

# Brown adipose tissue

## Recent insights into development, metabolic function and therapeutic potential

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Obesity is currently a global pandemic and is associated with increased mortality and co-morbidities including many metabolic diseases. Obesity is characterized by an increase in adipose mass due to increased energy intake, decreased energy expenditure, or both. While white adipose tissue is specialized for energy storage, brown adipose tissue has a high concentration of mitochondria and uniquely expresses uncoupling protein 1, enabling it to be specialized for energy expenditure and thermogenesis. Although brown fat was once considered only necessary in babies, recent morphological and imaging studies have provided evidence that, contrary to prior belief, this tissue is present and active in adult humans. In recent years, the topic of brown adipose tissue has been reinvigorated with many new studies regarding brown adipose tissue differentiation, function and therapeutic promise. This review summarizes the recent advances, discusses the emerging questions and offers perspective on the potential therapeutic applications targeting this tissue.

Obesity has now reached pandemic levels globally<sup>1</sup> and while the etiology and physiology are complex, the majority of weight gain in obese humans is characterized by an increase in adipose mass, and adipose tissue hypertrophy and lipid overload is believed to eventually precipitate other morbidities such as cardiovascular disease and type 2 diabetes.<sup>2</sup> In contrast to white adipose tissue (WAT), which not only stores energy in the form of triglycerides but also is recognized as an important endocrine and immune organ, brown adipose tissue (BAT) is specialized for energy expenditure. While WAT structure is characterized by a single, large lipid droplet and few mitochondria, BAT contains several small lipid droplets (multilocular), many mitochondria, and uniquely expresses uncoupling protein 1 (UCP1).<sup>3–8</sup> UCP1 is localized to the inner mitochondrial membrane and acts to uncouple oxidative phosphorylation from ATP production, thereby releasing energy as heat (termed thermogenesis). BAT plays a pivotal role in adaptive thermogenesis, a physiological process during which energy is dissipated in response to environmental changes, such as cold temperature and diet.<sup>9,10</sup> BAT is also

able to utilize both glucose and fatty acids in mitochondrial metabolism, however the thermogenic capacity of BAT is enormous. In humans, it has been estimated that as little as 50 g of BAT (less than 0.1% of body weight) could utilize up to 20% of basal caloric needs if maximally stimulated.<sup>11</sup> This energy expending role makes BAT an important potential tool for combating the complications of human obesity.

BAT is important for temperature regulation in small mammals. In humans, it is present in abundant quantity in newborns,<sup>12</sup> but it was traditionally believed that BAT was nonexistent or nonfunctional in adult humans. However, this dogma was recently reversed by evidence from nuclear medicine,<sup>13–20</sup> which showed active BAT in adult humans. Since then, there has been a flurry of new data surrounding BAT function and therapeutic potential.<sup>21–23</sup> The goal of this review is to summarize and offer perspective on these recent advancements in knowledge about BAT, from studies conducted in humans to rodent or in vitro models, with a special focus on recently published papers.

### The Importance of BAT with Cold-Exposure and for Seasonal Hibernating Mammals

The physiological importance of BAT, previously referred to as the ‘hibernating gland,’ is most strikingly observed in seasonal mammals, which require BAT’s thermogenic properties to maintain body temperature during periods of hibernation or torpor, and to mediate periods of arousal and re-warming from these decreased metabolic states. Hibernation is a period of heterothermia, where body temperature may drop from 35–37°C to 0°C, accompanied by a period of metabolic reduction.<sup>24</sup> The onset of hibernation is often triggered by shortening daylight cues reaching the brain, in conjunction with the brain’s own circadian rhythms. Torpor, on the other hand, is a short-term state of reduced physical activity and metabolism, and may be induced by reductions in environmental temperature or caloric restriction (or both).

A recent study measured liver and BAT gene expression in arctic ground squirrels during torpor, a hibernatory period of reduced ambient temperature which requires an 8-fold increase in energetic demand in order to maintain body temperature.<sup>25</sup> This study showed that in comparison to squirrels during warm summer months (i.e., not during torpor), hibernators had increased gene expression in pathways regulating fatty acid

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catabolism, ketogenesis and gluconeogenesis. By contrast, genes for fatty acid synthesis, amino acid metabolism, the urea cycle, glycolysis and lipid metabolism were decreased. Whether or not similar metabolic pathways are regulated in non-hibernation conditions of increased BAT thermogenesis remains to be determined.

BAT UCP1 also plays an important role in arousal from hibernation or torpor. Despite the importance of UCP1 for thermogenesis and energy expenditure, it has previously been shown that UCP1 deficient mice (UCP1<sup>-/-</sup>) are cold sensitive, but do not become obese on a high-fat diet at room temperature, although they do have an impaired thermogenic response after cold or  $\beta$ 3-adrenergic stimulation.<sup>26</sup> However, when room temperature is increased (to 27°C or 30°C, the latter of which is thermoneutral for mice), the resistance to diet-induced obesity is abolished.<sup>27,28</sup> In a recent study utilizing this UCP1<sup>-/-</sup> model, bouts of torpor under conditions of 48 h food deprivation and cold-exposure lasted significantly longer if induced during the dark-phase. Mice without UCP1 also had fewer daily bouts of torpor, took longer to arouse from torpor and required 60% more energy to do so.<sup>29</sup> During hibernation, BAT increases in mass and displays higher UCP1 expression. Stimulation of BAT  $\beta$ 3-adrenergic receptors with CL316,243 resulted in faster arousal from hibernation, while a  $\beta$ 3-adrenergic receptor antagonist produced the opposite effect.<sup>30</sup> These studies indicate that UCP1 and  $\beta$ 3-adrenergic signaling are required for changes in energy metabolism with diet and cold, a mechanism that is potentially similar in seasonal and non-seasonal mammals.

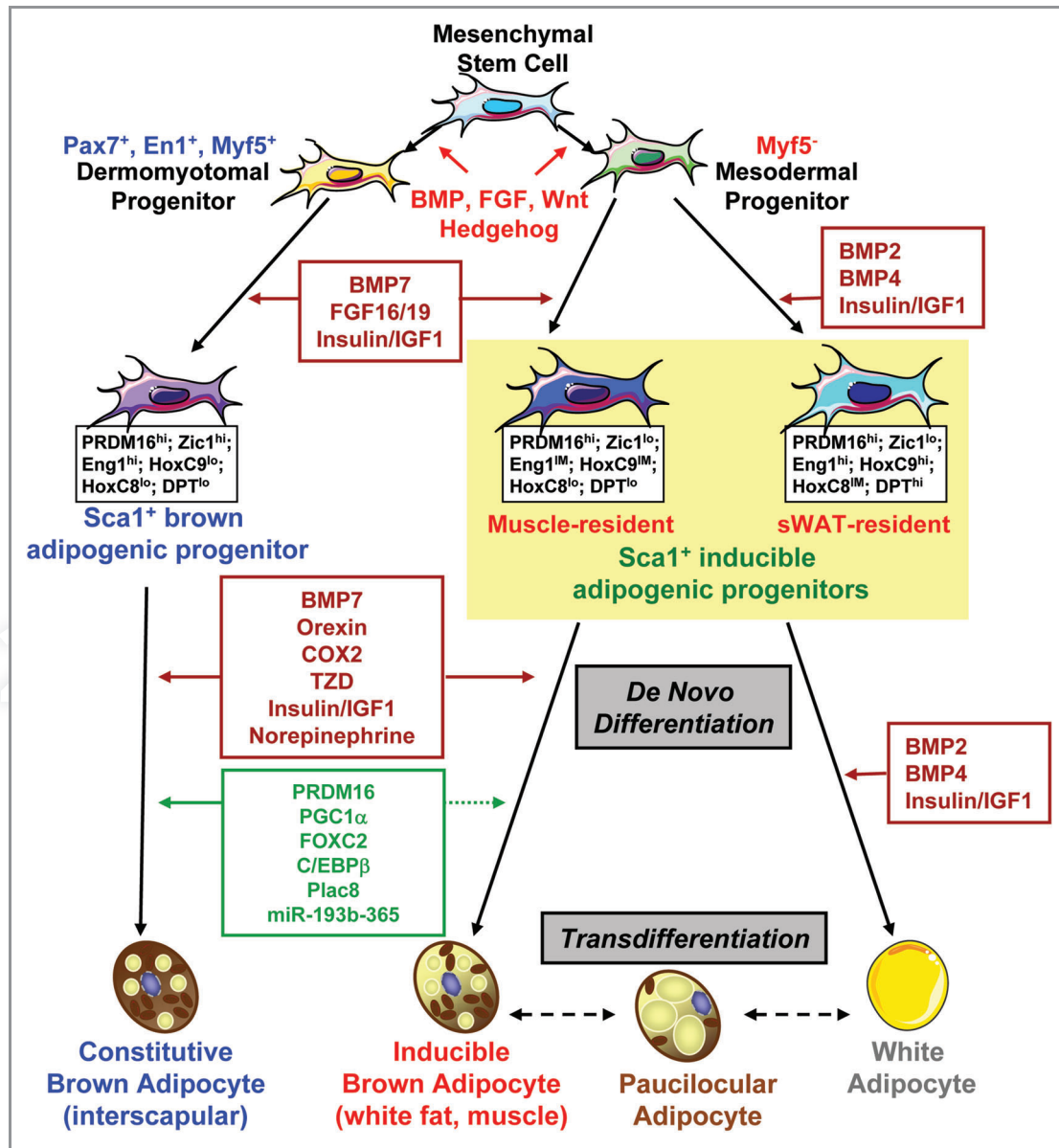
Melatonin may offer one insight into this similarity. Melatonin influences recruitment of brown adipocytes as well as their metabolic activity. In response to light cues received by the retina and pineal gland, melatonin production is upregulated, and short photoperiods (less light) have similar effects on BAT as cold environmental temperature.<sup>31</sup> It is now appreciated that melatonin may not only transmit information about photoperiods, but also about temperature and food availability, suggesting that rodent hibernatory models of seasonal changes in BAT thermogenesis may be indicative of BAT physiology in situations of cold or diet-induced thermogenesis as well (reviewed in ref. 31). Additionally, it is clear from rodent models of seasonally activated BAT that inputs via the central nervous system (CNS) are of utmost importance. Similarly, cold-exposure and other situations that stimulate BAT in non-seasonal models also involve pathways which originate in the CNS.

### Developmental Origin of Brown Fat

In rodents, brown fat is located in an interscapular fat pad, as well as smaller pads in other anatomical regions (such as perirenal and perivascular), and brown adipocytes are also dispersed throughout skeletal muscle and white fat. Recent fate-mapping studies combined with cell sorting analysis revealed that in rodents there are distinct progenitors that give rise to fat cells located in different anatomical locations of the body (Fig. 1). The myf5-expressing progenitors give rise to skeletal muscle and preformed brown adipocytes, which are found in the interscapular and perirenal regions,<sup>32</sup> and brown fat precursor cells express a myogenic gene

signature,<sup>33</sup> suggesting that brown fat and skeletal muscle share a common developmental ancestry. Indeed, during embryonic development, cells expressing transcription factors that are known to mark dermomyotome are found to give rise to the interscapular BAT. Homeobox transcription factor engrailed 1 (EN1)-expressing cells give rise to dermis, muscle and brown fat.<sup>34</sup> Selectively marking the somatic Pax7-expressing cells at embryonic day 9.5 (E9.5) demonstrates that Pax7-expressing cells can give rise to dorsal dermis, some muscle and brown fat. After E12.5, marked Pax7-expressing cells become lineage-restricted to skeletal muscle,<sup>35</sup> suggesting that early Pax7-expressing cells are multi-potent, but become committed to the myogenic lineage as embryonic development proceeds. However, not all of brown fat cells are derived from precursors expressing myogenic markers. In adult life, brown fat cells located in the non-classic sites, such as WAT and skeletal muscle, are derived from the myf5 negative progenitors.<sup>36</sup> These non-classic brown adipocytes have been given different names, such as 'brite cells'<sup>37</sup> and 'beige cells',<sup>38</sup> designations that reflect their recruitable and inducible nature. We have recently identified and prospectively isolated a sub-population of adipogenic progenitors (Sca-1<sup>+</sup>/CD45/Mac1<sup>-</sup>; referred to as Sca-1<sup>+</sup> progenitor cells, ScaPCs) residing in murine brown fat, white fat and skeletal muscle. Using the myf5 lineage tracing reporter mice, we and others have demonstrated that ScaPCs derived from skeletal muscle and subcutaneous WAT develop from cells that have never expressed myf5.<sup>39</sup> Nevertheless, it is likely an oversimplification to divide into only myf5 positive and myf5 negative lineages, because progenitors derived from different tissues possess unique molecular expression signatures and adipogenic capacities, further supporting the notion that brown fat depots located in different anatomical locations arise from distinct developmental origins (Fig. 1). Another non-classic brown fat depot is the perivascular fat. Perivascular adipose tissue around the thoracic region has been found to express gene profiles highly similar to the interscapular BAT,<sup>40</sup> but the developmental origin of this BAT depot remains to be determined. Recently, growing evidence has indicated that increased 'browning' in WAT can counteract diet-induced obesity, suggesting that inducible brown adipocytes may be potential targets for developing anti-obesity therapies. We review the recent evidence on browning in WAT in detail in the next section.

Cell fate determination in pluripotent stem/progenitor cells is controlled by the integration of cell intrinsic factors with extrinsic cues supplied by the surrounding microenvironment, known as the stem cell niche.<sup>41</sup> Prototypical stem cell niches include the stem cells, stromal support cells, soluble factors, extracellular matrix, blood vessels and neuronal inputs. The identification and characterization of niches within tissues and how niches support specific stem cell function remain key topics in understanding tissue development and homeostasis. Several developmental signaling molecules implicated in the evolution of mesodermal tissue have been shown to impact early stages of fat development (Fig. 1). These include members of the transforming growth factor- $\beta$  (TGF- $\beta$ ) and bone morphogenetic protein (BMP) family, the fibroblast growth factor (FGF) family, the wingless (Wnt) family, the hedgehog family, and others. The exact effects of these



**Figure 1.** Regulation of brown adipocyte development. Brown adipocytes located in different anatomical locations of the body arise from different developmental origins. While the Pax7<sup>+</sup>/En1<sup>+</sup>/Myf5<sup>+</sup> dermomyotome progenitor gives rise to interscapular brown fat, a distinct myf5<sup>-</sup> tissue resident progenitor serves as the common precursor for white adipocytes and systemic brown adipocytes. With the stimulation of appropriate developmental cues, these progenitors become committed to the adipocyte lineage. The Sca-1<sup>+</sup> progenitor cells isolated from interscapular brown fat serve as constitutively committed brown fat precursors, and Sca-1<sup>+</sup> progenitor cells from skeletal muscle and subcutaneous white fat are highly inducible to become mature brown adipocytes. These precursors possess unique molecular signatures that allow designation of the distinction of cellular origin. Agents that can promote brown adipocyte differentiation include norepinephrine, insulin/IGF1, thiazolidinedione (TZD), cyclooxygenase 2 (COX2), orexin, BMP7 and others. At the molecular level, a number of transcriptional/post-transcriptional regulators have been shown to specify or enhance brown fat phenotype, such as PRDM16, PGC1 $\alpha$ , FOXC2, C/EBP $\beta$ , Plac8 and miR-193b-365. The brown adipocytes in white fat may come from de novo differentiation and/or transdifferentiation. Dashed lines in this figure indicate links that are only partially established.

factors depend on the concentration, stage of differentiation, cell-cell interactions, and nature of the extracellular matrix. While TGF- $\beta$  inhibits adipocyte differentiation in vitro,<sup>42</sup> TGF- $\beta$  expression in fat is paradoxically increased with obesity in rodents and humans.<sup>43,44</sup> We have demonstrated that in contrast to the roles of BMP2 and BMP4 in development of white fat, BMP7 serves as a potent inductive signal for brown adipogenesis.<sup>45</sup> Some

members of the FGF family, such as FGF 1, 10, 16, 19 and 21, have been implicated in adipose development. In particular, FGF 16, 19 and 21 are specifically involved in brown fat formation.<sup>46-48</sup> Wnt signaling is known to suppress adipogenic and favor myogenic or osteogenic differentiation in MSCs.<sup>49</sup> Finally, while the anti-adipogenic role of the hedgehog pathway has been established,<sup>50</sup> a recent *Drosophila* genome-wide screen

identified hedgehog as a determinant of brown vs. white cell fate.<sup>51</sup>

### Molecular Control of Brown Fat Development

Despite the distinct functions of brown and white fat, these two cell types share a similar transcriptional cascade for adipocyte differentiation. This is a process involving adipogenic transcription factors PPAR $\gamma$  (peroxisome proliferator-activated receptor- $\gamma$ ) and CCAAT/enhancer-binding proteins (C/EBPs; for reviews see refs. 52–54). Importantly, a number of nuclear factors that specify or enhance brown fat phenotype have been identified. Before the initiation of the adipogenic program, the preadipocytes need to be released from suppression and become committed to terminal differentiation. Among the known inhibitors of preadipocyte-adipocyte transition, proteins of the retinoblastoma (Rb) family and necdin, a growth repressor functionally resembling Rb, selectively inhibit brown preadipocyte differentiation.<sup>55–58</sup> Consistent with these findings, Calo et al. recently demonstrated that deletion of both Rb and tumor suppressor p53 in primitive mesenchymal cells shifts the tumor spectrum away from osteosarcoma to the brown fat tumor hibernoma.<sup>59</sup>

After release from suppression, the adipose precursors initiate a transcriptional cascade to turn on lipid synthesis and other adipocyte specific programs. A number of transcription factors and co-regulators appear to play important roles in specifying brown fat cell fate or modulation of the expression of thermogenic genes, especially UCP1. Nuclear co-repressor RIP140 directs histone and DNA methylation to silence UCP1 expression and suppress mitochondrial biogenesis in white adipocytes.<sup>60,61</sup> Nuclear receptor liver X receptor (LXR) can suppress UCP1 gene expression by binding to the LXRE element of the UCP1 promoter and recruiting co-repressor RIP140.<sup>62</sup> Thus, LXR KO mice display increased energy expenditure and UCP1 expression.<sup>63</sup> The zinc-finger containing protein PRDM16, which is expressed at higher levels in brown compared with white adipocytes,<sup>64</sup> has been shown to drive differentiation of white preadipocytes or myoblasts into functional brown adipocytes. This effect depends on the interaction of PRDM16 with nuclear coactivator PGC-1 $\alpha$  and transcription factors PPAR $\gamma$  and C/EBP $\beta$ , while binding of PRDM16 to CtBP-1 and CtBP-2 suppresses expression of white fat-selective genes.<sup>65,66</sup> In addition, the forkhead family transcription factor forkhead box C2 (FOXC2) can induce expression of the R1 subunit of cAMP-dependent protein kinase A (PKA), thereby sensitizing cells to  $\beta$ -adrenergic stimulation and promoting brown adipogenesis.<sup>67</sup> Recently, Plac8, a 12.5 kDa protein containing a cysteine-rich domain, has been found to play a critical role in promoting brown adipogenesis via induction of C/EBP $\beta$  expression.<sup>68</sup>

Recently, microRNAs (miRNAs) have emerged as important regulators of diverse biological processes and pathologies, including cell fate decision. The role of miRNAs in brown adipogenesis has just begun to emerge. Certain myogenic miRNAs have been shown to be enriched in BAT compared with WAT.<sup>69</sup> Sun et al. have recently shown that the miR-193b-365 cluster is required for brown fat differentiation from the

myf5<sup>+</sup> progenitors, and that miR-193b-365 expression was regulated via the PRDM16-PPAR $\gamma$  transcriptional cascade;<sup>70</sup> however, whether this miRNA cluster could regulate the formation of the inducible brown adipocytes arising from the myf5<sup>-</sup> lineage is unknown.

### 'Browning' in White Adipose Tissue

Recent years have brought a greater appreciation for potential beneficial effects of acquiring brown fat cells in non-classic BAT locations, such as WAT and skeletal muscle.<sup>71</sup> Obesity-resistant strains of mice contain higher amounts of brown adipocytes dispersed in their WAT and muscle,<sup>72–75</sup> and physiological stimuli, such as cold exposure and sympathetic activation, are also known to induce brown adipogenesis in white depots.<sup>76</sup> Over the past two years, several studies reported that mouse models with increased UCP1-positive brown adipocytes in WAT are protected from high-fat diet-induced obesity. Transgenic expression of PRDM16 in fat tissue using the  $\alpha$ 2 promoter induced the formation of brown adipocytes in subcutaneous but not epididymal fat, and the transgenic mice exhibited increased energy expenditure, limited weight gain, and improved glucose tolerance in response to a high-fat diet.<sup>77</sup> Similarly, transgenic mice with overexpression of FOXC2 in adipose tissue induced the emergence of brown fat cells in WAT. This interconversion of white to brown adipose was recently shown to be reliant on the C/EBP $\alpha$  signaling pathway, which acts with co-repressors to reduce the expression of certain visceral WAT genes in order to promote BAT genes.<sup>78</sup>

Hormone-sensitive lipase (HSL) null mice have increased UCP1 expression and enhanced mitochondrial activity in WAT,<sup>79</sup> while mice with adipose triglyceride lipase (ATGL, also known as desnutrin) ablation display a conversion of BAT to a WAT phenotype,<sup>80</sup> suggesting that these two lipases may have opposite effects in adipose cell fate decision. A lipid droplet protein, perilipin, was recently demonstrated to induce a brown-like phenotype in WAT upon its overexpression, thereby reducing lipid-droplet size.<sup>81</sup> However, mice deficient in another lipid droplet protein, fsp27, also display brown fat properties in WAT.<sup>82</sup> Interestingly, mice living in an enriched environment with increased social interactions, novel objects and physical activity also displayed a brown-phenotype occurring in WAT, which was reproduced upon hypothalamic overexpression of brain-derived neurotrophic factor (BDNF).<sup>83</sup> BDNF has also been recently linked to ventromedial hypothalamus control of energy expenditure, through sympathetic activation of BAT.<sup>84</sup> As detailed below, the central nervous system appears to play an important role in the induction of brown fat cells within WAT.

Pharmacological agents that regulate different biological pathways have also been demonstrated to induce browning in WAT. For example, synthetic PPAR $\gamma$  agonists, such as the thiazolidinedione (TZD) family members, are also able to induce a brown phenotype in WAT.<sup>85</sup> At the cellular level, Petrovic et al. have demonstrated that TZDs can induce brown adipogenesis in adipose precursors isolated from white fat.<sup>86</sup> Furthermore, cyclooxygenase (COX)2, a rate-limiting enzyme in prostaglandin (PG

synthesis, can promote de novo BAT in WAT and increased energy expenditure, and also exerts anti-obesity effects in high-fat fed mice.<sup>87</sup> COX2 induces UCP1 expression specific to inguinal WAT, as expression is not induced in classic interscapular BAT.<sup>88</sup> Interestingly, co-injection of brown adipogenic factor BMP7 and  $\beta$ 3-adrenergic receptor agonist CL316,243 to mice maintained on a high-fat diet resulted in significant increases in the expression of brown fat marker genes UCP1 and CIDEA in both WAT and interscapular BAT. These data suggest that BMP7 may act in concert with other brown adipogenic agents to promote the formation of energy-dissipating brown adipocytes from tissue-resident brown fat cells.

The exact cellular and molecular mechanisms contributing to the browning phenomenon in WAT have not been fully elucidated. Two potential mechanisms have been proposed and each is supported by experimental evidence (Fig. 1). First, the presence of brown fat cells in WAT may come from de novo differentiation. Indeed, as described in the sections above, several brown fat inducers, such as TZD, BMP7 and COX2, can induce de novo brown fat differentiation from tissue resident progenitors. Second, the brown fat cells in WAT may come from the existing white adipocytes, a process called transdifferentiation. Recent evidence for this includes observation of an 'intermediate' cell type in cold-exposed WAT, which has 'paucilocular' lipid droplets and mitochondria characteristic of both WAT and BAT.<sup>89</sup> These two mechanisms are not exclusive to each other; in fact, it is likely that both mechanisms may co-exist in the body and different stimuli may preferentially activate one pathway over the other. Importantly, several findings also indicate that induction of brown adipocytes in WAT is directly reliant on the sympathetic nervous system. Therefore, it appears that complex signaling and neuronal inputs converge to either induce or maintain a BAT phenotype, providing potential in-roads for converting WAT to BAT.

### New Physiological Functions of Brown Fat

In addition to thermogenesis, recent studies have demonstrated that BAT is involved in triglyceride clearance and glucose disposal, serves as a source of adipokines, and possesses distinct inflammatory function compared with WAT. For example, Bartelt et al. recently demonstrated a new function of BAT in triglyceride clearance and glucose disposal, a process which involves whole-particle uptake of triglyceride-rich lipoproteins through activation of the cell-surface fatty acid translocase CD36.<sup>90-92</sup>

White adipose tissue secretes many cytokines, hormones, and other factors which are collectively termed 'adipokines,' leading to the classification of adipose tissue as an endocrine organ. While it is expected that BAT secretes many of the same factors which it also expresses (adiponectin, for instance), there are also several factors which may be uniquely secreted by BAT alone, which we are calling BATokines, for BAT-derived adipokines. Several BATokines have already been demonstrated in the literature, including FGF21, which is induced upon cold and adrenergic stimulation.<sup>93-95</sup>

Furthermore, BAT secretes other factors such as IL6 and neurotrophic factors such as BDNF and NGF,<sup>96-99</sup> which may play unique roles in BAT vs. WAT. For example, BAT is more highly innervated by the sympathetic nervous system and contains a more richly developed vasculature. Therefore, the paracrine and autocrine environment of BAT may have evolved to respond uniquely to various adipokines, given the metabolic functions of BAT.

White adipose tissue readily becomes infiltrated with immune cells and macrophages upon high-fat feeding and obesity, believed to be the trigger of the inflammation observed in obese adipose tissue. BAT, by contrast, does not appear to accumulate pro-inflammatory macrophages with a high-fat diet, though this may depend on mouse strain or diet conditions.<sup>40</sup> The authors of this study postulate that the high metabolic rate of mitochondria-rich BAT allows tissue to readily utilize free fatty acids through  $\beta$ -oxidation, whereas the overload of free fatty acids (lipotoxicity) in WAT may be the precipitating event leading to the influx of immune cells, including pro-inflammatory macrophages.

Interestingly, another group has similarly demonstrated that macrophages in BAT do not develop the same chemokine and cytokine expression profile as those in WAT.<sup>100</sup> Together, these studies indicate that the microenvironment provided by different adipose depots likely influence whether or not the tissue recruits immune cells such as macrophages, and whether these tissues become inflamed. They further suggest that the microenvironment of BAT may be protective against the pro-inflammatory state which may lead to insulin resistance, and interventions which convert white depots to BAT may be protective against this effect.

### Mechanisms of Increased Metabolic Activity in BAT

The traditional view of BAT is that its metabolic activity (both  $\beta$ -oxidation and thermogenesis via UCP1) is regulated via input from the sympathetic nervous system (SNS). SNS input to BAT results in the release of the catecholamine neurotransmitter norepinephrine (NE), which binds to adrenergic receptors in BAT. BAT expresses G-protein coupled adrenergic receptors (classified as  $\alpha$ 1- $\alpha$ 3 or  $\beta$ 1- $\beta$ 3), and while NE is believed to be the main catecholamine from sympathetic nerves which acts on these receptors, there is emerging evidence that epinephrine may also affect PGC1  $\alpha$  and UCP1 expression in BAT.<sup>101</sup> Activation of the  $\beta$ 3-adrenergic receptor stimulates cAMP production and PKA activation. It is believed that the  $\beta$ 3-adrenergic receptor isoform is the main isoform in mouse BAT, while  $\beta$ 1 may be the predominant isoform in human BAT. Indeed, a recent study found that non-shivering thermogenesis (activated in the absence of shivering thermogenesis only after several weeks in the cold) in  $\beta$ 3-adrenergic receptor knockout mice is still activated, and the knockout mice are able to survive 7 weeks or more. This period was characterized by upregulation of UCP1 and the  $\beta$ 1 adrenergic receptor in BAT, indicating that these KO mice may be a good model for human BAT thermogenesis where  $\beta$ 1 appears to be the main responsible isoform.<sup>102</sup>

Downstream of cAMP and PKA, HSL is phosphorylated and activated, as well as perilipin A.<sup>105</sup> Normally, perilipin A protects the lipid droplet, but after phosphorylation and activation it induces fatty acid cleavage. HSL also promotes fatty acid release from triglycerides. The newly available fatty acids are brought to the mitochondria by the carnitine shuttle, where they can activate UCP1 as well as be metabolized through  $\beta$ -oxidation (pathways summarized in Fig. 2).

For BAT thermogenic function, there is a synergy between sympathetic inputs and thyroid hormone action (reviewed in ref. 104). Thyroid hormones (as both the T3 and T4 forms, which are more or less active respectively) are transported into brown adipocytes, where T4 is further converted to T3 by type 2 deiodinase (DIO2), followed by T3-driven expression of genes such as UCP1.<sup>105</sup> The thyroid hormone is essential for adaptive thermogenesis, as demonstrated by hypothyroid mammals which succumb quickly to reduced environmental temperature. However, a recent study clarified that this metabolic response of hypothyroid animals is temperature-specific and does not occur at ambient room temperatures.<sup>106</sup> Relevant to BAT thyroid

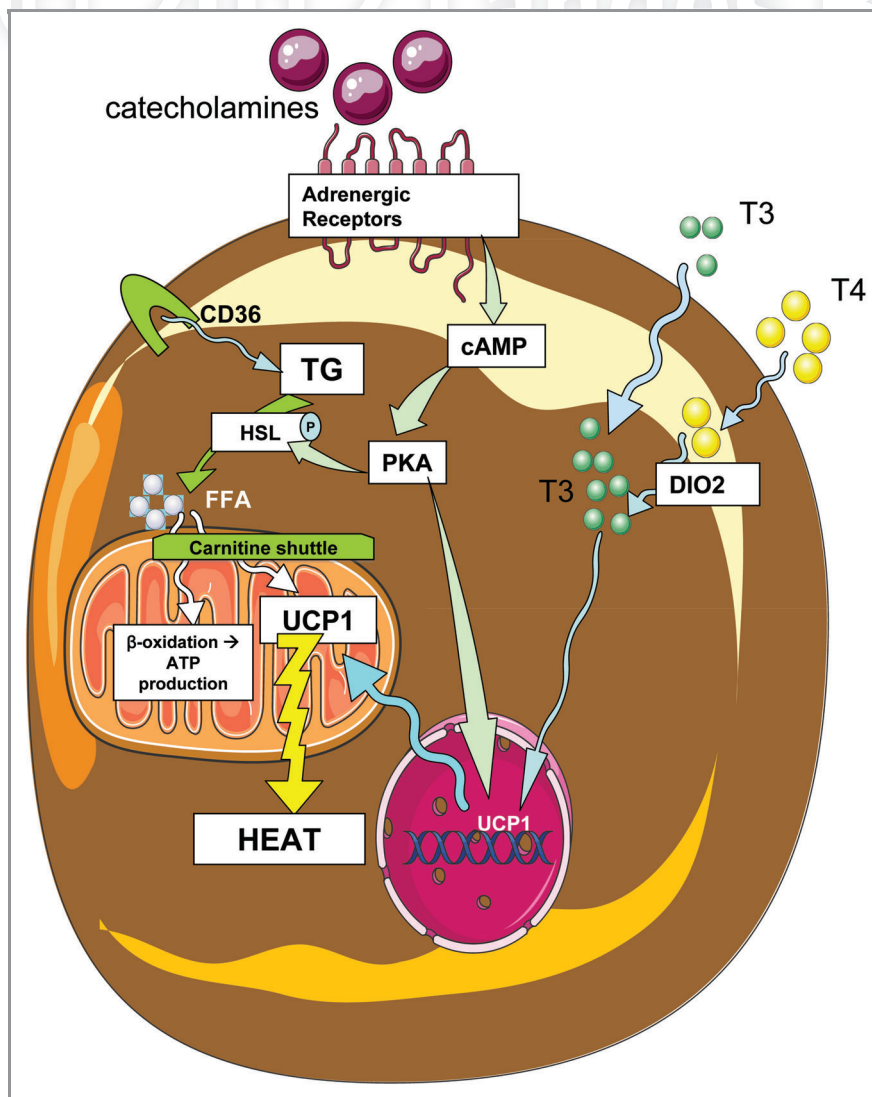
hormone signaling, the  $\beta$  isoform of the thyroid receptor has recently been demonstrated to mediate T3 regulation of UCP1.<sup>107</sup> Other pathways are also implicated in the increased expression of UCP1, such as the PKA and protein kinase G (PKG) pathways, and the p38-CREB pathway.<sup>108-110</sup>

Recently, several novel factors have been implicated in the regulation of these energetic processes in BAT. Sympathetic input to BAT can be removed through surgical or chemical denervation of the tissue. A recent study demonstrated that unilateral BAT denervation not only reduced UCP1 expression, but also reduced activity of AMPK, the cellular energy sensor which is activated in BAT upon  $\beta$ -adrenergic receptor stimulation.<sup>111</sup>

The regulation of fuel supply in BAT may also be a point of metabolic control. As mentioned above, BAT is involved in triglyceride clearance, through whole-particle uptake of triglyceride-rich lipoproteins, and Vergnes et al. have shown that FABP3 (the heart-type fatty acid binding protein) is required for BAT fatty acid oxidation and cold tolerance, despite FABP4 (or ap2) being the most abundant FABP in BAT.<sup>112</sup> Mitochondrial activity and energy expenditure in BAT are therefore complex processes with multiple points of potential regulation, from sympathetic/catecholamine stimulation of adrenergic receptors, to UCP1 and other genes involved in thermogenesis, to mitochondrial activity and fuel utilization.

### Central Nervous System (CNS) Control of BAT Function

The brain remains the most important organ for control and coordination of systemic energy balance. Several brain regions, including the hypothalamus, are involved in the central regulation of appetite and energy



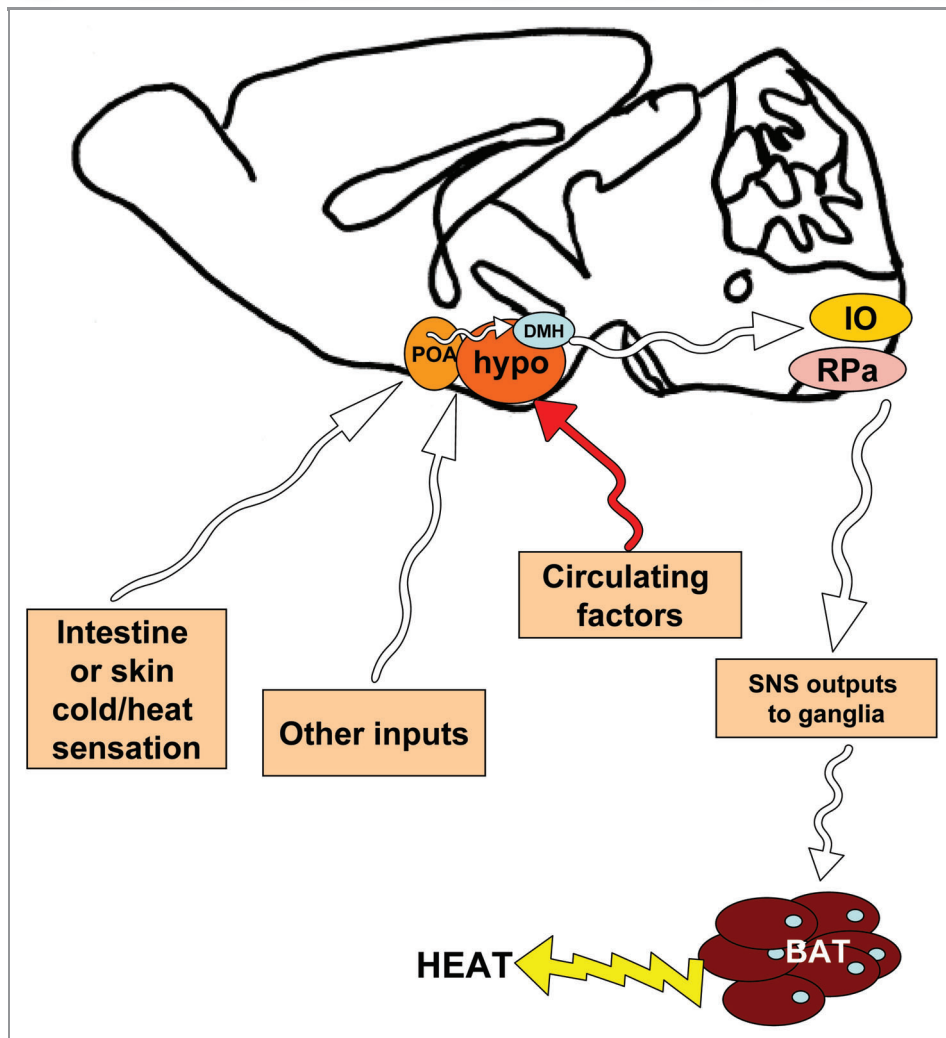
**Figure 2.** Brown adipose activity in response to sympathetic input and thyroid hormone. Catecholamines bind to adrenergic receptors on the surface of brown adipocytes, initiating signaling cascades that include cAMP and protein kinase A (PKA), which then phosphorylates and activates the enzyme hormone sensitive lipase (HSL), which then cleaves triglycerides (TG) into free fatty acids (FFA). Triglycerides enter the cell by uptake of triglyceride rich lipoproteins via CD36 transport, and fatty acids then enter the mitochondria through the carnitine shuttle. Mitochondrial fatty acids may be oxidized via  $\beta$ -oxidation, or serve to activate UCP1 thermogenesis. Additionally, thyroid hormones T3 and T4 enter the cell, and T4 is further converted to T3 by type 2 deiodinase (DIO2). T3 is then able to affect mitochondrial activity and nuclear transcription of genes that affect energetics, including UCP1. PKA also affects nuclear transcription of UCP1, a protein which acts in the mitochondria to uncouple oxidative phosphorylation from ATP production, resulting in heat generation.

expenditure, including the activation of sympathetic output to adipose depots. For example, when cold is sensed by the pre-optic area (POA) of the brain, signals are relayed to the dorsomedial hypothalamus (DMH), then the GABAergic neurons of the rostroventral medulla in the brainstem, followed by sympathetic outflow to the tissue<sup>113-116</sup> (summarized in Fig. 3). Several hypothalamic signaling pathways have been identified which can increase sympathetic output to adipose tissue, including leptin receptors in the ventromedial hypothalamus (VMH) and POA, as shown through retroviral tract tracing from BAT to the hypothalamic leptin-receptor neurons.<sup>117</sup>

In response to diet or cold, hypothalamic pathways like those described above lead to sympathetic activation of BAT, as well as increased sympathetic input to WAT depots, which in turn respond by upregulating brown-adipocyte genes such as UCP1. The exact CNS pathways involved in this communication with adipose depots to control peripheral energy expenditure continue to be elucidated, but much of the neural connections and neurotransmitters have already been identified. Recently several candidate signaling molecules have been identified with a CNS role in sympathetic outflow to adipose. For instance, the sirtuin

deacetylase SIRT1 in pro-opiomelanocortin (POMC) neurons appears to be responsible for 'browning' of perigonadal WAT in response to cold or diet.<sup>118</sup> Similarly, neurons in the paraventricular hypothalamus (PVH) appear to also inhibit sympathetic outflow to adipose tissue. Upon activation of PVH neurons by NMDA (an agonist for the NMDA glutamate receptor), no effect on BAT thermogenesis was observed, and in fact NMDA treatment to the PVH reversed BAT activation that had been stimulated by cold exposure. In addition to this, DMH neuropeptide Y (NPY) appears to be an inhibitory signal for adipose expression of UCP1, as its knockdown produces an increase in UCP1 expression in inguinal and BAT depots.<sup>119</sup> However, previous studies have demonstrated that the DMH is involved in activation of BAT thermogenesis.<sup>120</sup>

The same group of researchers has also recently identified orexigenic projections from hypothalamus to raphe pallidus, a region of serotonergic neurons in the medulla that plays an important role in sympathetic activation of thermogenesis.<sup>121,122</sup> Indeed, previous studies have shown that orexin infusions to the lateral ventricle affect sympathetic outflow and BAT thermogenesis.<sup>123-125</sup> Interestingly, Sellayah et al. recently showed that the



**Figure 3.** Simplified schematic of neural pathways in the mouse brain which affect sympathetic outflow to brown adipose tissue. Using rodent models, neuroscientists have begun to identify which neural pathways are involved in signaling temperature status (such as cold) to the brain, followed by sympathetic stimulation of brown adipose tissue in order to initiate thermogenesis. Some of these findings are summarized in this mid-sagittal view of a mouse brain, but for a complete review see Morrison et al. Cold temperature is sensed by the pre-optic area (POA), rostral to the hypothalamus (hypo). The POA sends signals to the hypothalamus, including the dorsomedial hypothalamus (DMH). Other hypothalamic nuclei are also involved in relaying various signals related to energy status, in response to various neural inputs and circulating factors. Neural outputs from the hypothalamus reach the inferior olive and GABAergic centers in the raphe pallidus (RPa) in the medulla of the brain stem. From here, sympathetic outputs are activated and send afferents to the sympathetic ganglia, followed by the brown adipose tissue, where catecholamine neurotransmitters are released from sympathetic nerve terminals, to act on adrenergic receptors there. White arrows represent neural pathways.

neuropeptide orexin, which is known to stimulate feeding and arousal in the hypothalamus, is involved in BAT differentiation and function. This was exhibited in orexin knockout mice which display impaired thermogenesis and smaller BAT depots, due to decreased ability of progenitor cells to differentiate. Additionally, in vitro studies showed that orexin was able to have a direct effect on BAT differentiation by inducing expression of PRDM16 and PGC1 $\alpha$ .<sup>126</sup> It is currently not clear if orexin's effects are solely mediated by CNS pathways and sympathetic nervous system activity,<sup>127</sup> or whether orexin is reaching the brown adipocytes (either via sympathetic neuron secretion or through the circulation) to act on BAT directly. Indeed, brown adipocytes have recently been shown to express orexin receptors.<sup>128</sup> However, orexin is a neurotransmitter and its expression has not yet been shown in sympathetic nerve terminals in BAT, it is not expressed in brown adipocytes, and its levels in the circulation are low, leaving open the question regarding the physiological pathways by which orexin regulates BAT.

Taken together, nearly every region of the hypothalamus has been implicated in some manner in the regulation of sympathetic outflow to adipose tissues and the resulting regulation of thermogenesis and energy expenditure; however a complete understanding of the circuitry involved remains to be fully described.

### Lessons from Human Brown Fat

Recently, several studies concurrently demonstrated that human BAT is present and active in adult humans. These findings reignited research on human BAT, which had essentially been ignored and believed to be inactive beyond the infant stage when it is important for maintenance of body temperature in the setting of high surface-to-volume ratio with no capacity for shivering. These studies have further clarified conditions in humans which correlate with increased (cold-exposure) or decreased (obesity) BAT activity, as measured by imaging modalities such as 18F-fluorodeoxyglucose (FDG)-positron emission tomography (PET) combined with X-ray CT (CT). BAT activity also declines with age, from 50% activity in subjects in their 20s, down to 10% for subjects in their 50s and 60s.<sup>129</sup> In accordance with this, it was also found that BAT is more prevalent in children than adults, and BAT activity increases into adolescence when it may play a specific metabolic role.<sup>130</sup>

Additionally, more recent studies have confirmed that circulating catecholamines such as epinephrine and norepinephrine also activate BAT.<sup>131</sup> While it is enticing to consider adrenergic stimulation as a means to increase BAT mass and activity in humans, thereby increasing energy expenditure and decreasing body weight, adrenergic stimulation through various pharmaceutical means results in non-specific activation in other tissues and undesirable, or even dangerous, side effects (reviewed in ref. 132). Interestingly, a recent study showed that blockade of  $\beta$ -adrenergic receptors in fact does not inhibit cold-induced thermogenesis in humans, an effect thought to be due to the differing roles of  $\beta$ -adrenergic isoforms in BAT vs. skeletal muscle, another thermogenic tissue.<sup>133</sup> This finding was surprising and important, taking into account that the three  $\beta$ -adrenergic

receptors ( $\beta$ 1– $\beta$ 3) are considered the most important for thermogenic effects, illustrated by the obesity observed in mice with total depletion of the  $\beta$ -adrenergic receptors.<sup>134</sup>

Instead of therapeutic approaches which may induce sympathetic drive to BAT in order to increase its activity, another approach is to induce tissue-resident progenitor cells in human BAT to differentiate, thereby increasing the total mass of human BAT. A recent study has provided promising evidence for this direction, demonstrating that progenitor cells derived from PET-CT positive BAT are able to differentiate to brown adipocytes in vitro, in contrast to PET-CT negative subcutaneous white tissue progenitors which did not develop into brown adipocytes.<sup>135</sup>

Increasing BAT mass and activity not only provide increased energy expenditure to potentially combat obesity, but BAT also serves to improve lipid and glucose homeostasis (reviewed in ref. 132). Cold may not be the only parameter to activate BAT in adult humans, as BAT also has the capacity to act as a glucose sink. Comparing cold to insulin stimulation, Orava et al. measured BAT perfusion as well as glucose uptake, and observed that insulin leads to increased glucose uptake independent of perfusion, while cold leads to increased thermogenesis correlated with perfusion rates,<sup>136</sup> thereby indicating two distinct mechanisms of BAT stimulation. Other signals beyond cold and adrenergic stimulation include leucine deficiency, which leads to decreased abdominal fat mass and has now been associated with increased BAT UCP1 expression and thermogenesis-related energy expenditure due to increased sympathetic innervation.<sup>137,138</sup>

It remains to be determined whether rodent BAT studies are translatable to understanding the biology of human BAT. Thus far, human BAT has been found to display a unique distribution, but an overall similar morphology and gene expression profile (including high UCP1 and type 2 deiodinase) as the mouse. However, a recent comparison of BAT vs. WAT in humans showed that many genes enriched in human BAT differ from those found in murine interscapular BAT.<sup>139</sup> Localization of human BAT is another difficulty, and PET-CT is the most common method for this in humans, where many small BAT depots are interspersed with WAT. However, recent data indicate that PET-CT is able to localize only dense regions of BAT, and BAT can be found in PET-CT negative fat biopsies. Also, given the mechanism of PET-CT (i.e., essentially a measurement of tissue glucose uptake) and the fact that BAT also utilizes fatty acids as fuel, not all BAT activity may be detected using this method. Overall, recent research regarding human BAT has provided the promise that this metabolically active tissue may be coaxed to increase in size or activity in order to boost energy expenditure and combat obesity. However, given the difficulty in obtaining BAT samples from human adults, most future research into the function of BAT may come from rodent models.<sup>140</sup>

### The Promise of Brown Adipose Tissue

As presented in this review, research into the development, function and control of BAT has now reached an exciting pace following the re-discovery of BAT in adult humans. Given the



global pandemic of obesity and the projection for epidemic rates of co-morbidities like diabetes, as well as the limited success rate with various interventions to treat and prevent obesity, knowledge about BAT and its promise as a potential therapeutic agent are exciting areas for biological and translational research. BAT activity may be increased in order to elevate whole-body energy expenditure, either through sympathetic or other activation of UCP1 and mitochondria pathways, increased differentiation from progenitor cells, or stimulation of a brown phenotype in WAT depots. Interestingly, transplantation of as little as 0.1–0.4 g of BAT into the visceral cavity of recipient mice is able to prevent weight gain and improve glucose homeostasis in diet-induced obese mice.<sup>141</sup> Given its huge capacity for energy expenditure, newly identified effects on fatty acid and glucose metabolism, as well as potential resistance from infiltrating pro-inflammatory macrophages, increasing the amount and function of brown fat may not only combat obesity, but may also prevent

type 2 diabetes and other metabolic disorders. Therefore, future research regarding BAT function will further our understanding of its unique physiology as well as its therapeutic promise.

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