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Brown adipose tissue: development, metabolism and beyond

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Abstract

Obesity represents a major risk factor for the development of several of our most common medical conditions, including type 2 diabetes mellitus, dyslipidemia, non-alcoholic fatty liver, cardiovascular disease, and even some cancers. While increased fat mass is the main feature of obesity, not all fat depots are created equal. Adipocytes found in white adipose tissue contain a single large lipid droplet and play well-known roles in energy storage. By contrast, brown adipose tissue is specialized for thermogenic energy expenditure. Due to its significant capacity to dissipate energy and regulate triglyceride and glucose metabolism and its demonstrated presence in adult humans, brown fat could be a potential target for the treatment of obesity and metabolic syndrome. Undoubtedly, fundamental knowledge about the formation of brown fat and regulation of its activity is imperatively needed to make such therapeutics possible. In this review, we integrate the recent advancements on the regulation of brown fat formation and activity by developmental and hormonal signals in relation to its metabolic function.

Introduction

Brown adipose tissue, owing to its inherent capacity to convert excess food energy into thermal energy, has become a focus of biomedical research as a means to counter the spread of obesity [1,2]. This pathological form of overweight is associated with increased risk for numerous metabolic disorders, such as diabetes, cardiovascular complications, atherosclerosis, and other dyslipidemias [3]. Due to its involvement in many aspects of metabolic control, brown adipose tissue (BAT) could potentially help to ameliorate these metabolic perturbations [4]. Excess energy from food is stored in the form of triglycerides within numerous white adipose tissue (WAT) depots throughout the body [3]. This organ possesses a high degree of plasticity, and a wide range of total body fat content, from less than ten percent to more than 50% of total body weight, has been observed [5]. Sedentary life-styles and the general availability of processed, energy-dense food in modern societies have led to an alarming epidemic of metabolic diseases that represents a serious challenge for medical care systems worldwide.

Brown adipocytes are equipped with abundant mitochondria, the so-called powerhouses of the cell, and uniquely express uncoupling protein (UCP)-1. UCP1 dissipates the proton gradient of the inner mitochondrial membrane that is formed as a result of oxidative phosphorylation of nutrients. This process, also known as thermogenesis, generates heat

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instead of adenosine triphosphate (ATP) [6]. In mouse models, the absence of UCP1 has been associated with increased body weight due to loss of brown fat-mediated thermogenesis [7,8]. The transcriptional control of brown adipocyte formation has been analyzed extensively and has been reviewed in great detail elsewhere [9–14]. In brief, the transcription factors peroxisome proliferator-activated receptor gamma (PPAR γ) [15] and the CCAAT/enhancer-binding proteins (specifically C/EBP α / β / δ) [16] have been established as essential parts of the transcriptional cascade that precedes the formation of mature, fully differentiated adipocytes. It is generally accepted that these transcription factors direct the differentiation of both brown and white adipocytes. The transcriptional events specifying a brown adipogenic program are regulated by the nuclear co-activator PPAR γ coactivator 1 α (PGC1 α) [17] and the transcription factor PR domain containing 16 (PRDM16), which physically interacts with C/EBP β [18,19]. Interestingly, recent evidence suggests that modulators of PPAR γ binding activity may specify brown adipogenic lineage transcriptional activity. The NAD-dependent deacetylase SirT1 could promote browning of white fat by deacetylating PPAR γ . This modification is essential for recruitment of PRDM16 to PPAR γ and initiate brown fat specific program [20]. Transcription factor EBF2 (early B-cell factor 2) acts to recruit PPAR γ to genes that are selective for brown adipogenic processes, thereby also affecting the commitment of myogenic and white adipogenic progenitors to the brown adipose lineage [21]. A critical component of these cascades is the activation of *de novo* mitochondrial biogenesis and the expression of functional components that are essential to thermogenesis, such as UCP1.

In recent years, along with the widely publicized re-discovery of metabolically active brown adipose tissue in adult humans [22–25], the common view that brown adipocytes represent a mere variation of the more prevalent white adipocytes has been challenged profoundly. In humans, the presence of BAT throughout life has been well documented for some decades, although an age-related decline of total BAT-mass is evident [26]. Expression of the key brown fat marker, UCP1, was subsequently also observed in human BAT further supporting the notion that human adults are in possession of functional brown fat depots [27]. First evidence for the role of the sympathetic nervous system and its neurotransmitter, norepinephrine (NE), in the process of brown fat-activation in adult humans was gained from patients with pheochromocytoma, a cancer that is accompanied by high levels of circulating NE and excessive formation of brown adipocytes in fat depots in the vicinity but also at distal locations from the tumor [28,29].

A key finding of the more recent renaissance of BAT has been the observation that two developmentally distinct types of brown adipocytes exist in mammals: the classical, or constitutive BAT (cBAT) that arises during embryogenesis [30]; and the beige [31] or brite (brown-in-white) [32] adipose tissue, that is recruited postnatally within WAT [33] or skeletal muscle [34], and which has therefore also been referred to as recruitable BAT (rBAT) [35]. It is now clear that the developmental origin of cBAT is distinct from white fat and rBAT, placing its ancestry closer to skeletal muscle [36]. Furthermore, the formation of rBAT appears to occur through two different pathways, depending on the fat-depot: either transdifferentiation of mature white to brown adipocytes (and vice versa) [37], or through induction of differentiation of brown-adipogenic progenitors [38–40].

These findings highlight an important theme of brown adipocyte biology which is characterized by a high degree of plasticity in brown adipose tissue formation. Secreted and circulating factors act as autocrine, paracrine, and/or endocrine signals to regulate these processes on the levels of (brown) adipogenic lineage specification during embryogenesis, the differentiation of brown adipocytes, and the regulation of the thermogenic activity of mature brown adipocytes (summarized in Figure 1). The aim of this review is to summarize the current state of the field, focusing on the brown adipogenic effects of secreted

physiological signals that act locally or in circulation, and the role of BAT in systemic energy metabolism.

Embryonic development of brown adipose tissue

It is generally accepted that adipocytes, both brown and white, arise from progenitor cells of the embryonic mesoderm, the only exception being the minor fat depots of the skull, which arise from stem cells of the ectodermal neural crest [41]. Adipocytes thus share a developmental origin with other mesodermal tissues, such as skeletal muscle, bone, and other connective tissues [3,42]. In the mouse, clearly distinguishable pads of cBAT are first recognizable on or after day 15.5 of embryonic development (E15.5) which then rapidly expand until birth (Figure 2) [43]. During this stage, developing cBAT appears to arise from a highly proliferative mass of fibroblast-like cells in the interscapular region [44,45]. Interestingly, lipid accumulation can only be observed in a small subset of the brown adipocytes during the early embryonic stages of cBAT-formation, but increases markedly after birth (Figure 2). Since definite markers that label progenitors exclusively giving rise to BAT, but not WAT or other mesodermal tissues such as skeletal muscle, are currently lacking, most research in this area has so far been confined to analysis of generally adipogenic progenitors that can give rise to brown and white fat, regardless of their developmental origin. Accordingly, it is now established that adipogenic progenitor cells in brown and white fat depots are located within the mural cell compartment, also known as pericytes, anatomically located within the vasculature of the adipose tissue [46,47]. Whether this is also true for adipogenic cells within other tissues remains to be determined, although they appear to share surface marker characteristics with adipose tissue-resident progenitors, such as stem cell antigen (Sca)1, CD29, CD34, and platelet-derived growth factor (PDGFR) α [39,48–50]. Despite their endothelium-associated location [46,47], their developmental ancestry from endothelial progenitors is still not entirely resolved, as lineage tracing methods have yielded inconclusive data, which may be due to differences in the methodological approaches [47,51].

Transcriptional control of adipogenic progenitor cell commitment has also been linked to the zinc finger proteins (Zfp) 423 and Zfp521. Zfp423 acts as a positive regulator of progenitor cell commitment to an adipogenic lineage, at least in part by regulating expression of the master regulator of adipogenesis, peroxisome proliferator-activated receptor (PPAR) γ , and by amplifying the adipogenic effects of the BMP signaling pathway [52]. Interestingly, Zfp423 acts as a definite marker for committed adipogenic cells isolated from different adipose tissue depots that reside within the adipose tissue vasculature, further corroborating the importance of this location as the niche for adipogenic progenitor cells [53]. Zfp521 has recently been identified as a negative regulator of adipogenesis that appears to act upstream of its paralog, Zfp423 [54]. At the molecular level, Zfp521 inhibits commitment to the adipogenic lineage by binding and sequestering the pro-adipogenic factor Ebf1 [55].

Among the two distinct types of brown fat, cBAT has been studied in greater detail regarding its developmental ancestry. These studies collectively indicate a close relation of cBAT to skeletal muscle rather than white adipose tissue [11,30,32,56–58]. Accordingly, it was first observed that progenitors isolated from cBAT, but not WAT, express a myogenic gene expression signature [58]. Specifically, cBAT progenitors express high mRNA-levels of myogenic transcription factors such as *myf5*, *myoD* and *myogenin*. Expression levels of these genes decline as the cells differentiate into mature brown adipocytes. Subsequently, it was demonstrated that the transcription factor PRDM16 is a key regulator of a developmental switch between the myogenic and brown adipogenic cell fate lineages [18,30]. Overexpression of PRDM16 in a myoblast cell line led to a change of cell fate and preferential differentiation into UCP1-expressing brown adipocytes, whereas RNAi-

mediated down-regulation of PRDM16 in brown pre-adipocytes resulted in the formation of mature myotubes. Similarly, formation of cBAT was attenuated in PRDM16-deficient mice, concomitant with reduced expression of common brown fat markers and increased expression of myogenic transcription factors [30]. How this mechanism is involved in the early cell fate decision processes of embryonic development of cBAT remains to be investigated in molecular detail. Interestingly, this study also demonstrated the presence of a common lineage of progenitor cells that expresses the myogenic transcription factor Myf5 and that can give rise to interscapular cBAT as well as skeletal muscle [30]. This notion is further supported by an independent study where Pax7-driven lineage tracing was used to demonstrate a common progenitor cell population in early embryonic stages that diverges into two distinct lineages, i.e. uniquely myogenic or brown adipogenic lineages, around E11.5 [57]. This is also consistent with earlier reports that are of somewhat serendipitous nature, generally referring to an unspecified form of adipose tissue rather than distinguishing between brown or white fat in any detail: In a mouse model lacking *myogenin*, a general defect in skeletal muscle formation was observed. Interestingly, the knockout mice displayed increased amounts of adipose tissue in the interscapular region [59]. Similarly, in animals with a genetic deletion of both *myf5* and *myoD*, excessive amounts of adipose tissue in place of skeletal muscle were observed [60]. While the underlying mechanisms remain unclear, the inability of the common myo-adipogenic progenitor to undergo myogenic differentiation may promote those progenitor cells to follow a brown adipogenic lineage.

More recently, it was reported that some depots of white adipose tissue partially derive from the Myf5⁺ lineage. This study shows that progenitors derived from the interscapular and retroperitoneal white fat depots contain significant proportions (around 50%) of white adipocytes that derive from the Myf5⁺ lineage while other, more classical depots of white fat, such as inguinal/subcutaneous and epididymal WAT, do not contain adipogenic cells of the Myf5⁺ lineage [61]. This observation indicates a high degree of heterogeneity among the different populations of adipogenic cells and strongly suggests the presence of yet-to-be-identified local signals that act to specify individual cell fate. In rodents, the major depots of rBAT-containing white adipose tissue (WAT) develop after birth [3]. Initial studies have established that a distinct developmental origin of these two types of fat is likely, as postnatal white adipocytes do not arise from a brown adipocyte-like progenitor [62]. However, not much is known about developmental distinction between rBAT and the surrounding white fat or skeletal muscle. While cBAT arises from the Myf5-expressing progenitors, it appears clear that muscle-resident rBAT derives from a Myf5⁻ lineage [30,39,48]. Despite their anatomical localization within skeletal muscle, these cells are clearly non-myogenic [48,50,63], possess a high adipogenic potential and require inductive signals to differentiate into brown adipocytes [39]. Less is known about the existence of progenitors for rBAT within WAT. Transdifferentiation of a phenotypically indistinguishable white adipocyte is likely to be a potential mechanism [37,64–66], although some evidence indicates that differentiation of progenitor cells into rBAT following inductive cues occurs [39]. Interestingly, depot-specific differences have been reported on whether transdifferentiation or direct proliferative expansion and differentiation of progenitors into rBAT takes place [38]. Given the high degree of plasticity within adipose tissue depots, it seems likely that both processes may exist in parallel to some extent. Importantly, both processes are initiated by inductive cues either through central pathways or from locally secreted or endocrine factors.

Recent evidence has established the intriguing idea that a subset of white adipocytes may have brown adipogenic potential, suggesting that distinct subpopulations of beige/brite adipocytes and even pre-adipocytes occur in white adipose tissues [40]. A recent study by Wu et al. analyzed clonal adipogenic progenitor cell lines and demonstrated that a subpopulation of cells possesses the potential to differentiate into beige adipocytes. The

beige cells express low level of UCP1, but it can be greatly increased by forskolin. Whether this process is based on lineage determination steps that occur during embryogenesis remains to be determined. The authors further identify the cell surface markers CD137 and TMEM26, among other markers that may help to define and prospectively isolate this subpopulation from different adipose tissues.

Morphogens: Developmental fate regulation of brown adipose progenitors

Morphogens regulate the developmental patterning during embryogenesis. Concentration gradients of certain morphogens and other secreted signals may also be involved in the lineage commitment of brown adipogenic progenitors during various developmental stages [67]. Accordingly, morphogenic signals of (1) the Wnt- (named after the Wingless and INT proteins in *Drosophila*), (2) the bone morphogenetic protein- (BMP), (3) the fibroblast growth factor- (FGF), and (4) the Hedgehog-signaling pathways have recently been implicated in the formation of brown adipocytes and may do so at early embryonic stages where multipotent populations of mesodermal progenitors occur (Figure 1) [68–73]. During the early stages of embryogenesis, a subset of cells within the central dermomyotome expresses the homeobox transcription factor engrailed-1 (En1), and their developmental fate is regulated by activation of Wnt-signaling: Using developmental lineage tracing, it was shown that the cells of the early embryonic dermomyotome are En1⁺, and give rise to cBAT in addition to skeletal muscle and dermis, indicating that a tripotent population of En1⁺ progenitor exists during early stages of embryogenesis. In addition, activation of the Wnt-transducer, β -catenin, was required to induce expression of dermo-1 and subsequent dermal differentiation of a subset of En1⁺ cells located close to the surface ectoderm [68]. Whether the Wnt-signaling cascade also plays a functional role in the fate specification of the residual En1⁺-lineage into myogenic versus brown adipogenic cells in the developing embryo is not known. However, the En1 study suggests that activation of Wnt-signaling may inhibit the development of myogenic progenitors, thereby also reducing the frequency of brown adipogenic progenitors. This is also consistent with the notion that Wnt-signaling negatively affects (brown) adipogenesis postnatally [70,74].

Emerging evidence has implicated BMP-signaling in the control of brown (and white) adipogenesis [67,73,75]. The ligands of this signaling pathway, the so called BMPs, were initially identified due to their ability to induce bone formation [76,77]. Since then, it has become clear that BMPs function as morphogens that direct the development of multiple organ systems during embryogenesis [78]. While previous findings have implicated a general role of BMPs in adipogenic processes, their role in early embryonic lineage commitment of different adipogenic cell populations is less clear. Several studies have demonstrated that BMP2 and BMP4 are involved in the commitment of multipotent progenitors to the white adipogenic lineage [75,79–81]. However, a recent study has also implicated BMP4 in the browning of mature white adipocytes [82], suggesting that individual BMPs may have distinct windows of activity to regulate brown adipocyte physiology. Importantly, it was recognized that BMP7 induces commitment of multipotent mesenchymal progenitor cells to the brown adipogenic lineage and drives differentiation of brown pre-adipocytes as compared to other BMPs that are rather white adipogenic [73]. Animals completely lacking BMP7 die shortly after birth due to kidney failure [83]. Interestingly, one of the major defects observed in this mouse model is a marked reduction of cBAT mass and function, indicating that BMP7 is a critical promoter of embryonic cBAT development [73]. The BMP-signaling cascade, and particularly its downstream signaling through the type 1A BMP-receptor, BMPRI1A, is a critical regulator of cBAT-development. Ablation of BMPRI1A in the Myf5⁺ lineage resulted in a marked paucity of cBAT due to impaired expansion of brown adipogenic progenitors during embryonic development, further highlighting the critical importance of BMP-signaling for brown fat development

[45]. Further studies are needed to establish the molecular mechanisms involved in the BMP-mediated regulation of progenitor cell commitment to the cBAT-lineage.

Like BMPs, other members of the TGF β have also been implicated in the regulation of BAT. Circulating levels of TGF β , for instance, correlated with obesity, and deletion of Smad3 promoted the browning of WAT, suggesting that some signaling of the TGF β -family may negatively affect brown adipocyte development [84]. Emerging evidence further supports a differential role for some members of the TGF β -family. Administration of a soluble form of the type IIB activin receptor, where the extracellular domain of this receptor was coupled to a human IgG Fc-domain (ActRIIB-Fc), rescued the negative effects of high fat diet on cBAT and additionally resulted in marked recruitment of rBAT in visceral WAT [85]. Mechanistically, the authors demonstrate that high-affinity ligands of ActRIIB, the growth and differentiation factors (GDF)-3, -8, and -11, as well as activins A and B, are negative regulators of brown adipogenesis and are depleted following administration of ActRIIB-Fc. Low-affinity ligands, on the other hand, such as BMP7, GDF5, and GDF7, were pro-brown adipogenic and were less affected by ActRIIB, thus permitting the emergence of rBAT within WAT by promoting expression of PGC1 α and UCP1 [85]. Taken together, these data support the notion that members of the TGF β -superfamily have very distinct regulatory effects on brown adipogenesis and modulation of the profile of these circulating ligands may prove a feasible tool to affect brown adipogenesis.

Among the fibroblast growth factor proteins, fibroblast growth factors 16 (FGF16) and 19 (FGF19) have been implicated in the regulation of embryonic cBAT-development. It was found that FGF16 is predominantly expressed in developing cBAT in rat embryo, where a high proliferation rate was observed that was regulated by FGF16, acting through its receptor FGF receptor 4 (FGFR4) [69]. Interestingly, cold exposure decreased FGF16 expression in adult cBAT, suggesting that the role of FGF16 in brown adipogenesis is restricted to embryonic developmental processes. Similarly, increased expression of FGF19 resulted in increased energy expenditure and decreased body fat content that could be related to increased cBAT-mass, suggesting that this FGF might also be involved in the development of cBAT [72]. Of note, FGF19, like FGF21, can also activate thermogenic activity of adult cBAT [86], an effect that will be discussed in more detail later in this review.

Lastly, morphogens of the hedgehog signaling pathway have been linked to white versus brown adipocyte lineage determination. While activation of the hedgehog signaling cascade leads to activation of brown adipose metabolism [87], a genome-wide screening connects hedgehog-activity to the lineage determination of the brown versus white adipogenic lineages [71]. It remains to be determined whether this could also affect the progenitor composition in white adipose tissue and whether brown adipogenic processes can be altered by modulation of hedgehog signaling. Many other secreted proteins and circulating factors are likely involved in the embryonic processes of cBAT formation. Oftentimes, these signaling proteins are involved in several stages of brown adipogenesis, as is the case for BMP7, and a clear distinction between these processes is not always possible. External signals that regulate the differentiation/maturation of brown adipocytes will be discussed in more detail later in this review. In summary, much remains to be determined with regards to the mechanisms that determine an adipogenic fate for mesenchymal progenitor cells. Additionally, the identity of putative rBAT progenitor cells in WAT and skeletal muscle warrants further investigation, as this type of brown adipose tissue could well be an important contributor of brown adipocytes in adult humans.

Regulation of BAT-activity: Crosstalk with other organs by secreted factors

Numerous studies have implicated increased formation and/or activity of both types of brown adipose tissue in increased resistance to diet-induced obesity. While activation of cBAT-activity may be one mechanism to achieve this goal, it is the inducible formation of rBAT in white adipose tissue that has garnered extensive scientific interest in recent years. Genetic manipulation of the transcription factors that control brown adipocyte differentiation is of only limited relevance for the treatment of human obesity. The discovery and analysis of brown-adipogenic signals, either present in the circulation or acting as locally secreted paracrine factors, has therefore become a key aspect of brown fat research. The identification of such factors is of particular interest as the first generation of agents to target BAT as a treatment for obesity, the $\beta 3$ adrenergic receptor (ADRB3)-agonists, have not fulfilled their initial promise in clinical trials [88]. This section will therefore address recent advances and discoveries of novel physiological factors that may affect brown fat formation and activity, their underlying molecular mechanisms, and their physiological sources. An emerging theme is the notion that signals coming from multiple organs and tissue types are integrated to control the total mass and thermogenic activity of BAT (summarized in Figure 3). Each organ produces a specific signal (or a set of signals) that contributes to the overall regulation of BAT-activity. A greater understanding of the crosstalk between these individual sources of brown-adipogenic and/or thermogenic factors will help develop new therapeutic approaches that may prove effective for treating human obesity.

Neuronal control of BAT, both on the central and the peripheral levels, has been well-recognized as the key mechanism regulating the thermogenic activity of mature BAT. The sympathetic nervous system (SNS) is a critical controller of thermogenesis by acting on ADRB3, the adrenergic receptor subtype most abundantly expressed in adipocytes [89–91]. By SNS-mediated activation, the heat output of cBAT can be quickly adapted in response to rapid changes in ambient temperature [92,93]. At the same time, sympathetic input to white adipose tissue, for instance by cold exposure or by chemical agonism of ADRB3, results in the browning of white adipose tissue [37,94–97], a process that apparently replaces the more short term measure of cBAT-activation [45]. Several neuropeptides and hormones have been linked to the thermogenic activity of BAT. Brain-derived neurotrophic factor (BDNF) has recently been implicated in hypothalamic control of energy expenditure through BAT. Administration of BDNF in different areas of the hypothalamus, namely the ventromedial and the paraventricular nuclei, resulted in increased energy expenditure that could at least in part be attributed to increased BAT-activity [98,99]. More recently, it was also discovered that BDNF acts on the browning of white adipose tissue. Animals housed in a so called ‘enriched environment’ that provides stimulatory cues to different brain regions displayed greater browning of white fat and resistance to diet-induced obesity. This effect was dependent on BDNF [100]. Interestingly, and considering the specific role of BMP signaling in the developmental aspects of brown adipogenesis [39,67,73,101], it was recently recognized that BMPs may also act on a central level to regulate energy homeostasis. In this line, we were able to demonstrate that systemic administration of BMP7 resulted in activation of systemic energy expenditure and weight loss [102]. Importantly, this involved the central nervous system (CNS): Intracerebroventricular administration of BMP7 resulted in an mTOR-dependent inhibition of food intake, altogether establishing the importance of BMP7 for systemic energy balance. Likewise, hypothalamic BMP8b acts as a positive regulator of thermogenesis in BAT. Expression of BMP8b was responsive to metabolic cues such as fasting/re-feeding regimens, that down- and up-regulated BMP8b mRNA levels, respectively. Loss of BMP8b markedly reduced activation of AMP-activated protein kinase (AMPK-) signaling, thereby also reducing thermogenesis and overall metabolic rate. Central administration of BMP8b had the opposite effects, resulting in increased sympathetic influx

to and activation of cBAT, altogether providing compelling evidence that in addition to BMP7, BMP8b also affects the thermogenic capacity of brown adipose tissue [103]. In the same line, orexin regulates thermogenesis in BAT by inducing sympathetic activation of cBAT [104–107]. Importantly, it appears that orexin may also act in the periphery [104]: Loss of this neuropeptide resulted in decreased proliferation of brown pre-adipocytes and enhanced expression of the brown adipogenic transcription factors PRDM16 [19] and PGC1 α [17]. Other neuropeptides and hormones play an important role in the regulation of brown fat activity. Their specific activities and roles in different brain areas have been reviewed in more detail elsewhere [93,108].

As summarized in figure 3, several organs are known to secrete factors that can be linked to brown fat formation and thermogenic activity. Although not much is known about the molecular details, BAT itself is a source of several secreted signals, such as BMP7 (Y.H.T. and T.J.S., unpublished observation), BMP8b [103], FGF21 [109], and interleukin (IL-)6 [110], whose effects on brown adipocyte-physiology will be discussed later in this review. While BAT is also a source of typical adipokines, such as leptin or adiponectin, the contribution from different types of brown adipocytes to the systemic circulating levels of these proteins is unclear, but likely much smaller than from the more prevalent WAT.

One of the best-established mechanisms to control brown fat activity is through thyroid hormones, most notably T3, which is the most active thyroid hormone. Its precursor, T4, is taken up by brown adipocytes and converted into the active T3 by type 2 deiodinase (DIO2) following sympathetic stimulation [111]. Production and release of T3 is under tight control by the CNS, specifically the so-called hypothalamic-pituitary-thyroid axis, and a close synergistic interaction of T3 with adrenergic activation of brown fat has been closely analyzed [112]. Along with T3, the pancreatic hormone insulin is a major regulator of brown adipocyte formation and function. The role of the insulin signaling cascade in brown adipogenesis is well established and loss of the downstream signaling components has detrimental effects on brown adipose tissue function [113] that can also lead to a diabetic phenotype [114]. While T3 and insulin are classical and well-known mediators of brown adipogenesis, they have pleiotropic effects on multiple organ systems in the body. Therefore, the identification of novel circulating factors that act more specifically on brown fat is highly desirable.

FGF21 is produced and secreted by the liver, and also by cBAT after sympathetic activation [109,115,116]. Interestingly, this circulating factor is involved in multiple metabolic processes and appears to have beneficial effects on whole body metabolic balance [117,118]. Accordingly, it was also found that FGF21, by acting through the transcription factor PGC1 α , induces browning of white fat in an autocrine/paracrine manner [119]. Taken together, these observations indicate that FGF21 derived from cBAT may also act as a brown adipose-derived adipokine that regulates the recruitment of brown adipocytes in white fat as an adaptive mechanism to prolonged metabolic stressed, such as cold exposure. Moreover, FGF21 secreted from brown adipocytes may exert direct metabolic effects, thereby providing a novel mode of communication of BAT with other peripheral tissues that may not necessarily involve the CNS. Cardiac natriuretic peptides (NPs), in both the atrial (ANP) and ventricular (BNP) forms, can induce browning of WAT, as signified by elevated expression of BAT-markers, mitochondrial biogenesis and oxygen consumption in cultured adipocytes treated with either peptide. In mice, cold exposure resulted in increased circulating levels of NPs, and direct infusion of BNP induced energy expenditure of brown and white fat. Loss of the NP clearance receptor, NPRC, similarly resulted in decreased fat pad size of cBAT and WAT in mice, while expression of thermogenic genes was increased, suggesting that fat pad weight loss was due to increased oxidation of stored lipids [120]. Secreted from skeletal muscle, the myokine irisin has been identified as an important

mediator of white fat browning [121]. Interestingly, irisin secretion was induced by exercise as well as overexpression of PGC1 α , which mimics the physiological effects of exercise. Administration of irisin protected against diet-induced obesity and the development of glucose intolerance, altogether establishing the myokine irisin as a skeletal muscle-derived contributor to systemic control of energy expenditure and thermoregulation. An autocrine regulatory mechanism of browning was recently identified in white fat depots. Specifically, two studies identified cyclooxygenase-2 (COX2) as a critical regulator of this process within white fat. COX2 is a key enzyme in prostaglandin biosynthesis, and accordingly, treatment of mice of prostaglandins resulted in increased formation of rBAT [122,123].

In summary, emerging evidence suggests that multiple cell types produce a wide variety of different signals that act as positive regulators of thermoregulation. These involve central as well as peripheral mechanisms and are integrated by brown adipocytes to control not only temperature homeostasis, but also metabolic regulation. In this line, we were recently able to demonstrate that different depots of brown adipocytes are subject to systemically active crosstalk-mechanisms that control overall thermoregulation. Specifically, loss of cBAT by genetic ablation of BMPR1A in the Myf5⁺ lineage or surgical denervation resulted in increased browning of white fat. Thus, these findings highlight the existence of a sophisticated regulatory mechanism in the body that involves cBAT-to-brain and brain-to-WAT circuits to control BAT-activity [45].

The Role of BAT-mediated thermogenesis in anti-obesity

Although the main function of BAT is thermoregulation, an important aspect is the associated role as a negative regulator of obesity. Two major signals for non-shivering thermogenesis by BAT have been described: cold exposure and adaptive/facultative diet-induced thermogenesis (DIT), the latter representing the portion of DIT that is not related to the essential energy consuming processes of food metabolism [124,125]. The mechanisms regulating this type of DIT in humans still warrant further investigation, but evidence suggests that BAT-dependent DIT may act through enhanced adrenergic activation of BAT and depends on nutrient type [126–128]. In rodents, it was initially recognized that the major mediator of non-shivering thermogenesis is NE, which is secreted from the SNS upon cold-exposure [129,130], thereby activating the adrenergic system. Later on, this discovery led to the development of specific agonists of the major NE-receptor in adipose tissues, ADRB3 [131,132]. Unfortunately, after initially promising studies performed in rodent models, it was soon recognized that this effect was negligible in adult humans, which was either due to reduced ligand-binding ability or bioavailability to human ADRB3 [133,134]. It is generally accepted that the thermogenic activity of UCP1 is absolutely required for normal brown fat function. Surprisingly, studies conducted in mouse models with partial or complete UCP1-deficiency have yielded somewhat inconclusive results: In some models, deletion of UCP1 is accompanied not only by increased cold-sensitivity, but also obesity [135,136]. Other studies, however, were able to corroborate cold-sensitivity, but found no effect of UCP1-depletion on obesity [7,135,137]. This surprising lack of obesity in UCP1-deficient mice has been explained by a chronic activation of metabolism in mice housed under standard conditions: At ambient temperatures of approximately 21 °C, mice are under thermal stress, meaning that their basic metabolic rate is elevated in order to maintain normal body temperatures. This effect alone is sufficient to correct for the obesogenic effect of UCP1-ablation. In contrast, when these mice were maintained at thermoneutrality, i.e. an ambient temperature where increased basic metabolic rate is no longer required to maintain body temperature, loss of UCP1 resulted in a striking obesity phenotype [7]. These findings suggest that regulation of systemic thermogenesis is not exclusively controlled by the heat-generating activity of BAT. For instance, it was described that SNS-controlled non-shivering thermogenesis by skeletal muscle may play a significant

role in overall thermoregulation [138]. More importantly, the systemic control of these processes is ensured by a tight web of control mechanisms that involves different tissues types and that are likely integrated by central mechanisms involving the SNS [45]. In summary, the role of BAT-mediated thermogenesis in counteracting obesity appears well established at least in rodents. The discovery that systemic compensatory mechanisms are in place that regulate the overall mass of metabolically active BAT and its metabolic output warrants further investigation to determine whether pharmacological approaches maybe used to affect the delicate balance of this system.

The role of BAT in metabolic disease

While the natural focus of brown fat research lies on its capabilities as an anti-obesity target, emerging evidence suggests that brown adipocytes may be critical regulators of general metabolic homeostasis, affecting a number of metabolic complications. Specifically, it has been well documented that some mouse strains are more sensitive to diet-induced obesity and insulin resistance. This has at least in part been attributed to elevated recruitment of brown adipocytes in skeletal muscle and white fat [33,34]. Interestingly, the determination of rBAT in different strains of mice is determined by genetic components [139], and it is currently unclear to what extent circulating and/or locally secreted factors are involved in the strain-specific differences. A study from our lab suggests that cell-autonomous properties play an important role: Pre-adipocytes isolated from muscle and white fat of obesity-prone mice, specifically the mouse strain C57Bl/6, had lower brown adipogenic potential compared to cells isolated from the relatively obesity-resistant strains, 129-S1 and A/J [39], although these observations do not rule out autocrine mechanisms.

As discussed above, loss of insulin signaling in brown adipose tissue resulted in impaired insulin secretion from pancreatic beta cells, thereby affecting glucose homeostasis [114]. These findings strongly suggest the existence of a yet unidentified mode of interaction between brown adipocytes and insulin-producing pancreatic β -cells that remains to be characterized. These findings were extended in a model of increased BAT-mass by transplantation of cBAT-pads into the visceral cavity of mice. This resulted in a marked improvement of glucose homeostasis, that is, surprisingly, dependent on the cytokine interleukin (IL)-6 and secretion of FGF21 from the transplanted fat pad [110], whose beneficial metabolic properties are well established [118]. When cBAT was transplanted from IL6 $^{-/-}$ mice, the beneficial effect on glucose metabolism were no longer present. An additional function of brown adipose tissue may be the regulation of lipoprotein homeostasis, and specifically the clearance of triglyceride-rich lipoproteins (TRLs). A recent study demonstrates that after cold exposure, BAT is involved in the rapid clearance of TRLs that are used as fuel for thermogenesis. This process was dependent on the activities of lipoprotein lipase (LPL) and the transmembrane receptor, CD36, which is involved in fatty acid uptake from the blood stream [140]. Taken together, these findings may have uncovered an additional mechanism by which brown adipose tissue may be used to improve metabolic health apart from its well-known function in obesity control.

Human brown fat

Brown fat was once thought to have a functional role only in rodents and human infants, however, recent studies using 18-fluoro-labeled 2-deoxy-glucose positron emission tomography (^{18}F FDG-PET) in combination with computed tomography (CT) clearly demonstrated the presence of metabolically active brown fat in adult humans [22–25,141–144]. These findings rekindled research on human brown fat. Like rodent brown fat, human BAT also has extensive neuronal and vascular connections and abundant mitochondria. Interestingly, human brown adipose tissue is an amalgam of brown and white adipocytes. At

the physiological level, human brown fat displays differential rates of glucose uptake in response to cold versus insulin stimulation [145]. Cold-activated human brown fat takes up more glucose per gram of tissue than insulin-stimulated skeletal muscle, suggesting that human brown fat could contribute to glucose homeostasis. Regarding detection of human brown fat, while ^{18}F FDG-PET/CT has been proven to be a useful tool for monitoring BAT in humans, it still has limitations in terms of sensitivity and reflection of BAT functionality. In particular, increased FDG uptake may not be a direct measurement of brown fat activity. Thus, several imaging methods have been utilized to monitor brown fat mass and activity in humans. Using dynamic oxygen-15 (^{15}O) PET imaging, oxidative metabolism of brown fat was found to be highly variable in individuals with detectable BAT determined by FDG-PET [146]. PET studies using ^{11}C -acetate and ^{18}F -fluoro-thiaheptadecanoic acid (a fatty acid tracer) have been applied in human volunteers to determine BAT oxidative capacity and nonesterified fatty acid uptake, respectively, and these studies confirmed that brown fat serves as an important tissue for non-shivering thermogenesis in humans [147]. In addition to PET, chemical-shift water-fat magnetic resonance imaging (MRI) has been used to characterize human brown fat due to its unique cellular structure, fat content and vascularization [148].

More recently, a new focus in human brown fat research is to determine if humans possess both classical and recruited types of brown fat cells as seen in rodents. Similar to rodents, human infants indeed retain classical brown adipocytes in the interscapular region[149]], but these brown fat cells disappeared with age [26,150]. Using molecular markers originally identified in rodent cells, supraclavicular adipose depots in adult humans and some children are found to contain a significant portion of recruited beige cells [40,151]. Interestingly, deep neck fat from adult humans displays molecular signatures of classical brown fat and possesses high levels of oxygen consumption rate [152]. These findings suggest that the properties of human fat vary from location to location, hence detailed mapping of human brown fat from different anatomical sites in conjunction with functional characterization are needed to better understand human brown fat.

Concluding remarks

Certainly, increasing energy expenditure by targeting brown fat has become an attractive way to combat obesity. New knowledge gained from the both the human and rodent systems has advanced our understanding on brown adipose biology and provided important insight to the role of brown fat in metabolic regulation at physiological and pathological conditions. These efforts have opened up exciting new avenues for developing new anti-obesity therapies that may also help to treat obesity-related complications. However, while the findings up to date suggest promising opportunities for developing anti-obesity treatments through increasing brown fat-mediated energy expenditure, the safety issue needs to be considered. In particular, the cardiovascular effect of any potential drug, especially in subjects with chronic cardiovascular diseases, needs to be closely evaluated.

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Figure 1. Distinct stages of brown adipocyte formation are regulated by instructive signals acting through endocrine, paracrine or autocrine mechanisms

Lineage chart summarizing the external signals that regulate the formation of constitutive and recruitable brown adipocytes during distinct stages of their formation: (1) Stem/progenitor cell lineage determination during embryonic development (green background; 'Embryogenesis'); (2) Brown adipocyte differentiation from a committed progenitor cell into either constitutive or recruited brown adipocytes (violet background; 'postnatal Maturation'); (3) thermogenic activity of mature, i.e. fully differentiated brown adipocytes (orange background; 'Activity'). A common mesodermal stem cell with multi-lineage potential gives rise to fibroblast-like brown adipogenic progenitors of distinct lineages: Constitutive brown adipocytes of the common myo-adipogenic lineage, that also gives rise

to skeletal muscle, and a predominantly adipogenic progenitor, that can generate mature white and recruited brown/beige/brite adipocytes residing within white adipose tissue depots. Distinct molecular markers specific to the individual cell types are shown in orange. Transcriptional regulators are shown in blue. Secreted signals that are involved in lineage determination or brown adipogenic maturation processes are shown in red, summarizing signals that may act through endocrine pathways, i.e. produced and acting in distal locations such as the CNS, and signals that may act as locally secreted paracrine or autocrine factors.

Figure 2. Brown adipose tissue emerges during late stages of embryogenesis

a, b, c, d, Hematoxylin and eosin (H&E) staining of transversal sections of the interscapular areas of mouse embryos at different embryonic stages. Arrows indicate morphologically distinguishable cBAT (**a**: embryonic day (E)14.5; **b**: E15.5; **c**: E16.5; **d**: postnatal day (P)1); original magnification: 100x; cBAT-pads indicated by arrows; § indicates spinal column). **e**, Same section of E14.5 (panel **a**) at greater magnification shows that only developing skeletal muscle, but not cBAT, can be observed at this stage (original magnification: 400x). **f, g, h**, Perilipin-immunohistochemistry (IHC), a marker for lipid droplets in maturing adipocytes, was performed in the same embryonic stages where cBAT was first observed (**f**: E15.5; **g**: E16.5; **h**: P1; original magnification: 400x). Perilipin-positive developing brown adipocytes are shown in brown, examples indicated by arrowheads. All stainings were performed as described before and using the same materials [39].

Figure 3. Brown adipocyte physiology is controlled by crosstalk mechanisms through secreted factors from multiple anatomical sites

Different organs produce and secrete instructive signals that are integrated within anatomical locations of brown adipocytes to regulate the development, differentiation, and possibly the thermogenic activity of this type of cell, thereby affecting thermoregulation and systemic energy metabolism. Figure was produced using Servier Medical Art (<http://www.servier.com>).