige or classical BAT in the different physiological conditions, it should be possible to compare the entire transcriptome between these tissues. This is possible because the transcriptome of
human thermogenically verified BAT has been characterized (Perdikari et al., 2018).

However, a simple, direct comparison of all the relevant transcriptomes yields the unsurprising but not very elucidating conclusion that humans and mice are different species! Thus, correspondence between the adipose tissues in the two species cannot be established (de Jong et al., 2019). It transpires that the number of genes that characterize the different adipose tissues is low compared with the entire transcriptome of the tissues, a problem that has been encountered earlier (Breschi et al., 2016; Lin et al., 2014). What is perhaps somewhat unexpected is that these mouse versus human cells apparently in general carry transcriptional signatures that identify them as the respective species; however, an analysis of the molecular background of these species signatures is outside the scope of the present discussion.

**11 | A REMARKABLE SHIFT IN ‘MARKER’ GENE IMPLICATIONS: MOUSE CLASSICAL BROWN ADIPOSE TISSUE IS NOW SIMILAR TO HUMAN BROWN ADIPOSE TISSUE**

Given that a comparison between the total transcriptomes does not answer the question of the nature of human versus mouse adipose tissues, other strategies must be used. One of them is to revert to the ‘marker’ genes discussed earlier (Figure 2) for brite/beige versus brown adipose tissue. In standard mice, the expression pattern of these marker genes clearly indicated that mouse brite/beige adipose tissue was the adipose tissue most similar to human BAT (Figure 2). However, when the expression pattern of these marker genes is compared between thermogenically verified human BAT and inguinal and BAT from physiologically humanized mice, a rather clear but surprisingly different picture is revealed (Figure 5; de Jong et al., 2019). Classical BAT from physiologically humanized mice is now similar to human BAT (Figure 5; de Jong et al., 2019).

Thus, making the most physiologically relevant comparison, human BAT has characteristics similar to mouse classical BAT, rather than to mouse brite/beige adipose tissue. Human BAT is thus brown and not brite/beige adipose tissue (Figure 5) (de Jong et al., 2019).

A similar conclusion may be reached if adipose tissue-defining genes (as determined by the BATLAS gene list (Perdikari et al., 2018)) and genes related to thermogenic potential (as determined by the ProFAT computational tool (Cheng et al., 2018)) are instead compared (de Jong et al., 2019). Notably, the expression level of Ucp1 is very similar between BAT from physiologically humanized mice and true human BAT, whereas the Ucp1 expression level in brite/beige adipose tissue even from standard mice is some 100-fold lower.

Thus, despite the molecular challenge that lies in understanding why some cells in white adipose tissue may express Ucp1 (i.e. become brite/beige) and the possibilities implied for vastly increasing Ucp1 gene expression in these depots, it should be understood that the study of brite/beige adipose tissue is not a study of the mouse equivalent to human BAT. For translationally relevant studies of mouse adipose tissue to understand and affect human BAT, classical BAT (in physiologically humanized mice) should be the preferred tissue.
12 | DOES BROWN ADIPOSE TISSUE IN PHYSIOLOGICALLY HUMANIZED MICE RETAIN ITS THERMOGENIC COMPETENCE?

The BAT encountered in physiologically humanized mice looks very atrophied, being lipid filled and with few Ucp1-expressing cells, etc. It may therefore be questioned whether this tissue still retains its ability to become recruited. In particular, it may be asked whether it can be induced to attain UCP1 protein levels of the same magnitude as those observed in mice that have been directly acclimated to cold.

The answer to this question would seem to be yes (in agreement with in silico predictions from ProFAT; Cheng et al., 2018). When the physiologically humanized mice are exposed to cold for an extended time (~1 month), the total amount of UCP1 found in the tissue is at least at the same level as it is in cold-acclimated standard mice (standard mice that are transferred directly to the cold without first having been physiologically humanized). The tissue has, however, a somewhat different appearance, with larger lipid droplets (de Jong et al., 2019).

However, very remarkably, but again in agreement with in silico results from ProFAT, in the physiologically humanized mice that are chronically cold exposed, the brite/beige adipose tissue loses its ability to become thermogenic, and UCP1 protein is no longer found in the tissue, even in the cold-acclimated state. The reason for this loss of thermogenic capacity has been examined in cell cultures derived from young and older mice. Brown adipocytes isolated from classical BAT depots fully retained the ability to respond to noradrenaline stimulation with a large increase in Ucp1 gene expression even when they were isolated from older mice. However, the brite/beige adipocytes isolated from older mice had completely lost this ability (which was present in brite/beige adipocytes isolated from young mice; de Jong et al., 2019).

Thus, it may be envisaged that human BAT, although it appears atrophied, can still be recruited fully by chronic cold exposure (or perhaps by pharmacological means), even in middle-aged humans living at thermoneutrality and eating palatable food. This view is supported by the outcome of direct studies of human brown adipogenesis (Lee, Swarbrick, Zhao, & Ho, 2011).

13 | IS BROWN ADIPOSE TISSUE OF METABOLIC SIGNIFICANCE IN ADULT HUMANS?

The implication above that human BAT can be recruited by chronic cold, despite its atrophied appearance, might be considered to be of cursory interest. However, a main issue under debate is, of course, whether human BAT could be able to influence energy balance in humans. Can the small and apparently atrophied BAT encountered in adult humans affect the development of obesity in humans?

At present, there is no clear answer to this question. However, it should be remembered that the presence or absence of BAT (UCP1) in mice would seem to affect energy balance in mice living at thermoneutrality, where the tissue appears as atrophied as it does in humans. Thus, when they are exposed to a high-fat diet, mice lacking the thermogenic capacity of BAT have generally been observed in different laboratories to become somewhat more obese, more quickly than wild-type mice. Concordantly, exposure to the high-fat diet is paralleled by a small, but apparently metabolically significant, increase in total UCP1 protein in wild-type mice (Feldmann, Golozoubova, Cannon, & Nedergaard, 2009; Luijten, Feldmann, von Essen, Cannon, & Nedergaard, 2019; Rowland, Maurya, Bal, Kozak, & Periasamy, 2016; von Essen, Lindsund, Cannon, & Nedergaard, 2017; Winn et al., 2017).

It is thus still possible that human BAT, which is of the same nature as mouse BAT, might be able to have an ameliorating effect on the development of obesity in humans living in ‘physiologically humanized’ conditions, i.e. being middle-aged, living in thermoneutral conditions (owing to housing and clothes) and constantly exposed to unlimited amounts of tasty and calorie-dense foods.

ACKNOWLEDGEMENTS

We thank all co-authors of the main primary paper discussed here (de Jong et al., 2019), and particularly Pirjo Nuutila and Kirsi Virtanen (Turku), Wenfei Sun and Christian Wolfrum (Zurich), and Andrea Frontini and Saverio Cinti (Pavia/Ancona) for their valuable contributions to the original publication. The original work was supported by the Swedish Research Council, the Novo-Nordisk Foundation, The Magnus Bergvall Foundation and The Olle Engkvist Byggmästare Foundation.

COMPETING INTERESTS

None declared.

AUTHOR CONTRIBUTIONS

B.C., J.N. and N.P. wrote this symposium report. N.P. performed further analysis of gene expression data. All authors commented on this symposium report. All authors approved the final version and agree to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. All persons designated as authors qualify for authorship, and all those who qualify for authorship are listed.

ORCID

Barbara Cannon https://orcid.org/0000-0001-6594-2363
Jasper M. A. de Jong https://orcid.org/0000-0001-8044-5410
Alexander W. Fischer https://orcid.org/0000-0001-6717-9090
Jan Nedergaard https://orcid.org/0000-0003-2070-1587
Natasa Petrovic https://orcid.org/0000-0002-4435-9651
REFERENCES

Abreu-Vieira, G., Fischer, A. W., Mattsson, C., de Jong, J. M., Shabalina, I. G., Rydén, M., et al. (2015). Cidea improves the metabolic profile through expansion of adipose tissue. *Nature Communications*, 6, 7433.

Breschi, A., Djebali, S., Gillis, J., Pervouchine, D. D., Dobin, A., Davis, C. A., et al. (2016). Gene-specific patterns of expression variation across organs and species. *Genome Biology*, 17, 151.

Cannon, B., & Nedergaard, J. (2004). Brown adipose tissue: Function and physiological significance. *Physiological Reviews*, 84, 277–359.

Cheng, Y., Jiang, L., Keipert, S., Zhang, S., Hauser, A., Graf, E., et al. (2018). Prediction of adipose browning capacity by systematic integration of transcriptional profiles. *Cell Reports*, 23, 3112–3125.

Christensen, C. R., Clark, P. B., & Morton, K. A. (2006). Reversal of hypermetabolic brown adipose tissue in F-18 FDG PET imaging. *Clinical Nuclear Medicine*, 31, 193–196.

Cypess, A. M., Lehman, S., Williams, G., Tal, I., Rodman, D., Goldfine, A. B., et al. (2009). Identification and importance of brown adipose tissue in adult humans. *New England Journal of Medicine*, 360, 1509–1517.

Cypess, A. M., White, A. P., Vernochet, C., Schulz, T. J., Xue, R., Sass, C. A., et al. (2013). Anatomical localization, gene expression profiling and functional characterization of adult human neck brown fat. *Nature Medicine*, 19, 635–639.

de Jong, J. M., Larsson, O., Cannon, B., & Nedergaard, J. (2015). A stringent validation of mouse adipose tissue identity markers. *American Journal of Physiology-Endocrinology and Metabolism*, 308, E1085–E1105.

de Jong, J. M. A., Sun, W. F., Pires, N. D., Frontini, A., Balaz, M., Jespersen, N. Z., et al. (2019). Human brown adipose tissue is phenocopied by classical brown adipose tissue in physiologically humanized mice. *Nature Metabolism*, 1, 830–843.

Erikson, H., Krog, J., Andersen, K. L., & Scholander, P. F. (1956). The critical temperature in naked man. *Acta Physiologica Scandinavica*, 37, 35–39.

Feldmann, H. M., Golozoubova, V., Cannon, B., & Nedergaard, J. (2009). Ucp1 ablation induces obesity and abolishes diet-induced thermogenesis in mice exempt from thermal stress by living at thermoneutrality. *Cell Metabolism*, 9, 203–209.

Fischer, A. W., Cannon, B., & Nedergaard, J. (2018). Optimal housing temperatures for mice to mimic the thermal environment of humans: An experimental study. *Molecular Metabolism*, 7, 161–170.

Fischer, A. W., Cannon, B., & Nedergaard, J. (2019). No insulating effect of obesity, neither in mice nor in humans. *American Journal of Physiology-Endocrinology and Metabolism*, 317, E952–E953.

Fischer, A. W., Csiszás, R. I., von Essen, G., Cannon, B., & Nedergaard, J. (2016). No insulating effect of obesity. *American Journal of Physiology-Endocrinology and Metabolism*, 311, E202–E213.

Hany, T. F., Sharehpapagh, E., Kamel, E. M., Buck, A., Himms-Hagen, J., & von Schultess, G. K. (2002). Brown adipose tissue: A factor to consider in symmetrical tracer uptake in the neck and upper chest region. *European Journal of Nuclear Medicine and Molecular Imaging*, 29, 1393–1398.

Hill, R. W., Muhich, T. E., & Humphries, M. M. (2013). City-scale expansion of human thermoregulatory costs. *PLOS One*, 8, e76238.

Jespersen, N. Z., Larsen, T. J., Peijs, L., Daugaard, S., Homoe, P., Loft, A., et al. (2013). A classical brown adipose tissue mRNA signature partly overlaps with brite in the supraclavicular region of adult humans. *Cell metabolism*, 17, 798–805.

Kalivich, A. V., de Jong, J. M., Cannon, B., & Nedergaard, J. (2017). Ucp1 in adipose tissues: Two steps to full browning. *Biochimie*, 134, 127–137.

Keijer, J., Li, M., & Speakman, J. R. (2019). What is the best housing temperature to translate mouse experiments to humans? *Molecular Metabolism*, 25, 168–176.

Lean, M. E. (1989). Brown adipose tissue in humans. *The Proceedings of the Nutrition Society*, 48, 243–256.

Lean, M. E., James, W. P., Jennings, G., & Trayhurn, P. (1986). Brown adipose tissue uncoupling protein content in human infants, children and adults. *Clinical Science [London, England: 1979]*, 71, 291–297.

Lee, P., Swarbrick, M. M., Zhao, J. T., & Ho, K. K. (2011). Inducible brown adipogenesis of supraclavicular fat in adult humans. *Endocrinology*, 152, 3597–3602.

Lidell, M. E., Betz, M. J., Dahlqvist Leinhard, O., Heglin, M., Elander, L., Slawik, M., & Enerback, S. (2013). Evidence for two types of brown adipose tissue in humans. *Nature Medicine*, 19, 631–634.

Lin, S., Liu, Y., Nery, J. R., Urich, M. A., Breschi, A., Davis, C. A., et al. (2014). Comparison of the transcriptional landscapes between human and mouse tissues. *Proceedings of the National Academy of Sciences of the United States of America*, 111, 17224–17229.

Luijten, I. H. N., Feldmann, H. M., von Essen, G., Cannon, B., & Nedergaard, J. (2019). In the absence of UCP1-mediated diet-induced thermogenesis, obesity is augmented even in the obesity-resistant 129 mouse strain. *American Journal of Physiology-Endocrinology and Metabolism*, 316, E729–E740.

Nedergaard, J., Bengtsson, T., & Cannon, B. (2007). Unexpected evidence for active brown adipose tissue in adult humans. *American Journal of Physiology-Endocrinology and Metabolism*, 293, E444–E452.

Nedergaard, J., & Cannon, B. (2013). How brown is brown fat? It depends where you look. *Nature Medicine*, 19, 540–541.

Nedergaard, J., & Cannon, B. (2018). Brown adipose tissue as a heat-producing thermoeffector. *Handbook of Clinical Neurology*, 156, 137–152.

Perdikari, A., Leparc, G. C., Balaz, M., Pires, N. D., Lidell, M. E., Sun, W., et al. (2018). Batlas: Deconvoluting brown adipose tissue. *Cell Reports*, 25, 784–797 e784.

Petrovic, N., Walden, T. B., Shabalina, I. G., Timmons, J. A., Cannon, B., & Nedergaard, J. (2010). Chronic peroxisome proliferator-activated receptor γ (PPARγ) activation of epididymally derived white adipocyte cultures reveals a population of thermogenically competent, UCP1-containing adipocytes molecularly distinct from classic brown adipocytes. *Journal of Biological Chemistry*, 285, 7153–7164.

Rowland, L. A., Maurya, S. K., Bal, N. C., Kozak, L., & Persiason, M. (2016). Sarcolipin and uncoupling protein 1 play distinct roles in diet-induced thermogenesis and do not compensate for one another. *Obesity (Silver Spring)*, 24, 1430–1433.

Saito, M., Okamatsu-Ogura, Y., Matsushita, M., Watanabe, K., Yoneshiro, T., Nio-Kobayashi, J., & Tsuji, M. (2009). High incidence of metabolically active brown adipose tissue in healthy adult humans: Effects of cold exposure and adiposity. *Diabetes*, 58, 1526–1531.

Sanchez-Gurmaches, J., Hung, C. M., & Guertin, D. A. (2016). Emerging complexities in adipocyte origins and identity. *Trends in Cell Biology*, 26, 313–326.

Sharp, L. Z., Shinoda, K., Ohno, H., Scheel, D. W., Tomoda, E., Ruiz, L., & Kajimura, S. (2012). Human BAT possesses molecular signatures that resemble beige/brite cells. *PLoS One*, 7, e49452.

Timmons, J. A., Wennmalm, K., Larsson, O., Walden, T. B., Lassmann, T., Petrovic, N., et al. (2007). Myogenic gene expression signature establishes that brown and white adipocytes originate from distinct cell lineages. *Proceedings of the National Academy of Sciences of the United States of America*, 104, 4401–4406.

van Marken Lichtenbelt, W. D., Vanhommerig, J. W., Smulders, N. M., Drossaerts, J. M., Kemerink, G. J., Bouvy, N. D., et al. (2009). Cold-activated brown adipose tissue in healthy men. *New England Journal of Medicine*, 360, 1500–1508.

Virtanen, K. A., Lidell, M. E., Orava, J., Heglin, M., Westergren, R., Niemi, T., et al. (2009). Sarcolipin and uncoupling protein 1 in healthy adults. *New England Journal of Medicine*, 360, 1518–1525.

von Essen, G., Lindsund, E., Cannon, B., & Nedergaard, J. (2017). Adaptive facultative diet-induced thermogenesis in wild-type but not in UCP1-ablated mice. *American Journal of Physiology-Endocrinology and Metabolism*, 313, E515–E527.
Walden, T. B., Hansen, I. R., Timmons, J. A., Cannon, B., & Nedergaard, J. (2012). Recruited vs. nonrecruited molecular signatures of brown, “brite,” and white adipose tissues. *American Journal of Physiology-Endocrinology and Metabolism, 302*, E19–E31.

Westerterp, K. R. (2018). Exercise, energy expenditure and energy balance, as measured with doubly labelled water. *The Proceedings of the Nutrition Society, 77*, 4–10.

Winn, N. C., Vieira-Potter, V. J., Gastecki, M. L., Welly, R. J., Scroggins, R. J., Zidon, T. M., … Padilla, J. (2017). Loss of UCP1 exacerbates Western diet-induced glycemic dysregulation independent of changes in body weight in female mice. *American Journal of Physiology-Regulatory, Integrative and Comparative Physiology, 312*, R74–R84.

Wu, J., Boström, P., Sparks, L. M., Ye, L., Choi, J. H., Giang, A. H., … Spiegelman, B. M. (2012). Beige adipocytes are a distinct type of thermogenic fat cell in mouse and human. *Cell, 150*, 366–376.

Zhang, F., Hao, G., Shao, M., Nham, K., An, Y., Wang, Q., … Oz, O. K. (2018). An adipose tissue atlas: An image-guided identification of human-like bat and beige depots in rodents. *Cell Metabolism, 27*, 252–262.e253.

Zingaretti, M. C., Crosta, F., Vitali, A., Guerrieri, M., Frontini, A., Cannon, B., … Cinti, S. (2009). The presence of UCP1 demonstrates that metabolically active adipose tissue in the neck of adult humans truly represents brown adipose tissue. *FASEB Journal, 23*, 3113–3120.

How to cite this article: Cannon B, de Jong JMA, Fischer AW, Nedergaard J, Petrovic N. Human brown adipose tissue: Classical brown rather than brite/beige? *Experimental Physiology*. 2020;105:1191–1200. [https://doi.org/10.1113/EP087875](https://doi.org/10.1113/EP087875)