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Cell Metabolism
Perspective

The Browning of White Adipose Tissue: Some Burning Issues

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http://dx.doi.org/10.1016/j.cmet.2014.07.005

Igniting thermogenesis within white adipose tissue (i.e., promoting expression and activity of the uncoupling protein UCP1) has attracted much interest. Numerous "browning agents" have now been described (gene ablations, transgenes, food components, drugs, environments, etc.). The implied action of browning agents is that they increase UCP1, through this heat production, leading to slimming. Here, we particularly point to the possibility that cause and effect may on occasion be the reverse: browning agents may disrupt, for example, the fur, leading to increased heat loss, increased thermogenic demand to counteract this heat loss, and thus, through sympathetic nervous system activation, to enhanced UCP1 expression in white (and brown) adipose tissues.

To harness the thermogenic power of the uncoupling protein UCP1—and thus its ability to nullify the effects of extra energy intake—has attractive perspectives for human health. Not only would it enable us to stay or even to become slim even if an excess of food were eaten, but it would also counteract comorbidities such as type 2 diabetes. Particularly, recent years have seen an intense interest in the ability to "brown" what have traditionally been seen as white adipose tissue depots. The idea is to place the combustion machinery (i.e., UCP1) directly in the excess fat supply.

Until recently (Nedergaard et al., 2007), it was common wisdom that adult humans do not possess active brown adipose tissue. Therefore, the concept that certain white depots could develop brownish characteristics had a great advantage: this way of translating the power of brown fat into human health appeared much more feasible than reintroducing true brown fat into adult men. Additionally, observations that very large increases (100-fold) in the mRNA levels of UCP1 could be induced in rodents in certain white adipose tissue depots had made the browning process very attractive for therapeutic exploration.

In this Perspective, we discuss some of the burning issues concerning the browning process. We pragmatically define browning as any significantly increased UCP1 expression at the mRNA level occurring in what are normally considered as white adipose tissue depots. The resulting cells that express UCP1 may be referred to as beige (Ishibashi and Seale, 2010), brite (Petrovic et al., 2010), convertible (Loncar, 1991), ectopic (Lehr et al., 2009), inducible (Lee et al., 2011), or recruitable (Schulz et al., 2013). (This latter term is unfortunately often misread to indicate that classical brown adipose tissue is not recruitable. However, the opposite is true: classical brown adipose tissue is very recruitable in the sense that it increases its thermogenic capacity some 5-fold from 20°C to 5°C [Nedergaard and Cannon, 2013]). Here, we use a simple "browning" terminology.

Presently, Almost Everything Browns White Adipose Tissue

Some 50–100 different treatments have been demonstrated to induce browning: food components, drug substances, transgenes, gene knockouts, and enhanced or deteriorated living conditions. The list of browning agents is growing rapidly. For simplicity, we refer to any of these compounds or genetic modifications or ways of living as "browning agents." Thus, it should be kept in mind that the expression in this Perspective includes factors of a much broader nature than just drug substances. Several reviews have recently carefully listed these agents (Bonet et al., 2013; Wu et al., 2013), and we will not duplicate these efforts. Rather, we would like to suggest some "unifying hypotheses" for understanding why such a broad variety of agents can all result in browning. Because of the very rapid growth of literature, this Perspective cannot attempt to be comprehensive, and we apologize if we have overlooked contributions with a principal impact on the issues discussed.

Browning as a Sympathetic Event

The first report of the browning phenomenon is that of Young et al. (1984). The authors observed that areas in the parametrial adipose depot developed brown-fat characteristics when mice were acclimated to cold. Given that recruitment in classical brown adipose tissue is due to chronic stimulation by norepinephrine released from the sympathetic nerves innervating the tissue (Cannon and Nedergaard, 2004), the possibility that browning could also be induced by chronic adrenergic stimulation was examined. Indeed, chronic treatment with a β_3-adrenergic agent led to browning (Cousin et al., 1992; Ghorbani et al., 1997; Ghorbani and Himms-Hagen, 1997). In mice without β_3 adrenoceptors, browning was barely induced (Jimenez et al., 2003). Correspondingly, overexpression of β_1 adrenoceptors in white adipocytes was sufficient to induce browning (Soloveva et al., 1997). Thus, adrenergic stimulation of white adipose tissue definitely induces the browning process. Here, we examine to what degree this norepinephrine-induced, and thus centrally mediated, process is sufficient to explain the effect of (some of) the browning agents.

A Hierarchical Activation System

On the basis of the divergent physiological functions we normally ascribe to brown versus white adipose tissue, it would be expected that the innervation of these tissues would be qualitatively very different. Particularly, it would be thought that the

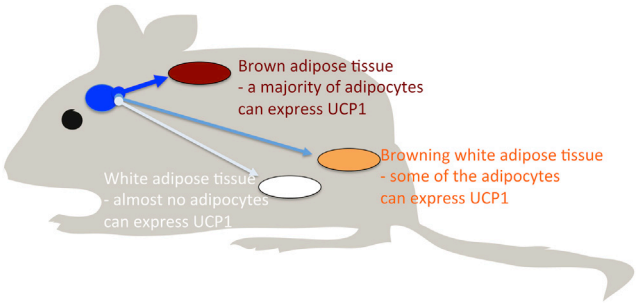

Figure 1. The Hierarchical Organization of the Activation of Brown and White Adipose Tissues

The nerve pathways stimulating brown and white adipose tissues have similar origins in the brain, but they are differently controlled; thus, with increasing exposure to cold, the nerves to brown fat will initially become active, but, with continued decreasing temperatures, the nerves to white-fat depots may also be successively engaged. The receiving depots contain different fractions of cells able to respond to norepinephrine stimulation with an increase in UCP1 gene expression, as indicated. The browning white adipose tissues are often those referred to as "subcutaneous" adipose tissue depots; the one normally studied is the inguinal depot. The depots least able to respond are those often referred to as "visceral"; the one normally studied is the epididymal depot. Thus, with increasing intensity of sympathetic stimulation, browning of the inguinal depot will be augmented in addition to recruitment of classical brown adipose tissue.

brain centers involved in the control of their activity would be distinct. However, studies where the innervation to brown versus white adipose tissue depots has been delineated tend not to identify distinct brown- versus white-fat-specific control centers in the brain (Bamshad et al., 1998, 1999). Basically, this would mean that an organization exists where similar brain events would evoke qualitatively similar activation of either tissue (Figure 1A). Although this is somewhat unexpected in several respects, it makes it possible to understand that browning of white adipose tissue may not be substantially different from recruitment of classical brown adipose tissue.

The parallel innervation does not mean that the sympathetic activation occurs to the same degree in different depots. Classical brown adipose tissue demonstrates small but significant UCP1 gene expression already at thermoneutrality (≈30°C). This may indicate that sympathetic stimulation can occur in the absence of a thermal signal. White adipose tissue is principally devoid of UCP1 at thermoneutrality, and cold acclimation leads to successive browning, with some UCP1 expression at "normal" animal house temperatures (≈20°C) and much more in the cold (≈5°C) (Waldén et al., 2012). Thus, a hierarchy may be formulated wherein increasing central stimulation successively activates first brown and then white adipose tissue depots. In contrast, experiments with injected adrenergic agents do not show this hierarchical system. Such injections directly induce browning with the bias that injections at normal animal house temperatures appear to have a much greater (relative) effect on white than brown adipose tissues. This is because, under normal experimental conditions, the classical brown adipose tissue is already physiologically adrenergically stimulated and therefore shows only comparatively modest relative effects of additional stimulation—but these effects may be quantitatively large.

This organization of the system is confirmed by classical observations that surgical removal of a given brown adipose tissue depot will result in the activation of other depots (Connolly and Carnie, 1982; Stephens et al., 1981). Also, in accordance with this, a substantial reduction of "classical" brown adipose tissue activity (molecularly introduced by eliminating the bone morphogenetic protein [BMP] receptor 1A from brown adipocyte precursors) results in "compensatory" browning of white depots (Schulz et al., 2013) with a regaining of thermogenic capacity. Thus, the brain will augment the intensity of nerve activity to the relevant adipose tissue depots to the exact extent required to generate the heat needed. If the intensity is sufficiently high, then browning via physiological means will occur.

It may be wondered whether the UCP1 gene in the white adipose tissue can be induced to be expressed without first being "unmasked" by another process. However, Boyer and Kozak (1991) observed that there were DNase hypersensitive sites in the upstream region of the UCP1 gene in both brown and white adipose tissues. These sites were not seen in nonadipose tissues, implying that the UCP1 gene in white adipose tissue is already "open" and merely needs, for example, adrenergic stimulation to become expressed. However, there is the difference that far fewer cells in white than brown adipose tissue have open UCP1 genes (see the in vitro discussion in the Supplement Information available online).

All in all, there is no reason to think that browning of white adipose tissue in the cold is a process that is fundamentally different from the standard recruitment of brown adipose tissue in the cold. Thus, it can be explained by increased sympathetic stimulation of either tissue.

An Efficient, but Very Indirect, Browning Agent: The Feeling of Cold

Given that the most notable physiological factor leading to browning is cold, pertinent but infrequently addressed questions are (1) do (some of) the animals that demonstrate browning feel cold to a greater extent, and (2) can this be the reason that browning occurs?

By this, we do not imply that their nervous system should have been altered by the treatment. Rather, they may experience the normal surroundings as much colder than do mice not exposed to the browning agents.

For a mouse, the temperature of a normal animal house is in itself a cold stress. As seen in Figure 2, at 20°C, a mouse loses so much heat that it needs to increase its heat production (and thus its food consumption as well) by about 50% in comparison to the heat production in the thermoneutral zone. This is the case for a normal wild-type mouse.

If a mouse loses its fur, then much of its insulation will also disappear. Shaved mice increase their metabolic rate at normal animal house temperatures by nearly 50% because of the increased heat loss (Hirata et al., 2011). Similarly, the genetically nude Balb/c mice have a metabolic rate ≈80% higher than normal Balb/c mice (Hirata et al., 2011). We have illustrated the consequence of this in Figure 2. It can be understood from this that shaving a mouse living at 20°C is equivalent to transferring an unshaven mouse from 20°C to 10°C. Also note that, at 20°C, the extra metabolism required to counteract the extra heat loss due to shaving is some 300% of that of the unshaven mouse. This indicates a requirement for 3-fold more thermogenesis in the shaven mouse.

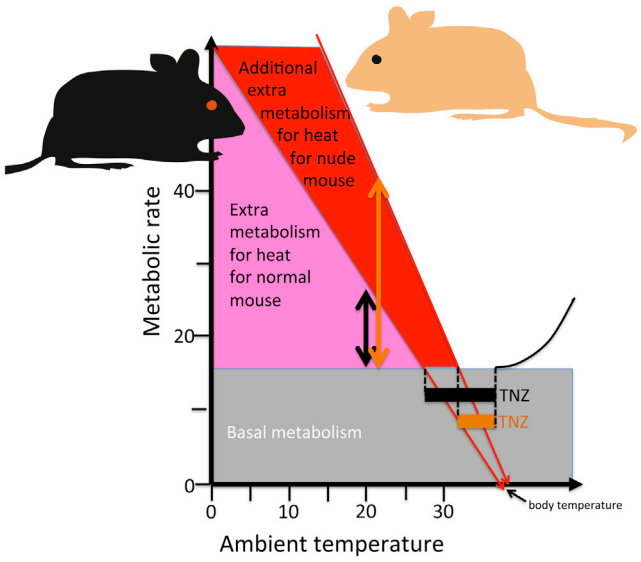

Figure 2. The Effect of Decreased Insulation on the Heat Needed to Counteract Cooling at Different Temperatures

When the environmental temperature is decreased, mice have to increase their metabolism in order to compensate for the increased heat lost. The response follows physical laws for heat transfer and is therefore linear, as illustrated in the pink triangle; the line extrapolates to body temperature (actual values are based on data in Golozoubova et al., 2004). The black double arrow indicates the extra heat necessary for living at ≈20°C as compared to living within the thermoneutral zone (TNZ), illustrated by the length of the black box. If mice lose some of their insulation because of (unintended and possibly unobserved) effects of browning agents, the heat loss at every temperature will be higher, as illustrated by the red area (the values shown are extrapolated from those reported for shaven and for genetically nude mice by Hirata et al., 2011). The extra heat needed in the nude mice to compensate for the heat loss at 20°C (skin-colored double arrow) is nearly three times as high as in normal mice; this means that the nude mice experience the environment at 20°C as being similar to that of normal mice at 10°C. Therefore, browning of white adipose tissue will take place for physical reasons. The effect of diminished insulation is also that the lower limit of the thermoneutral zone moves upward, as illustrated by the length of the skin-colored box; therefore, in browning-agent-affected animals, 30°C may no longer be thermoneutral. This way of representing metabolic data may be referred to as a Scholander plot (Scholander et al., 1950).

Thus, loss of insulation, through changes in the quality of the skin, fur, and hair, can be considered to be fully equivalent to being exposed to a colder environmental temperature—and cold is, as discussed above, a strong browning agent. Thus, the pertinent question becomes: can (some/any of) the reported browning effects be explained through mechanisms akin to shaving a mouse?

We have rather haphazardly examined the literature concerning the effect of some of the browning models on the skin, fur, and hair phenotypes of the mice. We found—as exemplified below—that it is not uncommon that the browning effect is associated with changes in insulation; strikingly, this is relevant for some of the most noted browning agents. Indeed, for some of these browning agents, also exemplified below, it is clear even from a photograph of the mouse that the mouse has a fur and hair insulation problem. This means that, in many cases, the browning is a secondary, but necessary, consequence of the enhanced heat loss rather than a primary molecular effect on the browning cells themselves. Importantly, such insulation problems cannot be sidestepped as being irrelevant additional effects of the browning agents: the point is that they can fully explain both the browning and any metabolic effects. Only if such effects are retained at the thermoneutral temperatures of the animals exposed to browning agent would the browning agent possess valid desirable properties for human therapy.

Some Browning Agents Are Simply Epilating

Several browning agents have the effect that the affected mice become fully nude (full alopecia) with time. This is clearly the case for *Phospholipase C delta knockout (KO)* mice that demonstrate full hair loss (Nakamura et al., 2003) and thus expected browning (Hirata et al., 2011) and for the *Vitamin D receptor KO*, which also fully loses its hair (Li et al., 1997) and shows the expected browning effects (Narvaez et al., 2009). The ablation of the *leucine-rich repeat containing G protein coupled receptor 4* leads at least to partial hair loss (Mohri et al., 2008) and browning (Wang et al., 2013). Also, *cyclooxygenase 2* (Cox2) is a browning agent that, when specifically overexpressed in the skin, leads to hair loss (Neufang et al., 2001). This skin-selective expression is accompanied by white adipose tissue browning (Vegiopoulos et al., 2010). It has been discussed that alterations in cyclooxygenase products in the skin would be molecularly transmitted to the white adipose areas in order to induce browning (Wu et al., 2013). However, such intricate mechanisms are probably not necessary to explain the induced browning: the increased heat loss is probably sufficient, and the transmission is physiological.

Thus, at least for these browning agents, an a priori explanation for the browning effect would be that the mice have lost insulation and consequently feel as though they were exposed to 10°C. Therefore, recruitment of thermogenic capacity, including browning, is induced through the sympathetic nervous system as it would be in wild-type animals living at 10°C. This does not exclude that cell-autonomous effects of the browning agent may be observed (as discussed below). However, such effects are principally of physiological or therapeutic interest only if the browning agent maintains its beneficial properties under conditions wherein the effects of the increased heat loss have been eliminated.

Some Browning Agents Curl the Hair

There may be more subtle effects of browning agents. Some browning agents may alter the function of the fur. These browning agents are often fatty acid metabolism related. Thus, mice with a KO of the stearyl CoA desaturase 1 (SCD1) (Miyazaki et al., 2001), elongation of very-long chain fatty acids 3 (Westerberg et al., 2004), and acyl-CoA binding protein (ACBP) (Neess et al., 2013) all demonstrate altered fur characteristics. In such mice, browning may be observed (Sampath et al., 2009). In agreement with the cyclooxygenase case discussed above, a mouse with a skin-selective SCD1 KO still shows adipose browning (Sampath et al., 2009). Indeed, artificial skin occlusion with Vaseline eliminates the metabolic effects of an SCD1 KO (Binczek et al., 2007) and an ACBP KO (Bloksgaard et al., 2014; Neess et al., 2013).

In Some Cases, Available Data Implicate that Skin and Fur Defects Could Explain Browning

In the cases above, a direct relationship between a browning agent and skin and fur problems can be identified. In other

cases, such a relationship can only indirectly be suggested. For instance, myostatin KO leads to browning that is not cell autonomous. Although other suggestions have been made (Shan et al., 2013), it should be noted that myostatin (unexpectedly) is expressed in the skin. Accordingly, the myostatin KO shows decreased wound healing (Zhang et al., 2012), implying a problem in the skin of these mice. Similarly, KOs of retinaldehyde dehydrogenase 1a1 (Kiefer et al., 2012) also induce browning. Retinaldehyde dehydrogenases are found in the skin and hair follicles (Everts et al., 2007), and their loss may affect skin insulation. More intricately, the expression of FOXC2 under the aP2 promoter leads to an increased blood vessel formation under the skin (Xue et al., 2008) and also browning (Cederberg et al., 2001). Concerning other browning agents, such indirect indications may exist, but it is of course not possible to guess with the use of this type of data whether the browning agent leads to increased heat loss. A comprehensive examination of all demonstrated browning agents with respect to insulation problems would certainly be warranted and may markedly shorten the list of browning agents such that only true browning agents would remain; i.e., those that cannot be explained as working through increased heat loss.

Those We Know that We Don't Know

The browning agents discussed above represent some of those where information on skin or fur effects can be found in the scientific literature. For many browning agents, such information is not presently available, but an absence of data clearly does not mean an absence of effects.

It is important to realize that we cannot, by guessing, eliminate the risk that a browning agent works by increasing heat loss. Who would think that a KO of protein tyrosine phosphatase 1B would increase heat loss? But it does—it doubles it (Alberts et al., 2006)—and this may well explain its effects on energy expenditure, obesity, etc. (Klaman et al., 2000).

The Tale of the Tail

A possibility for an altered heat loss that is not visually obvious is the involvement of the tail in the browning process. The tail is the major thermoregulatory organ for heat loss in the mouse and other rodents. When the mouse produces more heat than is needed, the blood vessels in the tail dilate, and more heat is lost. At normal animal house temperatures, the mouse needs to protect itself against heat loss, and the tail blood vessels are contracted. This means that, if an agent can overcome the physiological control of tail vasoconstriction and lead to vasodilation, then heat loss will increase, and the mouse will experience conditions akin to being exposed to a colder environment. Proof of principle for the significance of such a process can be seen in an α_1 thyroid hormone receptor mutant. This mutant shows enhanced thermogenesis (Sjögren et al., 2007), but this is not due to metabolic effects of the mutation as such. Rather, the mutation causes a loss of control of the tail blood flow, leading to increased heat loss and thus increased thermogenesis (Warner et al., 2013).

Demonstrating the Primary Site of Action of Browning Agents

It may correctly be advocated that the discussion above only implies that it is (often) alterations in the quality of insulation (skin, fur, and blood flow) that induce browning. In none of the above cases (except the thyroid hormone receptor mutation) has this been directly demonstrated. Demonstrating that a browning agent is a true browning agent necessitates demonstrating that the effect is not secondary to insulation effects. This principally requires that the metabolism should be studied in experiments of the Scholander type (Figure 2), which should be performed in order to elucidate possible loss of insulation.

In reality, all animal facilities do not possess full facilities for such insulation experiments. However, it should not be insurmountable to temporarily make an animal room warmer and examine the browning phenomenon under such conditions. If the extent of browning is diminished, it would be a clear indication that heat loss has been an important driver of browning. Such studies are rarely performed, although there are exceptions: Boon et al. (2013) found that BMP7 treatment induced substantial browning at 21°C, but this effect was totally eliminated at 28°C. There are several ways to interpret such results; a synergistic effect between sympathetic stimulation and the browning agent is one. However, BMP7 has been associated with the development of hair follicles (Zouvelou et al., 2009), and Boon et al. (2013) may thus exemplify the type of investigations that are necessary for examining whether a skin and fur alteration secondarily induces browning.

Early elimination of the risk that browning could be the result of these "banal" heat loss effects would be quickly rewarded in that academic or pharmaceutical enterprises would not pursue expensive experimental avenues that have this physiological but nonetheless secondary explanation as their background. A prudent attitude would be to confirm that the effects are not due to enhanced heat loss. This may significantly decrease the number of papers reporting browning phenotypes. However, it would perhaps provide the scientific society with some important bona fide candidates as true browning agents.

Thermoneutrality Is Not Just 30°C

For purely physical reasons, as seen in Figure 2, decreased insulation will also lead to an increased lower limit of the thermoneutral zone from about 28°C in normal mice to about 34°C in poorly insulated mice. This means that transferring mice to 30°C does not necessarily lead to satisfactory elimination of the enhanced heat loss. Thus, an observation that browning effects persist at 30°C (although at a lower level) (Flowers et al., 2011; Vegiopoulos et al., 2010) does not in itself demonstrate that enhanced heat loss cannot explain the browning effect. Given certain skin problems, it may even be that true thermoneutral conditions may not be attainable merely by increasing the temperature even further. For example, if water permeability is increased, then an increased temperature would lead to higher evaporation and thus additional heat loss. Under these conditions, sealing the skin as in Binczek et al. (2007), Bloksgaard et al. (2014), and Neess et al. (2013) would be able to reduce an enhanced heat loss.

The Feeling of Cold: Reversing the Cause and Effect

The outcome of the issues discussed above is that there is a large risk that, in browning investigations, the cause and effect are inverted. The line of thought often presented is that the browning agent induces more UCP1 and thus more heat

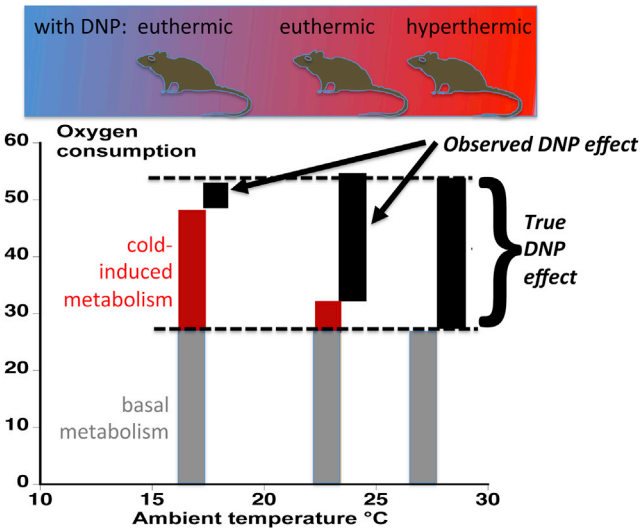

Figure 3. The Vanishing Effect of DNP

Dinitrophenol (DNP) is a classical artificial mitochondrial uncoupling agent, leading to extra heat production in practically all cells of the body. If (right-hand bar) DNP is injected into rats at thermoneutrality, it leads to a very marked increase in total rat metabolism (black box). However, seemingly confusingly, if DNP is injected into rats at lower environmental temperatures, then the effect of DNP successively disappears as the temperature is decreased (black boxes in middle and left-hand bars). In reality, the DNP effect does not disappear. Instead, what happens is the following: at lower temperatures, the rat has already been forced to increase its metabolism in order to compensate for the heat loss (red part of bars). When the extra heat induced by the DNP uncoupling is available (and, in all cases, it is the same amount as that observed at thermoneutrality [rightmost black box]), the rat ceases all other ongoing thermoregulatory heat production, and there is therefore an apparently lower effect of DNP at lower temperatures. At the lower temperatures, the body temperature is not affected by DNP, and the rats stay euthermic. Only at thermoneutrality is the extra heat not needed, and, if it cannot easily be disposed of, then the rats become hyperthermic. Thus, any "extra" heat production induced by exogenous activation of any process in brown fat or browned white fat will lead to reduced thermoregulatory thermogenesis and will, in principle, be invisible at normal animal house temperatures; only at thermoneutrality will such an extra effect be visible, and only at thermoneutrality would such extra heat production lead to hyperthermia. Based on data in Shemano and Nickerson (1958); see also Goldgof et al. (2014).

production (and, through this, protection against obesity). For many browning agents, the cause and effect may be the opposite: the browning agent leads to decreased insulation, which leads to increased heat loss, leading to increased need for thermogenesis in order to counteract the heat loss, resulting in browning, and the increased energy expenditure thus protects against obesity.

Not Really Cold, but Febrile

Observations of browning are sometimes reported in connection with reports of an increase in body temperature. This is normally taken as an indication that the browned white fat is producing extra heat and is thus functionally active. However, activation of browning white fat—or any thermogenic process—would not a priori be expected to result in an increase in body temperature, particularly not in a cool environment. Mice and rats, similarly to any mammal, are truly euthermic animals that strive to protect their body temperature and defend a centrally determined "set point" (this expression is not popular among thermal physiologists, but it remains a valid practical concept).

Under normal laboratory conditions, protection against extra internal heat production is easy, given that the animals (mice) are living at a temperature where they are cold (as indicated, 20°C is much below thermoneutrality). Any extra heat production would be compensated by an equal reduction in thermoregulatory thermogenesis and thus would be visible neither in the metabolic rate nor as an increase in body temperature. Experimentally, this can be seen in experiments where heat production is increased artificially by the classical uncoupling agent dinitrophenol (DNP) at different temperatures ("the vanishing effect of DNP"). As illustrated in Figure 3, there is almost no effect on metabolism or body temperature when DNP is injected at "normal" animal house temperatures. Only when injected at thermoneutrality does DNP lead to an enhanced metabolism and also increased body temperature when increased blood flow in the tail cannot off load the extra heat (Shemano and Nickerson, 1963) (see also Golgof et al., 2014, and similar observations may be made concerning the extra heat generated during exercise [Virtue et al., 2012]). Thus, this increase in body temperature is a hyperthermia rather than a fever; i.e., it represents a body temperature that is higher than the set point. Thus, this body temperature is not the one preferred by the animal and is observed only because the animal cannot dissipate the extra heat. Thus, increases in body temperature would not be expected as an effect of browning agents under normal laboratory conditions.

So why is an increase in body temperature often reported in connection with browning (e.g., for fibroblast growth factor 21 [FGF21] [Coskun et al., 2008] and melatonin [Jiménez-Aranda et al., 2013])? We would suggest that what is observed may not be hyperthermia but, in reality, a fever (or a pyrexia); i.e., an increase in body temperature set point elicited by the browning agent. A fever means that the animal will defend this higher temperature even when it can easily dispose of extra heat. Results may be obtained as those illustrated in Figure 4A wherein the body temperature of the browning-agent-treated animal during a circadian cycle tracks that of the untreated animal but at a constantly higher and parallel level. It is clear that this increased body temperature is not a hyperthermia (the mouse can clearly decrease its body temperature during its sleeping phase). Note that different definitions of fevers can be made; here, we use the broadest definition; i.e., that any defended higher body temperature represents a fever (this includes—but is not limited to—traditional fevers of the type that are mediated by prostaglandins [via COX1 and COX2] and thus sensitive to aspirin and similar drugs).

What is then the connection between browning and browning-agent-induced fevers? A thermoregulatory mechanism could again be relevant. Returning to the temperature/metabolism (Scholander) diagram, it can be seen that, as a consequence of an increase in set point, any temperature below the lower critical limit of thermoneutrality is experienced as more cold (Figure 5). Thus, the cause and effect may also here be the opposite of what is generally expressed; instead of the browning causing the increase in body temperature (hyperthermia), the browning is an effect of the defended increased body temperature (fever) and the ensuing feeling of being more cold at the same environmental temperature. If examination of the mice at a higher temperature diminishes the browning effect, then it

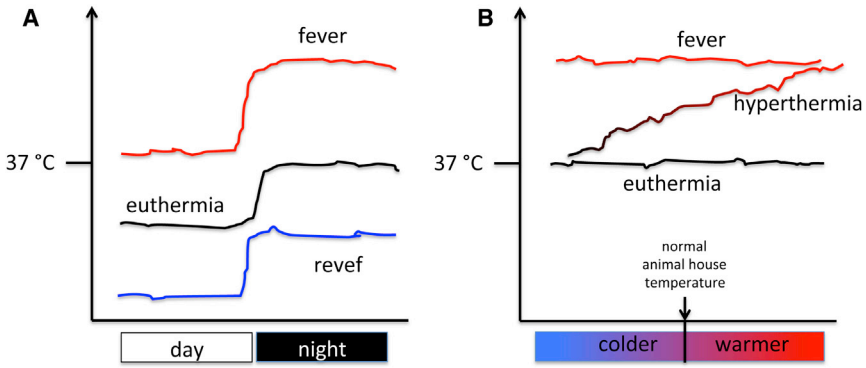

Figure 4. Fever, Hyperthermia, and "Revef"
(A) Fevers (defended body temperature increases) and "inverted fevers," revefs (or anapyrexia, defended body temperature decreases), are characterized by keeping day and night rhythms; a mouse showing a lowered body temperature during the day indicates that the mouse is not forced into an increased body temperature during the night because of overproduction of heat caused by, for example, a browning agent.
(B) Given that a fever is a defended increase in body temperature, the body temperature is principally unaffected by environmental temperature (top curve). A hyperthermia means that the animal cannot dispose of extra heat produced; however, by placing the animal at a lower environmental temperature, heat disposal will be easier and the body temperature would become lower (middle curve). Increases in body temperature at normal animal house temperatures are sometimes observed as an effect of a browning agent; the implication is that this is a hyperthermia that indicates extra heat production from the browned tissue. However, under these conditions, extra heat could readily be disposed of, and an increased body temperature is thus probably a fever induced by the browning agent. This can be tested by placing the mouse acutely at lower temperatures; if the increased body temperature is principally unaffected, then it is a fever, not a hyperthermia, indicating an effect of the browning agent on the central control of body temperature.

would support this interpretation. Examination of the body temperature as being a fever is also relatively simple: the animals should be exposed acutely to different environmental temperatures (e.g., between 15°C and 30°C). If there is no effect of altered ambient temperature on the increased body temperature, then it represents a fever rather than a hyperthermia (Figure 4B). Such studies may further transfer some browning agents to the list of secondary browning agents.

The opposite phenomenon would be evident if the browning agent induces what may be called a "revef"; i.e., an inverted fever (spelled backward), also referred to as an anapyrexia, that is defended decrease in body temperature set point. Following similar arguments, this would result in a diminished sympathetic drive to the white adipose tissue at any given environmental temperature. There are indications that, for example, FGF21 KO leads to both a revef/anapyrexia and a diminished browning (Fisher et al., 2012), which may thus be understandable as a consequence of the revef.

Stress: Positive and Negative

The nerves innervating the white adipocytes are part of the sympathetic nervous system. Normally, the outflow from any branch of the system is independently controlled, and, only under conditions of massively enhanced acute stress, would all sympathetic nerves be activated in parallel (in sympathy). Therefore, this stress activation may include the adipose branches.

A negative stress for a rodent would be to be suspended by a hindlimb (Yamashita et al., 1995) or the tail (Lew et al., 2009), immobilized (Gao et al., 2003; Harris et al., 2006; Murazumi et al., 1987), or exposed to foot shock (McGregor et al., 1994) or social defeat (Lkhagvasuren et al., 2011). In all these cases, there are indications that at least the nerves to brown fat are activated; the sympathetic nerves to white adipose tissue are also most likely activated, and browning may thus be induced.

A positive stress could be to be placed in an enriched environment, which does lead to browning (Cao et al., 2011). In general, increased alertness will activate the sympathetic nervous system most likely through an orexin-mediated pathway (Tupone et al., 2011). The implication of this is that it may not be surprising that any condition (browning agent) that may represent a general—positive or negative—stress may also be accompanied by browning. Although the central mechanisms underlying this are interesting, from the browning perspective, it would seem that this may be another example of increased sympathetic drive being responsible for the browning process. To examine whether this is the case, it could be investigated whether browning could be decreased by inhibition of the action of the sympathetic nervous system with, for example, the β-adrenergic antagonist propranolol. This was indeed the case for the browning effects of environmental enrichment (Cao et al., 2011). Similarly, other browning agents may act indirectly via the brain and sympathetic stimulation; e.g. thyroid hormone (T_3) (López et al., 2010).

A natural question is what is the purpose of stress-induced browning and brown-fat activation? It would not seem beneficial to activate heat production and nutrient utilization under conditions of stress. Perhaps there is no benefit: our Darwinian thinking tends to make us assume that all we observe must have a positive evolutionary explanation, but it really suffices that the effects are not acutely detrimental. Thus, stress leads to general sympathetic activation accompanied by increased fighting power, and the activation of browning may merely be an organizationally necessary but physiologically irrelevant effect of the general activation.

The Special Case of Exercise

During prolonged exercise, eating does not occur, and this means that the energy demands for exercise must be derived from available energy stores in the body. This would initially be muscle energy stores (mainly glycogen), but, during more prolonged exercise, this would not suffice, and the energy would necessarily have to come from the largest energy stores; i.e., the white-fat depots. Thus, exercise is associated with prolonged adrenergic stimulation of white adipose tissue depots in order to release fatty acids to the circulation (Ranallo and Rhodes, 1998). Therefore, it should inevitably be that browning occurs during exercise, and browning is indeed observed (Boström

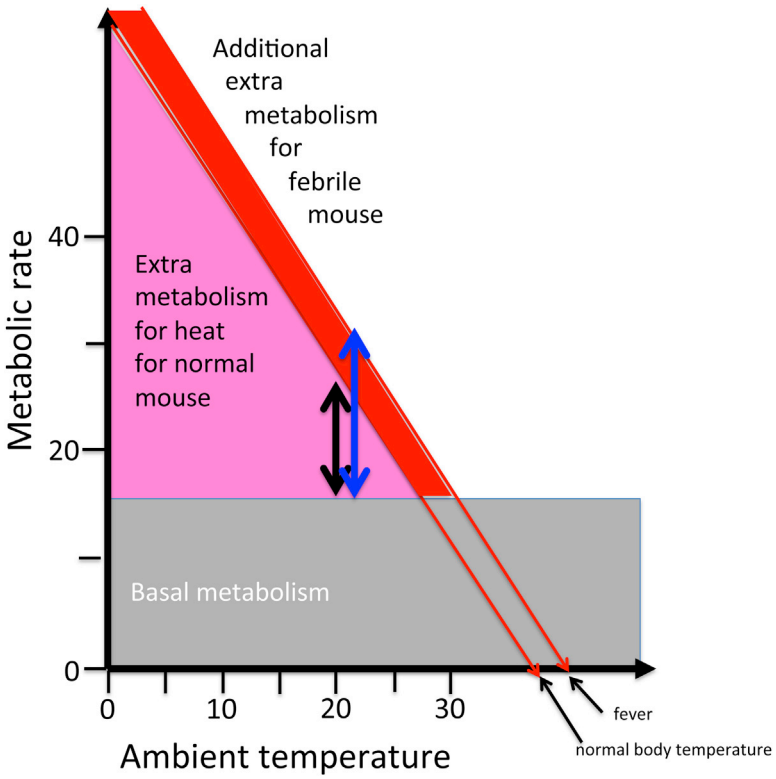

Figure 5. The Effect of Fever on the Heat Needed to Counteract Heat Loss at Different Temperatures
During fever, the defended body temperature is increased, as indicated on the x axes. Physically, this means that the line representing the insulation must be shifted to extrapolate to the increased defended body temperature, whereas the slope is unchanged, given that the insulation is the same. The consequence is that, at all environmental temperatures below thermoneutrality, the demand for extra heat production is increased (blue versus black double arrow). Thus, if a browning agent increases the body temperature set point (i.e., induces a fever), then this would lead to an increased demand for thermoregulatory thermogenesis (blue double arrow), and browning would occur as a response to this.

et al., 2012; Xu et al., 2011). However, there is evidence that also other mechanisms may be responsible (Boström et al., 2012).

Many Browning Agents Work through Stimulating the Sympathetic Nervous System

A conclusion from the discussion above is that many browning agents presently described may exert their actions through indirect mechanisms, leading to activation of the sympathetic nervous system and thus browning, essentially through accepted cellular mechanisms. However, this tenet has been fully experimentally proven in only a few of the above cases, and it is clearly possible that several browning agents exist that induce browning in a unique way. However, we would consider it prudent, before large enterprises are initiated scientifically or commercially based on observations of induced browning, to ascertain that the effects are not simply explainable by enhanced sympathetic activity due to cold or stress.

The Glitazones

In addition to the adrenergic pathway discussed above, only one alternative pathway for induction of UCP1 gene expression is generally accepted: glitazones or thiazolidinediones (TZDs; with rosiglitazone as the standard drug used). It is expected that rosiglitazone utilizes a pathway of physiological significance, but the nature of the "endogenous rosiglitazone" is not known (if it exists) and neither is the physiological relevance of this pathway. Rosiglitazone interacts with peroxisome proliferator-activated receptor γ (PPARγ) but does this in different ways, both classically adipogenically and by interacting with the ability of the Cdk5 kinase to phosphorylate PPARγ (Choi et al., 2010), leading to other effects. It may interact with other PPARs and also with some mitochondrial outer (Colca et al., 2004) and inner (Divakaruni et al., 2013) membrane proteins. It is not fully established which of these pathways is responsible for the browning effect. In contrast to the sympathetic activators discussed above, stimulation of browning by TZDs is not paralleled by an activation of the UCP1 that is induced; i.e., stimulation by TZDs does not in itself result in thermogenesis (UCP1 is not leaky [Shabalina et al., 2010], but it needs adrenergic stimulation to be activated). Given that the TZD effectors may be rather broad in their ligand requirements, it is not unlikely that several of the food substances reported to induce browning may use the TZD system (e.g., bofutsushosan [Akagiri et al., 2008], scallop shell powder [Liu et al., 2006], and several others). Given that the intracellular mechanism mediating browning by TZDs is not fully established, it is not straightforward to examine whether novel browning substances hijack the TZD pathway, but the effect of PPARγ antagonists could at least be tested before novel pathways are implied.

Are There Stimuli that Selectively Induce Browning?

The above discussions would imply that browning of white adipose tissue would always be paralleled by (or preceded by) recruitment of classical brown adipose tissue because of the hierarchical stimulatory arrangement of the sympathetic nervous system. Such browning agents may be considered to be of less principal interest than putative browning agents that selectively induce browning without recruiting brown adipose tissue.

In the—at the time, probably comprehensive—listing of browning agents presented by Wu et al. (2013), the authors meticulously tabulate 65 agents that induce browning. Then, they ask the important question of whether any of these agents selectively browns white adipose tissue or whether the browning is paralleled by recruitment of classical brown adipose tissue (i.e., in line with the hierarchical activation discussed above). Wu et al. (2013) conclude that only four agents have been reported to selectively induce browning: 4E-BP1 KO, IKKε KO, Cox2 skin overexpression, and TRPV4 antagonism (for another seven browning agents, brown adipose tissue was not examined). Examining the four studies, the impression gained is that even these exceptions have not been extensively corroborated; for the 4E-BP1 KO, only unchanged brown adipose tissue wet

weight seems to be reported (Tsukiyama-Kohara et al., 2001); for IKKε, unchanged brown adipose tissue UCP1 mRNA and protein levels (see below) (Chiang et al., 2009); for Cox2 skin overexpression, brown adipose tissue UCP1 mRNA levels were (only) doubled (see below) (Vegiopoulos et al., 2010); and, for TRPV4 antagonism, brown-fat UCP1 mRNA levels were unchanged (Wu et al., 2012).

In investigations of browning, it is often reasoned that, if a given browning agent induces UCP1 mRNA levels many-fold in white fat but has (nearly) no effect on UCP1 mRNA levels in classical brown adipose tissue, then the effect in white fat must be the important one. However, starting from mice at normal temperatures, brown adipose tissue recruitment may not reveal a (dramatic) increase in UCP1 mRNA levels nor even in UCP1 protein per milligram tissue protein. This is because, in mice living at normal animal house temperatures, these parameters are (nearly) saturated already, given that the tissue is already stimulated by the cold environment (Nedergaard and Cannon, 2013). For the tissue to increase its thermogenic capacity, it must expand its active mass; i.e., through increased cell proliferation and differentiation. Thus, only if the total amount of UCP1 protein in the whole tissue is monitored can it be concluded whether classical brown adipose tissue is also recruited. In white depots, large relative increases in, for example, UCP1 mRNA levels do occur, but levels start from absolute values of UCP1 mRNA that are some 100- to 1,000-fold lower than those in brown adipose tissue (Nedergaard and Cannon, 2013). Thus, a small relative increase in classical brown adipose tissue thermogenic markers may thermogenically outclass a large relative increase in the thermogenic markers of browning white adipose tissue.

Are the Metabolic Effects Really Caused by Browning?

Concerning the browning agents, reasoning is often forwarded as follows: browning agent X has metabolic effects (increases thermogenesis, makes the mouse slimmer, etc.), and browning agent X browns white adipose tissue; therefore, browning agent X makes mice slim because of browning in white fat. Clearly there could be other reasons for the metabolic effects than browning; it should be realized that many metabolic effects caused, for example, by decreased insulation would principally occur irrespective of whether brown-fat recruitment and browning took place or not. However, except for the browning induced by cold (Golozoubova et al., 2001) or high-fat diet (Feldmann et al., 2009), involvement of UCP1 in the metabolic effects has not been proven for any browning agent, and, in the cases of cold and high-fat diet, it is very likely primarily the UCP1 in the classical brown-fat depots that is quantitatively responsible for the metabolic effects.

To establish whether indeed browning is responsible for the phenomenon of interest, the metabolic effect should disappear if browning is prevented; i.e., mice should be engineered that do not express UCP1 specifically in the white adipose tissue depots. Such mice are not available today and would in any case probably not provide an unequivocal answer, given that compensation in other adipose depots would probably occur (cf. the compensation found in the myf5-BMPR1-KO mice) (Schulz et al., 2013). Given that this experimental approach not is avaiable, the necessity of UCP1 for the effect should at least be demonstrated. This is readily possible given that UCP1 KO mice exist (Enerbäck et al., 1997) and are commercially available. However, there can be some interpretation problems in the use of UCP1 KO mice. At normal animal house temperatures, UCP1 KO mice unexpectedly do not become more obese than controls, and, at this temperature, they are actually "protected" against the effects of obesogenic diets (Liu et al., 2003) (the reason for this effect is not clarified but may be related to the stress that the absence of nonshivering thermogenesis causes for mice living in the cold). Thus, the obesogenic effect of the absence of UCP1 is observed only at thermoneutrality (Feldmann et al., 2009). The experiment would then be to test the browning agent in UCP1 KO mice kept at thermoneutrality; if the slimming effect were to disappear, then it could at least be concluded that it was due to UCP1 activation (although the site could not be established through this).

Apparent, but Flawed, Increases in Metabolism

In many studies reporting slimming effects of browning agents, one of the main arguments that the slimming is due to the browning of white adipose tissues and the ensuing increased thermogenic capacity is data showing elevated mouse metabolism (oxygen consumption). However, it may often be observed that the metabolic data in these reports are obtained by dividing the metabolic rate (oxygen consumption) of each mouse by its total body weight (sometimes to the power of 0.75 or 0.66, but this does not alter the outcome). Given that obese and "normal" mice basically differ only in the amount of chemical triglycerides they have accumulated, the amount of metabolically active "lean" tissue is generally not different between slim and obese mice. This means that using total body weight as the divisor will result in an erroneous contribution to metabolism from chemically inert triglycerides, and this means that, with actual unchanged metabolism between obese and slim animals, the slim mice would appear to demonstrate an increased metabolism. This pseudophenomenon has been commented repeatedly over the years (Butler and Kozak, 2010; Cannon and Nedergaard, 2011; Himms-Hagen, 1997; Tschöp et al., 2012). The simplest way to avoid this problem is to express metabolism per total mouse (not dividing by anything); this is again because most browning agents only induce marginal alterations in the amount of metabolically active tissue (lean body weight). If data for body composition are available (e.g., through MRI analysis), then a more adequate procedure is to express the values per lean body weight (or lean body weight to the 0.75 power).

Even when adequately presented in this respect, the logic of cause and effect may easily become inverted. If the browning agent leads to an enhanced heat loss (as many do, as discussed above), any metabolic data collected at temperatures below thermoneutrality demonstrate only the stimulation of thermogenesis necessary to balance the heat loss and are not indications of free-running, agent-induced overproduction of heat. Only if an enhanced metabolic rate, that had been adequately calculated, were observable at thermoneutrality would it be evidence that the browning agent actually induced an overproduction of heat. We are currently not aware of any of the browning agents having been demonstrated to induce increased metabolism when analyzed in this way. This does not mean that this could not be possible. Even small but chronically maintained increases in metabolic rate, too small to be detected reliably in present

systems, could affect energy balance and thus counteract the development of obesity.

The Relationship between Slimming and Browning

Reports on many browning agents include data indicating that the agent also induces slimming, the implication being that it is the browning that causes the slimming. Again, considering enhanced heat loss as a possible cause of browning, an alternative scenario would be that the browning agent, through some other mechanism, causes slimming. It is generally assumed that increased fat layers (particularly in the subdermal or subcutaneous adipose depots) have an insulating effect, although convincing mouse data on this seem to be missing. If this is the case, then slimming would lead to increased heat loss under subthermoneutral conditions, and this increased heat loss would then be the driving force for the browning of the white adipose tissue. Again, this important distinction can only be made when heat loss is not a problem; i.e., by performing the experiments at the thermoneutral temperature of the treated mouse.

Furthermore, it could be expected that any increased metabolism in the cold would be compensated by increased food intake (i.e., that leptin or another adipostat mechanism would ensure sufficiently increased appetite to maintain body energy reserves). However, it appears that cold is to some extent exempt from the adipostat effect (discussed in Cannon and Nedergaard, 2009), even though we do not understand why the extra energy combusted in the cold is not fully compensated. Therefore, observations that animals that experience a browning-agent-induced increased cold stress (as discussed above) become slimmer should not be considered as indicative of a special action of browning agents.

Slimming is the result of degradation of triglycerides stored in white adipose tissue and combustion of the liberated fatty acids. Whereas the concept that UCP1 in the browning white adipose tissue could be the site of combustion is appealing, there is no a priori reason to presume that the combustion should take place directly in the white adipose tissue. Just as is the case during prolonged exercise (Ranallo and Rhodes, 1998), the fatty acids needed for combustion could equally well be transported to the energy-utilizing organ. During exercise, this would be muscle and, during cold exposure, it would be, to a high degree, brown adipose tissue. There are several observations that indicate that brown adipose tissue is well endowed to take up fatty acids from the circulation for combustion in the classical brown-fat mitochondria (Henkin et al., 2012; Wu et al., 2006).

Cell-Autonomous or Central

In the discussions above, the implication has been that browning may often be the result of central effects; i.e., that a browning-agent-induced increase in sympathetic stimulation can explain the browning in the white adipose tissue. However, many browning agents have been shown to have cell-autonomous effects; i.e., when added to cell cultures consisting of real adipocytes or of cell lines, they induce effects that can be understood in terms of browning. The interpretation of such studies has some complications.

The expression of UCP1 is not spontaneous in any brown or white fat cell. Although we think of brown adipose tissue as a tissue in which UCP1 is found, brown-fat precursor cells isolated from brown adipose tissue and grown in culture to adipocyte maturity express essentially no UCP1, nor do white-fat precursors (“essentially” means some 100- to 1,000-fold lower UCP1 mRNA levels than in active brown fat). However, when such cultures are stimulated with norepinephrine, a large increase in UCP1 mRNA levels is induced in both brown- and white-fat cells by some 100-fold (absolute levels in white fat do not reach those in brown fat, even if the increases are relatively larger, given that they start from lower levels) (Petrovic et al., 2008, 2010). In vivo, the browning process induced by cold similarly increases UCP1 mRNA levels in white adipose tissue by some 100-fold (Waldén et al., 2012).

These relative increases may be contrasted to the increases in UCP1 gene expression reported to be induced by many browning agents in cell culture studies. These increases are often statistically robust, but their absolute levels are often much lower than those induced by norepinephrine (or in the cold) and are often also much lower than what is observed in vivo by the same browning agent. In order to eliminate the risk of unspecific effects and be potentially physiologically significant, cell-autonomous effects would be expected to be close to those that can be induced by norepinephrine. To our knowledge, none or few of the presently tested browning agents fulfill such criteria. An “explanation” for these modest cell-autonomous effects could be that some of the agents interact with noncognate receptors, are therefore only partial agonists, and thus can only induce minor effects. Additionally, even if a browning agent shows robust effects in cell culture, the physiologically and therapeutically relevant question must still be whether a metabolic effect is observable in vivo at thermoneutrality, a property not demonstrated for any browning agent presently.

Concerning functional analysis of the browned cells, their thermogenic capacity, their possible specific roles, their origin, and the nature of human UCP1-containing cells, a short summary can be found in the Supplemental Information.

The Burning Issues

This Perspective has reviewed and pointed to some burning issues, mainly whether many of the browning results reported are secondary, being due to activation of the sympathetic nervous system, because heat loss has been increased.

If we require a true browning agent to (1) function through other mechanisms than those affecting heat loss, (2) lead to increased metabolism at thermoneutrality, (3) lead to decreased obesity, and (4) do this without recruiting classical brown adipose tissue, then it would seem that no true browning agent has been fully verified to date. Some of those suggested may eventually achieve this status, but there are presently no unequivocal candidates.

Concerning the consequences of the browning effect itself, there is presently (see the Supplemental Information) no consensus that browned white adipose tissue possesses physiological functions qualitatively different from those of classical brown adipose tissue or that the tissue is regulated in a way distinguishing it principally from classical brown adipose tissue. The quest for true browning agents, and exclusive browning effects may therefore go on.

SUPPLEMENTAL INFORMATION

Supplemental Information contains Supplemental Results and can be found with this article online at http://dx.doi.org/10.1016/j.cmet.2014.07.005.

ACKNOWLEDGMENTS

Our investigations into the function and significance of brown adipose tissue and related issues are supported by grants from the Swedish Research Council (NT and M). The authors are members of the DIABAT program within the European Union Seventh Programme and the Wallenberg Foundation's program on brown adipose tissue. The authors would like to thank members of the unit of Molecular Metabolism for stimulating discussions. J.N. is a member of the SAB and a shareowner in Ember Therapeutics. B.C. is a member of the SAB of Metabolic Solutions Development Cooperation.

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Cell Metabolism, Volume *20*

Supplemental Information

The Browning of White Adipose Tissue: Some Burning Issues

Jan Nedergaard and Barbara Cannon

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Supplementary material concerning cellular studies in browning white adipose tissue

Functional studies

Is the UCP1 in browned adipose tissue functionally active?

We have here defined browning as the observation of (much increased) UCP1 mRNA levels in white adipose tissue depots. However, these expression levels are still low (at best about 10-fold lower UCP1 mRNA levels than in active brown adipose tissue (Walden et al., 2012)), and it may therefore be questioned whether sufficient amounts of UCP1 protein would be present in the mitochondria to make UCP1-dependent uncoupling both visible and functional. However, mitochondrial preparations from browned inguinal white fat indeed show UCP1-dependent uncoupling, and the UCP1 is canonically regulated (Shabalina et al., 2013).

What is the quantitative significance of UCP1 in browning cells versus classical brown adipose tissue UCP1?

The relationship between total thermogenic capacity (≈ the total amount of UCP1) in the browned white adipose tissue versus that in classical brown adipose tissue is of interest in this connection. Using different estimating principles (total amounts of UCP1 protein, total amounts of UCP1 mRNA, extrapolations from oxygen consumption analyses (Shabalina et al., 2013)), we have arrived at the conclusion that the total thermogenic capacity from the browned white depots could amount to some 25% of the total UCP1-dependent thermogenic capacity when browning is at its most recruited (i.e. in 129Sv mice in chronic cold). Concerning thermogenic capacity and UCP1 content per cell, studies of isolated brown versus brite fat cells have arrived at similar conclusions (Okamatsu-Ogura et al., 2013). The capacity may thus be substantial but it is not thermogenically dominating.

Are there special functions of the browned cells? and does it matter?

An underlying issue concerning the browning process is clearly whether the browned white-fat cells have other functions than classical brown-fat cells? and/or are they differently regulated. We, and others (Giralt and Villarroya, 2013), would tend to conclude that there is presently no demonstrated distinct function of the browned white-fat cells; whether the regulation may be different can also be discussed (see above). A problem in this content is the difference between the absolute levels of UCP1 in classical brown and browning white tissues. For instance, a deletion of Prdm16 in all adipose tissues leads to a practically total elimination of UCP1 mRNA in browning tissue but only to a minor (-25 %) decrease in UCP1 mRNA in classical brown fat (Cohen et al., 2014). However, as the total amount of UCP1 is so much higher in classical brown fat, it is uncertain to which extent the altered metabolic traits observed can be ascribed to diminished browning or to diminished classical brown fat – or both.

An additional problem in the present situation is that interest is now so focused on the browning cells that classical brown adipose tissue is sometimes no longer examined. Also, when additional effects are sought, there is a tendency to neglect the possibility that classical brown adipose tissue is equally involved. It has e.g. been demonstrated that overexpression of FoxC2 – that leads to browning – is beneficial for the skeleton, and based on cell culture studies this is suggested to be due to the browned white adipose tissue (Rahman et al., 2013); classical brown adipocytes were, however, not examined.

So who are the browning cells?

"Brown fat cells could arise in the parametrial fat pad by recruitment from precursor cells or by trans-formation from existing white fat cells. Our current measurements do not allow us to draw any firm conclusions about the way in which these cells arise." This is a statement from the first description of the browning process (Young et al., 1984), and now, 30 years later, this conclusion still principally holds: we cannot yet draw firm conclusions about the origin of the browning cells.

In the 1980'ies, the browning phenomenon was not cell-biologically surprising, because the general notion at that time was that white fat cells and brown fat cells were similar, i.e. the white-fat cells could in general be "pushed" to become brown-fat cells. This general notion was maintained, even though in-vitro experiments had demonstrated that precursor cells isolated from brown or white depots and cultured under identical conditions had distinct morphology (Nechad et al., 1983) and differed qualitatively in their ability to express UCP1. Later studies (Atit et al., 2006;

Seale et al., 2008; Timmons et al., 2007) indicated that classical brown-fat cells derive from a lineage distinct from that of white-fat cells, in being derived from a common muscle/brown-fat progenitor (myf5-positive cells).

The first level of discussion concerning the browning white cells is whether all cells within a white adipose tissue depot possess the ability to brown. Although arguments in favor of this have been forwarded (Rosenwald et al., 2013; Rosenwald and Wolfrum, 2014; Smorlesi et al., 2012), it is our contention that molecular studies indicate that (at least) two types of adipocytes can be identified in white adipose tissue. It may also be noted that the browned cells tend to appear in clusters, implying a clonal origin.

Secondly, even accepting that the browning occurs in specific precursors in the white adipose tissue depots, there is an ongoing discussion as to whether the browning cells are true brown-fat cells, in having a myf5 origin – or they are not. Initial studies found cells of myf-positive background only in classical brown-fat depots (Seale et al., 2008) but later studies have indicated that a fraction of cells of myf5 origin can be observed in certain white adipose tissue depots (Sanchez-Gurmaches and Guertin, 2013). While it would be simple if it were so that the myf5-expressing progenitors were those that browned, there are in fact indications that these cells may be less subject to browning than myf5-negative cells (Shan et al., 2013). There are other suggestions and demonstrations concerning the origin of the browning cells (Lee et al., 2012; Long et al., 2014; Wang and Scherer, 2014; Wu et al., 2012) but these Perspectives will not attempt to judge these issues. Thus, "Our current measurements do not allow us to draw any firm conclusions about the way in which these cells arise", as was stated 1984.

What is human brown adipose tissue: brown or browned white?

Recent years – since 2007 (Nedergaard et al., 2007) – have seen an acceptance of the presence of active "brown adipose tissue" in adult humans. The tissue is found at locations similar to those of classical brown adipose tissue in rodents – except that the interscapular depot – a major depot in rodents – is almost absent, at least in adult humans (Lidell et al., 2013). In an influential paper, Wu et al. (Wu et al., 2012) suggested that human brown adipose tissue is really browned white adipose tissue.

The issue, as currently formulated, is essentially whether human brown adipose tissue is of myogenic origin or not. The technical problem is that in humans the cells cannot be lineage-traced, so the conclusion must be based on molecular "markers" – and thus be dependent on marker quality. Based on other markers and on deeper samples of human brown adipose tissue, present data would tend to indicate that human brown adipose tissue is probably both classical and also browned white adipose tissue, depending on location (Cypess et al., 2013; Jespersen et al., 2013; Lidell et al., 2013; Nedergaard and Cannon, 2013). In these discussions, rodent brown adipose tissue sampled in recruited states has generally been the norm for true brown adipose tissue. Perhaps the relationship in adult humans between classical brown and, potentially browning, white adipose tissue is much closer to that of a mouse at thermoneutrality exposed to a high-fat/cafeteria diet for a prolonged time.

But does it matter whether it is brown or browned? Matter it does only if it becomes established that the browning cells in white adipose tissue possess properties that can ascribe to them a special role, or that they are principally differently regulated from classical brown-fat cells. As can be understood from these Perspectives, we are not in a situation today where either of these two qualifiers has been un-equivocally demonstrated. Until such data appear, the issue is of minor translational significance.

In extension of this issue, it has been discussed that the large amounts of adipose tissue in adult semi-obese subjects could harbor significant UCP1 activity. It can clearly not be excluded that the white adipose tissue in such humans could contain 1 % of the UCP1 (per gram) of classical brown but that there would be 100 times more…- in which case, of course, the browning of white adipose tissue in adult humans could be of considerable metabolic interest.

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