White, Brown, Beige/Brite: Different Adipose Cells for Different Functions?

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Brown adipose tissue (BAT) is a major site of nonshivering thermogenesis in mammals. Rodent studies indicated that BAT thermogenic activity may protect against obesity. Recent findings using novel radiodiagnosis procedures revealed unanticipated high activity of BAT in adult humans. Moreover, complex processes of cell differentiation leading to the appearance of active brown adipocytes have been recently identified. The brown adipocytes clustered in defined anatomical BAT depots of rodents arise from mesenchymal precursor cells common to the myogenic cell lineage. They are being called "classical" or "developmentally programmed" brown adipocytes. However, brown adipocytes may appear after thermogenic stimuli at anatomical sites corresponding to white adipose tissue (WAT). This process is called the "browning" of WAT. The brown adipocytes appearing in WAT derive from precursor cells different from those in classical BAT and are closer to the white adipocyte cell lineage. The brown adipocytes appearing in WAT are often called "inducible, beige, or brite." The appearance of these inducible brown adipocytes in WAT may also involve transdifferentiation processes of white-to-brown adipose cells. There is no evidence that the ultimate thermogenic function of the beige/brite adipocytes differs from that of classical brown adipocytes, although some genetic data in rodents suggest a relevant role of the browning process in protection against obesity. Although the activation of classical BAT and the browning process share common mechanisms of induction (eg, noradrenergic-mediated induction by cold), multiple novel adrenergic-independent endocrine factors that activate BAT and the browning of WAT have been identified recently. In adult humans, BAT is mainly composed of beige/brite adipocytes, although recent data indicate the persistence of classical BAT at some anatomical sites. Understanding the biological processes controlling brown adipocyte activity and differentiation could help the design of BAT-focused strategies to increase energy expenditure and fight against obesity. **(*Endocrinology* 154: 2992–3000, 2013)**

Since the 1970s, brown adipose tissue (BAT) has been increasingly recognized as the main site of nonshivering thermogenesis in mammals, and there are indications, as well as some debate, that it is also the site of the process called "diet-induced thermogenesis." The thermogenic processes elicited in BAT occur via a unique biochemical property of the mitochondria in brown adipocytes, wherein the brown adipocyte-specific protein, uncoupling protein-1 (UCP1), physiologically uncouples the respiratory chain. Brown adipocytes thus harbor numerous highly oxidative, naturally uncoupled mitochondria that actively oxidize metabolic substrates for the sole purpose of dissipating chemical energy as heat. In response to cold- or overfeeding-triggered activation of the sympathetic nervous system, the thermogenic activity of BAT is induced via distinct cellular processes. They include the rapid activation of the existing UCP1 and transcriptional induction of the genes encoding UCP1, components of the enzymatic machinery responsible for oxidizing metabolic substrates, and components of the cellular machinery re-

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Abbreviations: BAT, brown adipose tissue; C/EBP, CCAAT-enhancer binding protein; PET, positron emission tomography; PGC-1α, peroxisome proliferator–activated receptor-γ-coactivator-1α; PPAR, peroxisome proliferator-activated receptor; PRDM16, PR domain–containing protein-16; UCP1, uncoupling protein-1; WAT, white adipose tissue.

sponsible for the active uptake of lipids and glucose from the circulation, to sustain oxidation and thermogenesis. Complex processes of cellular hypertrophy and hyperplasia, which enlarge the organism's thermogenic capacity, are also induced (see below). Experimental studies in rodents have led researchers to propose that diet-induced thermogenesis in BAT may protect against obesity. Ablation of BAT and genetic invalidation of the UCP1-encoding gene have been shown to sensitize organisms to obesity in several experimental settings, whereas genetic models of rodent obesity often show abnormal inhibition of BAT activity (for review, see Ref. 1).

The recent recognition of active BAT in adult humans (see below) and indications that BAT activity may be negatively associated with obesity have led to recently renewed interest in BAT biology. However, new studies and the recognition of unexpected complexities in brown adipocyte differentiation have yielded numerous novel concepts and terms, which could cause confusion in this intensely evolving area. Because dozens of excellent reviews have examined BAT biology in the last few years (1–4), the present review does not intend to provide an exhaustive view of the field. Instead, we examine the major emerging views on BAT-associated energy expenditure processes and brown adipocyte biology and seek to clarify the current nomenclature.

White Adipose Tissue (WAT) and BAT, Near and Far

In nearly all mammalian species, brown adipocytes cluster in defined anatomical depots of BAT, at least during the early stages of development. Rodents and small mammals in general possess defined BAT depots throughout the animal's lifetime, such as in the scapulae (interscapular, cervical, and axillary depots) and thoracic (mediastinal depot) areas of mice and rats. WAT also develops in multiple anatomical sites, and there are known metabolic and endocrine differences between subcutaneous and visceral WAT depots. BAT develops significantly earlier than WAT (ie, during fetal life). The development of subcutaneous WAT begins in utero but primarily occurs after birth, when specialized fat storage cells are needed to provide fuel during fasting periods. Hyperplastic and hypertrophic white adipocyte processes occur during WAT development and throughout the organism's lifespan, with the energy balance ultimately governing the sizes of the WAT depots (for reviews, see refs. 5, 6).

Traditionally, brown adipocytes have been discussed in the context of their morphological and biochemical similarities to white adipocytes, both of which have intracellular fat stores (multilocular and unilocular, respectively) as an intrinsic feature of their cell identity. However, once researchers found that brown adipocytes function in energy expenditure, in total opposition to the specialized role of white adipocytes in energy storage, the supposed close similarity and common cell lineage of these cells appeared somewhat paradoxical. Since then, researchers have outlined unanticipated complexities in development and differentiation of brown and white adipocytes. Both adipose tissues arise from a mesodermal origin, but white and brown adipocytes are now believed to originate from different mesenchymal stem cell lineages (see below). In contrast to this difference in the preadipocyte commitment, however, their subsequent adipogenic differentiation involves a shared transcriptional cascade that mainly involves peroxisome proliferator-activated receptor (PPAR)-γ and CCAAT/enhancer-binding proteins (C/EBPs) (7, 8). Indeed, PPARγ is indispensable for the development of all types of adipose cells (9, 10). C/EBPα functions to maintain PPARγ expression, and both cooperatively regulate gene transcription to promote and maintain the differentiated state of adipocytes (ie, lipid metabolism, glucose metabolism, and insulin sensitivity). The absence of C/EBPα in mice prevents the development of white, but not brown, adipose depots, indicating that a lack of C/EBPα can be compensated for in brown fat development probably via C/EBPβ (11, 12). Recently, considerable effort has been devoted to identifying specific transcriptional mechanisms that govern brown vs white adipose-related gene regulatory networks. The factors involved include PR domain–containing protein-16 (PRDM16) (13) and peroxisome proliferator–activated receptor γ-coactivator-1α (PGC-1α) (14, 15). PRDM16, but not PGC-1α, was shown to specifically confer brown fat cell identity (13, 16). In fact, PRDM16 binds and coregulates C/EBPβ, PPARγ, PPARα, and PGC-1α to promote brown fat–specific gene induction (13, 17, 18). PGC-1α, which also coactivates PPARγ and PPARα, is involved in regulating mitochondrial biogenesis, oxidative metabolism, and thermogenesis (14, 19). Interestingly, most factors that induce or repress the "browning" program (see below) act through modulation of PGC-1α activity. Although most of the molecular actors that promote adipogenesis (eg, PPARγ) commonly function in the differentiation of both brown and white adipocytes, recent studies using a chromatin immunoprecipitation sequence–based approach found that numerous PPARγ binding sites are specific to either BAT or WAT (20). The mechanistic basis for the selective binding of PPARγ to target genes in different adipose tissues is poorly known, but it appears to involve the interaction with coregulators. For instance, PRDM16 and the early B cell factor-2 were found to re-

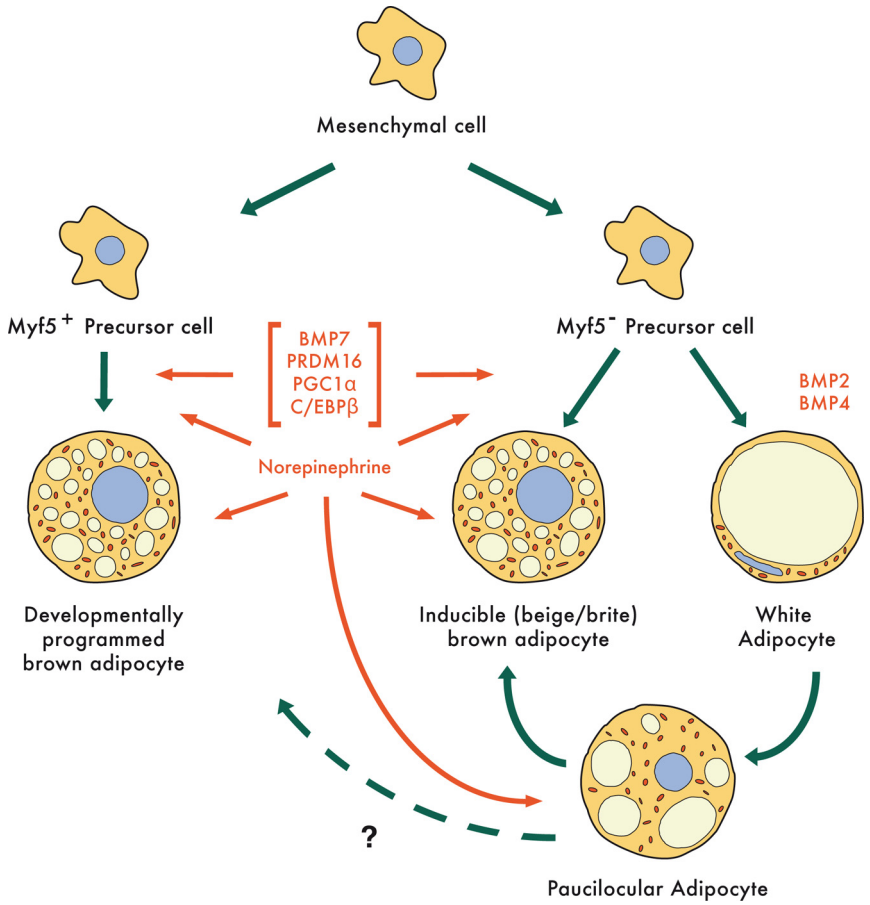

Figure 1. Schematic representation of the main pathways of differentiation of brown adipocytes. Myf5$^+$ progenitor cells, common to skeletal muscle cell lineage precursors, give rise to developmentally programmed brown adipocyte differentiation. A distinct population of Myf5$^-$ cells give rise to inducible (beige/brite) brown adipocytes as well as to white adipocytes. Several soluble molecules and intracellular factors act at distinct points of these processes (norepinephrine, members of the bone morphogenic protein [BMP] protein family, PRDM16, PGC-1α, and C/EBPβ are highlighted; for a complete description, see Ref. 3). In addition, there is evidence of the capacity of white adipocytes to interconvert into inducible brown adipocytes through transdifferentiation, being paucilocular adipocytes (UCP1-positive with lipid droplet distribution intermediate between brown and white adipocytes), intermediate forms of adipocytes transitioning from white to inducible, beige/brite adipocytes.

cruit PPARγ specifically to BAT-selective genes (13, 21), whereas other factors, such as TLE3, have been reported to recruit PPARγ to activate specifically white adipocyte differentiation (22).

The Browning Process

The so-called "browning" of WAT is the process by which brown (UCP1-expressing, multilocular) adipocytes appear at anatomical sites characteristic of WAT. This phenomenon occurs after a thermogenic stimulus, such as prolonged cold exposure (23), which also causes the recruitment of BAT at the classical anatomical sites (eg, interscapular depots). The browning process can be mimicked by chronic treatment with β_3-adrenergic receptor activators (24).

Recent studies have established that the cell lineage of these brown adipocytes that appear in the former WAT depots differs from that of brown adipocytes present in the BAT depots that arise via developmentally programmed processes. Contrary to previously held beliefs, researchers showed that the brown adipocytes present in BAT depots were more closely related to skeletal muscle precursor cells than to white adipocyte precursors (16, 25). In fact, brown adipocytes and myocytes share a common precursor that expresses the myogenic lineage marker Myf5. In contrast, the brown adipocytes that develop in WAT via the browning process were found to come from Myf5-negative cells that more closely resemble white adipocyte precursors (16). Cell lineage studies have suggested that resident Myf-negative mesenchymal precursor cells present in WAT depots can differentiate into these brown adipocytes in response to thermogenic stimuli. Today, researchers typically use the terms "beige" and "brite" (from "brown in white") to designate brown adipocytes that appear in WAT after permanent thermogenic induction, whereas those present in the standard BAT depots are often called "classical," "constitutive," or "developmentally programmed" brown adipocytes (26–28).

In recent years, researchers have sought to establish the intra- and extracellular molecular actors that govern the differentiation of each precursor type into the classical or beige/brite, brown adipocytes. To date, more than 50 molecules have been identified and their action mechanisms have been defined to some extent (for an updated, extensive review, see Ref. 3). Several members of the bone morphogenic protein family (29), in addition to transcription factors such as C/EBPβ and PRDM16 (17) and the PGC-1α coregulator, appear as main actors at distinct steps of the commitment and differentiation processes (Figure 1). However, the literature does not present a totally unanimous view of these processes, and the events

underlying the appearance of brown adipocytes at distinct anatomical sites and under distinct environmental stimuli are far from fully established.

Some groups claim that the browning process (in whole or in part) arises via the transdifferentiation of white adipocytes into beige/brite adipocytes (30). "Paucilocular" adipocytes (UCP1-positive with lipid droplet distribution intermediate between that of brown and white adipocytes) have been reported and suggested to represent intermediate forms of adipocytes transitioning from white to beige/brite adipocytes (31, 32), and recent data based on genetic labeling of adipose cells support the existence of bidirectional interconversion processes between beige/brite and white adipocytes (33). On the other hand, some reports indicated that β_3-adrenergic activation induces browning through 2 different processes; white to brown transdifferentiation in inguinal WAT, but proliferation and further differentiation of precursors in epididymal WAT (34). Finally, even the use of the Myf5-positive vs Myf5-negative criterion to distinguish between developmentally programmed brown adipocytes and beige/brite adipocytes has been challenged to some extent by the finding that Myf5-positive precursors may also differentiate to white adipocytes (35, 36).

How Distinct Are Brown and Beige/Brite Adipocytes?

Two concepts are currently being considered for the distinction between brown and beige/brite adipocytes, regardless of the original cell lineage: differential inducibility and differential function.

Recent studies have emphasized the inducible character of the beige/brite cells vs the more constitutive character of the brown adipocytes present in BAT. However, this should be considered an oversimplified (if not wrong) concept. Since the 1980s, it has been well known that BAT depots are extremely plastic in their response to thermogenic stimulation (37). Chronic cold stimulation elicits hyperplastic and hypertrophic processes in classical BAT depots; the proliferation and differentiation of precursor cells (preadipocytes and interstitial cells) into brown adipocytes is enhanced, increasing BAT mass (37, 38). This process is often referred to as "recruitment" of BAT (1). Thus, thermogenically activated classical BAT depots contain brown adipocytes that are derived from the adrenergic signaling-mediated differentiation of precursor cells induced by thermogenic activation occurring in adulthood.

On the other hand, studies in multiple experimental settings suggest that the inducibility of the brown adipocyte phenotype in WAT depots may have been overemphasized, largely because the parameters indicative of BAT-related thermogenesis (UCP1 expression and mitochondrial respiratory activity) show very low basal levels in WAT under regular conditions. Thus, their induction by thermogenic triggers can lead to a fold induction that appears extreme, regardless of the ultimate absolute levels of BAT activity indicators in WAT depots (39). In the future, researchers should consider this experimental complexity when evaluating new activators of brown adipocyte thermogenic activity and comparing their effects on classical BAT and the browning process (see below).

In terms of differential function, although there has not yet been a precise bioenergetic analysis of beige/brite cells, the available data indicate that they have all the morphological and molecular characteristics of classical brown adipocytes present in BAT depots (multilocularity, expression of UCP1, and increased mitochondrial respiratory equipment) and are thus likely to have intrinsically similar functions. Even though the current terminology stresses the differences in cell lineage and anatomical placement, the evidence suggests that the beige/brite cells function as true thermogenic brown adipocytes. Researchers have recently identified differential expression of several genes in classical vs beige/brite brown adipocytes that can be used to distinguish between the 2 cell lineages. These genes encode proteins with very distinct cellular functions, including transcription factors (eg, Zic1 and Tbx15), metabolism-related proteins (eg, Slc27a1), and proteins associated with inflammatory pathways (eg, CD40 and CD137) (28, 40, 41). Their differential expression is still observed when the adipocytes are differentiated, but further research is needed to establish whether these proteins, beyond their usefulness as cell lineage markers, confer relevant functional differences between classical brown adipocytes and beige/brite adipocytes.

Some authors have speculated that one possible differential feature of beige/brite cells is their capacity to make adipose depots more plastic and able to switch between energy storage vs thermogenic activity, because of their capacity to shift from a "white"-type morphology (large depot of lipids stored as a single lipid droplet) to a "brown"-type phenotype (multiple lipid droplets, and energy dissipation associated with uncoupled mitochondrial respiration) (3). This rationale is plausible, but the actual ability of a brown adipocyte to burn energy (in BAT or WAT depots) only relies on intracellular lipid stores for a short time. After a few hours of strong thermogenic activation, brown adipocytes burn energy mainly through the oxidation of lipids (and possibly glucose) coming from blood via active lipoprotein lipase activity and the subsequent oxidation of circulating triglycerides (42–44). En-

dogenous fat stores should be considered short-term "security" stores that are intended to sustain thermogenesis while blood flow, lipoprotein lipase activity, and other processes are activated.

A relevant observation about the specific impact of the browning process on the energy balance comes from the awareness that mouse strains showing an increased tendency to become obese show decreased capacities for browning of WAT depots compared with those of obesity-resistant strains (45). However, their BAT size, activity, and capacity to recruit classical BAT in response to thermogenic activation is similar (45, 46). This result suggests that the browning process is especially relevant for effective protection against obesity. However, we do not yet have a precise explanation for this phenomenon in the context of whole-body energy balance.

Finally, a key aspect of the systemic physiological impact of brown adipocyte activity is its capacity to substantially clear circulating triglycerides and glucose (42, 47, 48). This will need to be investigated in detail to further understand the potential differences between the 2 types of brown adipocytes in this processes.

Novel Activators of BAT and the Distinct Types of Brown Adipocytes

As mentioned above, the first studies on the hormonal signaling capable of inducing BAT activity/recruitment and the browning of WAT indicated that noradrenergic activation, which mimics the thermogenic effects of cold, could promote both processes to some extent (49, 50). Pharmacological activation of the sympathetic nervous system does not appear to be useful for promoting weight loss via increased BAT activity due to negative side effects (51). Other agents, such as the PPARγ activators, thiazolidinediones, can recruit BAT existing depots and promote the browning of WAT (52–54) but require concomitant noradrenergic activation to result in effective thermogenic induction (55). In recent years, researchers have identified multiple novel nonadrenergic soluble molecules that are capable of inducing BAT activity and/or the browning of WAT (56). Although some of them act indirectly by modulating sympathetic activation and subsequent noradrenergic pathways, several appear to have direct effects on brown adipocytes and/or the browning process. Some of these agents (eg, fibroblast growth factor-21 and the cardiac peptides atrial natriuretic peptide and B-type natriuretic peptide) appear to have common inductive effects on BAT activation and WAT browning (57–59). In contrast, irisin, a recently identified hormonal factor released by muscle, appears to affect the browning process but not classical BAT activation (60). The cyclooxygenase-2–mediated local generation of prostaglandins may also be differentially involved in promoting the browning process (61). Bmp8b, a recently identified novel activator of BAT activity, acts mainly by sensitizing existing BAT to sympathetic signaling (62).

What About Humans?

The current intense interest in BAT biology largely stems from the recent identification of active BAT in adult humans. In humans and other large mammalian species (unlike rodents) BAT was traditionally thought to be restricted to the neonatal and early childhood periods (63, 64). Positron emission tomography (PET)–scanning technologies were recently developed to detect metabolically active sites for oncology diagnosis, based on the uptake of radiolabeled nonmetabolizable glucose derivatives. Side observations (65) and further specific use of PET scans to analyze BAT activity (66–68) showed that active BAT is present in adult humans at discrete anatomical sites, especially in the upper trunk (ie, in cervical, supraclavicular, paravertebral, pericardial, and, to some extent, mediastinal and mesenteric areas). These BAT sites were in agreement with earlier histological reports from human necropsies (69). The adipose depots were subsequently identified as bona fide BAT via microscopic analysis of cell morphology and expression of the BAT-specific marker gene, *UCP1* (67, 70, 71). Moreover, cold exposure enhanced the PET scanning–determined activity of BAT (72), whereas propranolol treatment abrogated BAT activity in humans (73), suggesting that, similar to observations made in rodents, BAT activation occurs mainly through β-adrenergic–mediated signaling. However, recent observations have been somewhat contradictory, perhaps due to differences in the dosing protocol, regarding the extent to which sympathomimetics (eg, ephedrine) are able (74) or unable (75, 76) to induce human BAT activity. Observations from several laboratories indicate that the extent of human BAT activity in patients is inversely associated with obesity, age, and type II diabetes (77), and weight loss after bariatric surgery is associated with increased BAT activity in obese patients (78). It is not known whether high BAT activity is the cause or consequence of a lean phenotype, either in basal conditions or after bariatric surgery. Further research is needed to establish the potential cause and effect relationships underlying these observational associations.

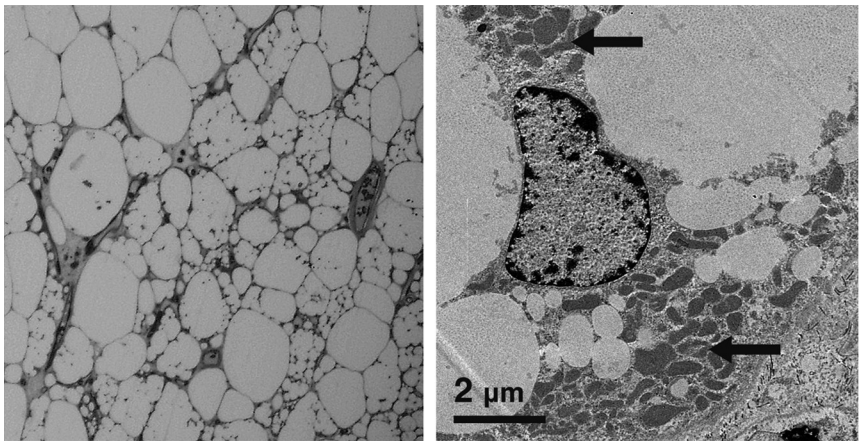

Figure 2. Induction of brown adipocytes in adipose depots of adult humans. Examples of brown adipocytes found in the visceral adipose tissue of a human patient with pheochromocytoma. A, Light microscopy, indicating the presence of multilocular brown adipocytes, interspersed among other unilocular white adipocytes. B, electron microscopy; arrows show the high mitochondrial content characteristic of the brown adipocyte phenotype.

BAT in Humans: Classical? Beige/Brite? Both?

The results from in vitro cell biology studies and direct analyses of BAT biopsy samples from the anatomical areas mentioned above of adults and children led researchers to propose that BAT depots are largely composed of beige/brite cells (26, 39). Studies examining the expression levels of rodent-validated marker genes showed that human BAT coexpresses *UCP1* and marker genes characteristic of beige/brite cells (*CD137*, *TMEM26*, or *TBX1*) rather than those of classical, developmentally programmed, brown adipocytes (28, 40, 41).

The capacity of adult humans to develop the appearance of brown adipocytes in WAT depots was recognized relative early by the study of patients with pheochromocytomas. In the 1980s, researchers observed numerous brown adipocytes (multilocular, highly enriched in mitochondria, and UCP1-expressing) in the visceral fat of patients with pheochromocytomas at sites where only WAT was present in the absence of the disease (79–81) (Figure 2). This finding was attributed to the tumor-mediated release of catecholamines and subsequent local adrenergic activation, which is a trophic factor for BAT development. This phenomenon indicated the capacity of human WAT depots to develop brown adipocytes in response to a permanent stimulus (in this case, of a pathological nature). Molecular marker gene analysis supports the fact that the brown adipocytes in these patients show the beige/brite phenotype (F. Villarroya, A. Frontini, and S. Cinti, unpublished observations).

Based on the above findings, we might ask whether classical brown adipocytes truly exist in humans. As in rodents, we might expect that the BAT present in very early stages of human development could be composed of developmentally programmed, classical, brown adipocytes. A recent report found that when the anatomical areas of BAT in adult humans are analyzed in early neonates, the expressed genes reflect a beige/brite cell lineage (41). However, very recent data indicate that the interscapular BAT of human neonates and some anatomical sites in the neck of adults shows a classical, developmentally programmed, BAT phenotype (82, 83, 84). We recently observed that, in human neonates, the 2 types of brown adipocytes coexist: classical brown adipocytes in the interscapular area and beige/brite in visceral fat (Kopecky, J. and F. Villarroya, unpublished observations).

In summary, we now know that adult humans predominantly have inducible beige/brite, brown adipocytes in their BAT depots, and additional brown adipocytes can develop in adult human WAT under proper stimulation. Therefore, the future development of BAT-focused pharmacological and/or nutritional strategies to increase energy expenditure and fight against obesity seems to be feasible. The human-focused assessment of novel hormonal factors reported to be especially active during the browning process in rodents may be especially promising in this context.

Future Directions

Clearly, multiple basic and biomedical issues need to be addressed in the context of BAT-related research. Further studies will be needed to establish the relative physiological contributions of the distinct BAT-related activation processes to the organism's overall adaptive energy expenditure. In rodents, it seems possible that neither the traditional view (that adaptation to thermogenic activation relies almost entirely on the activation and recruitment of existing BAT) nor an overstated view of the recent research (that inducible brown adipocytes in WAT depots are largely responsible for thermogenic adaptation in response to environmental changes) will turn out to be balanced perspectives. However, even if the energy expenditure associated with the browning process turns out to be moderate (39), the browning process is likely to be physiologically significant. It does not seem biologically reasonable to maintain such complex cell differentiation or transdifferentiation processes for relatively minor consequences in total energy expenditure terms. In any case,

precise experimental designs will be needed to obtain a full qualitative and quantitative understanding of these processes. Other emerging concepts, such as the potential endocrine role of brown adipocytes, are being recognized through various experimental approaches, ranging from direct assessments of the outflow of specific endocrine factors by BAT (eg, fibroblast growth factor-21) (85) to the evidence of metabolic effects of BAT transplantation that may not be explained by intrinsic energy expenditure processes (86, 87). Thus, BAT may significantly influence overall metabolism not only by local energy-burning processes but also by releasing endocrine signals that can act on distant organs and tissues, potentially spurring new considerations with respect to how much BAT is actually needed for its activity to have systemic consequences.

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