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Brown, Beige, and White: The New Color Code of Fat and Its Pharmacological Implications

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Abstract

Brown adipose tissue (BAT) was previously regarded as a special type of fat relevant only for defending hibernating animals and newborns against a cold environment. Recently, BAT has received considerable attention following its (re)discovery in humans. Using glucose tracers, multiple laboratories independently found metabolically active BAT in adults. The enormous metabolic powers of BAT in animal models could make it an attractive target for antiobesity therapies in humans. Here, we review the present knowledge on the role of BAT in energy homeostasis and metabolism, focusing on signaling pathways and potential targets for novel therapeutics. We also shine light on ongoing debates, including those about the true color of brown fat in adults, as well as on the requirements for translation of basic research on BAT into clinical medicine.

INTRODUCTION

White adipose tissue (WAT): major site for energy storage; also functions as an endocrine organ by secreting adipokines (e.g., leptin)

Brown adipose tissue (BAT): specialized tissue converting nutrient energy into heat; energy-combusting properties could be used to reduce body weight

Nonshivering thermogenesis (NST): generates heat to adapt to cold environments and maintain body temperature; main function of BAT

Uncoupling protein 1 (UCP1): expressed in mitochondria of BAT; disrupts the proton gradient, thereby generating heat instead of ATP

Obesity is caused by an imbalance between energy intake and energy consumption that leads to an abnormal increase in body fat. It is an enormous burden not only for the individuals affected but also for society and health-care systems. Obesity has been expanding in recent decades throughout Western societies and all over the world (1), reaching pandemic dimensions: Globally, more than 2.1 billion people are overweight [with a body mass index (BMI) between 25 and 30], and more than 735 million people are obese (BMI > 30) (2).

Obesity increases the risk of comorbidities, which include metabolic syndrome, type 2 diabetes mellitus (T2DM), cardiovascular disease, and certain types of cancer (3). According to the World Health Organization, at least 2.8 million adults die each year as a result of being overweight or obese (4).

The two types of adipose tissue are white and brown fat (5). White adipose tissue (WAT) is the largest energy store in humans. WAT also performs endocrine functions and secretes adipokines and cytokines such as adiponectin and leptin. The other type of fat, brown adipose tissue (BAT), is distinguished by its ability to dissipate energy in the form of heat. BAT plays an especially important role in newborns, who use this tissue for nonshivering thermogenesis (NST) to defend themselves against a cold environment. NST is different from thermogenesis derived from the shivering of skeletal muscle. Recently, researchers in Europe, Japan, and the United States independently demonstrated that adults have metabolically active BAT (6–9).

In light of the obesity pandemic, new pharmacological strategies are needed to rebalance energy homeostasis. Obese subjects have significantly less active BAT than do lean people (6). Researchers have therefore postulated that adult BAT may play a role in obesity and might be a promising target for novel antiobesity therapies.

THE ROLE OF BAT IN ENERGY EXPENDITURE AND METABOLISM

The major function of BAT is NST as a defense against a cold environment. In addition, BAT may also be activated by a high fat diet (HFD) and may play a role in diet-induced thermogenesis (DIT) (10).

Brown adipocytes (BAs) produce heat efficiently because they contain many mitochondria and multiple small lipid droplets, which give them a multilocular appearance—in contrast to the unilocular white adipocytes (WAs) (**Figure 1**)—and a high lipolytic rate. In addition, BAT is richly vascularized with up to five capillaries per adipocyte (see 11 and references therein), which, together with its high mitochondria content, results in a brown color. The relatively large number of vessels supplies lipids and glucose that are converted to heat upon cold stimulation and helps to dissipate thermoenergy via the bloodstream.

BAT-dependent NST is based on the uncoupling of mitochondria, which is achieved by a unique, cold-inducible protein: the uncoupling protein 1 (UCP1) (12–14). UCP1 belongs to a family of mitochondrial carriers and disrupts formation of adenosine triphosphate (ATP), leading to a direct conversion of nutrient energy into heat (15). UCP1 activity is constitutively inhibited by purine di- and triphosphate nucleotides (12). In response to cold, UCP1 is activated by free fatty acids that are mobilized in BAs by lipolysis. Thus, lipolysis not only provides fuel for BAT mitochondria but also induces their uncoupling by directly activating UCP1.

Researchers have demonstrated the metabolic power of BAT in different mouse models of obesity and lipid disorders. Activation of BAT by cold (5°C) exposure for 7 days resulted in a 55% loss of body fat mass in mice exposed to a HFD, even though the cold-exposed mice took in 50% more food (16). However, BAT must first be activated by cold exposure to efficiently function

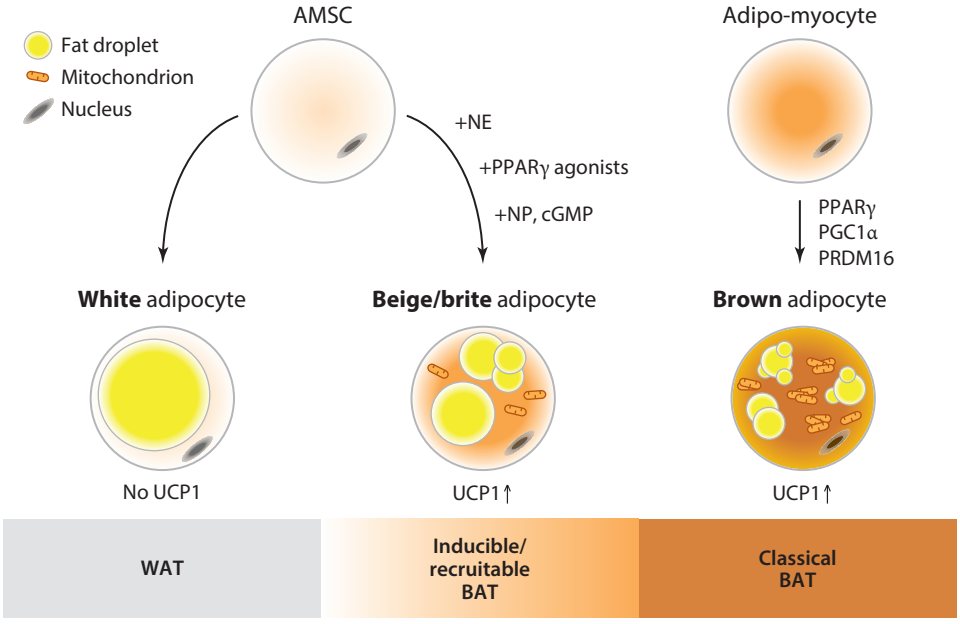

Figure 1

Colors of adipose tissue. White adipocytes descend from AMSCs and are characterized by unilocular fat droplets and the absence of UCP1 expression (*left*). Beige/brite adipocytes also descend from AMSCs and can be induced by certain stimuli, as indicated. These cells express UCP1 and compose inducible BAT (*center*). In contrast, classical brown adipocytes are derived from adipo-myocytes (*right*). Brown adipocyte mitochondria express UCP1, and fat droplets are multilocular in these cells. Abbreviations: AMSC, adipose tissue–derived mesenchymal stem cell; BAT, brown adipose tissue; cGMP, cyclic guanosine monophosphate; NE, norepinephrine; NP, natriuretic peptide; PGC1α, peroxisome proliferator–activated receptor γ coactivator 1α; PPARγ, peroxisome proliferator–activated receptor γ; PRDM16, PR domain containing 16; UCP1, uncoupling protein 1; WAT, white adipose tissue.

as an energy sink. Once BAT is activated, calorie uptake in BAT is even higher than in muscle, liver, and WAT (17). Jörg Heeren and colleagues (17) found that activated BAT takes up ~50% of dietary triglycerides and glucose from blood. Positron emission tomography (PET) studies in humans suggest that fully activated BAT could dissipate energy equivalent to ~4.1 kg WAT over one year (9).

DIT is another mechanism to activate BAT, but it is highly controversial (18, 19). In humans, energy expenditure (EE) increases with body mass, and severe obesity is associated with an increase in EE (20). After a single high-calorie, carbohydrate-rich meal, lean men increased glucose uptake into BAT. However, no direct relation between BAT and DIT was found (21). This might be because of glucose uptake in other tissues (e.g., muscle), varying degrees of metabolically active BAT in the individuals tested, or both. Overall, the magnitude of BAT-derived EE induced by high caloric diets in humans was much smaller than cold-induced energy dissipation.

Energy expenditure (EE): energy used for physical activity, food digestion, breathing, and heat production; energy intake in excess of EE leads to weight gain

BROWN FAT IN HUMANS

Interscapular BAT is the major site of BAT in newborn humans and in rodents (18). Histomorphological autopsy studies of human infants revealed the presence of BAs not only in the interscapular region (22) but also in the mediastinum, renal and suprarenal fat depots, and para-aortic and neck

areas. Recent postmortem magnetic resonance (MR) imaging studies of human infants, combined with gene expression and histological analyses, confirmed the presence of BAT in the interscapular and supraclavicular regions (23).

BAT is widely distributed in all areas of the human body in the first decade of life (22). With age, BAT gradually disappears (22, 24); the more peripheral areas, such as the interscapular area, are the first to lose BAs. Because of the scientific focus on interscapular BAT, it was generally accepted that adults possess no active BAT, and the BAT depots in deeper areas, such as the neck, mediastinum, and around the kidneys, were neglected.

In 2009, PET/computer tomography (PET/CT) imaging based on cold-induced uptake of radioactively labeled glucose [2-deoxy-2-(^{18}F)fluoro-D-glucose (FDG)] detected metabolically active BAT in human adults, which led to a revival of scientific interest in BAT (25, 26). These PET studies demonstrated cold-induced glucose uptake in the supraclavicular, anterior neck, and paraspinal regions (6, 7). Biopsies confirmed the presence of UCP1-positive BAs in these fat depots (8, 9). BAT activity depends on age (BAT declines in older subjects), sex (BAT prevalence, BAT mass, and FDG uptake are higher in women), BMI, and ambient temperature (18). Although the prevalence of BAT in the FDG-PET/CT studies was only <10% (6), experimental studies using cold exposure to maximally stimulate BAT found metabolically active BAT in 50% to 100% of (mostly young) subjects (8, 27).

In summary, FDG-PET/CT studies reveal that adults possess metabolically active BAT, which is activated by cold, is found preferentially in winter (as compared to summer), is reduced in older subjects, and inversely correlates with BMI and white fat mass.

2-deoxy-2-(^{18}F)fluoro-D-glucose (FDG)-PET/CT: radioactively labeled glucose molecule taken up by metabolically active tissues (e.g., BAT and tumors); used in PET/CT scans

Cyclic guanosine monophosphate (cGMP): second messenger generated by guanylyl cyclases; activates lipolysis in human adipocytes and recruits constitutive and inducible BAT in mice

Natriuretic peptides (NPs): brain NP (BNP), atrial NP (ANP), and C-type NP (CNP) bind to particulate guanylyl cyclases that produce cGMP

Inducible BAs: also called beige/brite adipocytes; occur in WAT, express UCP1, and dissipate energy

COLORS AND TYPES OF BROWN ADIPOCYTES: MOLECULAR AND FUNCTIONAL CHARACTERISTICS

BAT is derived from the central dermomyotome that also gives rise to muscle and dermis (28). In contrast to WAs, BAs express a myogenic signature during differentiation (29). Seale et al. (30) showed in mice that cells expressing the transcription factor Myf5 could develop into muscle cells or BAs but not WAs. However, a recent study (31) indicates that the situation is more complex: A subset of WAs also arises from Myf5-positive precursors, and the metabolic significance of Myf5-positive or Myf5-negative origins is unknown. In addition to classical interscapular BAs, a second type of BA that resides in WAT depots has been identified and termed beige or brite (brown in white) cells (**Figure 1**). Beige cells share several characteristics with classical BAs, including multilocular fat droplets, a high mitochondrial content, and expression of a brown-like gene program, e.g., *UCP1* (26, 32, 33). Beige cells can be induced by cold and a broad spectrum of hormones and drugs (see 26, 32, and 33 and references therein) that include peroxisome proliferator–activated receptor γ (PPARγ) agonists and β-adrenergic agonists as well as substances that increase cyclic guanosine monophosphate (cGMP): natriuretic peptides (NPs) (34), and inhibitors of phosphodiesterase 5 (PDE5) (e.g., sildenafil) (35) (see the section titled Pharmacological BAT-Centered Therapies for details). Thus, the beige cells in WAT can also be defined as inducible BAs that constitute inducible BAT (**Figure 1**).

Different WAT depots exhibit a varying capacity for browning, i.e., they have varying occurrences of inducible BAs. Inguinal/subcutaneous WAT (iWAT) and perirenal depots have a greater propensity for the formation of brite cells, whereas the epididymal/perigonadal WAT (eWAT) is less susceptible to browning (see 36 and references therein). Similarly, human preadipocytes isolated from iWAT are more prone to browning than cells isolated from mesenteric or omental WAT (37). Depot-specific factors appear to determine not only the efficiency of browning but also the mechanism by which inducible BAs are formed. The vast majority of inducible BAs in iWAT

are derived from conversion of existing WAs (transdifferentiation) (38, 39). In contrast, almost all inducible BAs in eWAT newly differentiate from progenitors expressing stem cell antigen 1 (Sca-1), CD34, and platelet-derived growth factor receptor α (PDGFRα) (38). Interestingly, a bidirectional interconversion between WAs and inducible BAs can occur under certain stimuli (40).

Several studies indicate that inducible BAT influences whole-body metabolism and protects mice against diet-induced obesity (DIO) (36, 41–43). In contrast, inhibition of browning in mice on HFD resulted in late-onset obesity, hepatic steatosis, and insulin resistance (44).

Classical and inducible BAT are difficult to distinguish because of their molecular and functional similarities. In mice, *ZIC1*, *HOXC9*, and *TCF21* are the most specific markers for classical BAT, inducible BAT, and WAT, respectively (45). The situation is less clear in humans. The detection of beige cell markers in supraclavicular fat depots in human adults (46–48) spurred the idea that human adult BAT is composed of inducible BAs and not of classical BAs (47). Localization is apparently an important determinant for the type and color of fat: Recent studies (e.g., 49) found that superficial fat in the neck area shares features with WAT, whereas the more deeply localized fat has the characteristics of classical BAT. These recent data are in good agreement with earlier histological studies (e.g., 22), which demonstrated that BAT persists in the more deeply situated areas of the body but tends to be lost in more superficial areas much earlier in life during the aging process than in deeper regions.

In summary, beige/brite cells constitute an inducible type of BA that is competent in EE and make an important contribution to whole-body metabolism, at least in mice. From a therapeutic point of view, the debate about the true color of BAT in human adults appears to be more of academic interest than of practical and clinical consequence, as both inducible and constitutive BAs are similar in terms of energy dissipation.

Constitutive or classical BAs: brown adipocytes present in the interscapular BAT depots at birth; population diminishes in humans with age

Transient receptor potential (TRP) channels: cation channels expressed in sensory neurons activated by cold or capsinoids

β_3-adrenoceptor: G protein–coupled receptor; is highly expressed in brown adipocytes, is activated by norepinephrine, and stimulates adenylyl cyclase

SIGNALING PATHWAYS THAT REGULATE BAT

A complex network of hormones and signaling pathways regulates the differentiation and function of BAT (12, 24, 50–52). The vast majority of these signaling components have been discovered in cells isolated from mouse, rat, and hamster. Although some have been tested in animal models of genetic obesity or DIO, little is known about their role in humans, and human BA models are urgently needed. Here, we focus on those signaling pathways with therapeutic relevance; some of these are being tested in initial clinical trials (**Table 1**).

Hormones

Cold temperatures stimulate transient receptor potential (TRP) channels in peripheral sensory neurons, initiating a central activation of the sympathetic nervous system (SNS). SNS nerve fibers innervate BAT and release the catecholamine norepinephrine (NE). To date, researchers have focused mainly on NE and β-adrenergic activation of BAT (24), but cotransmitters released together with NE from SNS nerve terminals may also play a role in BAT regulation. In addition, a cold environment induces activation of tissue macrophages in BAT and WAT, which produce catecholamines and increase EE (53).

NE activates β-adrenoceptors on BAs. Although BAT expresses all three types of β-adrenoceptors (β_1, β_2, and β_3), the β_3-adrenoceptor predominates in mature BAs (24). Ablation of β_3-adrenoceptors in mice resulted in a modest metabolic phenotype (54), but mice that lacked all three adrenoceptors (β-less mice) were unresponsive to cold-induced stimulation of BAT (55). Apart from acute effects on lipolysis and BAT activation, extended cold exposure produces

Table 1 Physiological and pharmacological approaches as well as published and ongoing human clinical trials investigating BAT function and regulation

	Intervention/analysis	Patients/probands	Results	NCT number and/or reference(s)[a]
1. Physiological approaches				
BAT activation	Analysis of existing PET/CT scans	Healthy subjects	FDG uptake: ↑ in women, ↑ at low outdoor temperatures, ↓ with age	6
		Patients with adrenal tumors	Not yet published; will investigate effects of catecholamine overproduction on BAT	NCT01949714
	Cold exposure (16–19°C), single 2-hour exposure	Healthy subjects	FDG uptake: supraclavicular and paraspinal; BAT activity correlates negatively with BMI	7–9
	Cold exposure to an extent that it gives an unpleasant feeling, 1 hour daily for 6 weeks	Healthy subjects	Not yet published	NCT01797328
	Cold exposure (4°C), 20 min daily for 4 weeks	Healthy men, BMI 18–24	Not yet published	NCT01898949
	Cold exposure at −30°C, −60°C, and −110°C, 1 to 3 times a week for 4 months	Obese subjects (BMI > 30), nondiabetic	Not yet published	NCT01312090
	VNS treatment	Epilepsy patients	VNS: ↑ EE, ↑ BAT activity	NCT01491282, 153
2. Pharmacological approaches				
Thyroid hormones	Thyroid hormone replacement therapy, i.e., levothyroxine, trade name Euthyrox®	T2DM patients with hyperthyroidism	Not yet published; will investigate the effects of thyroid hormone treatment on T2DM patient metabolism and BAT activity	NCT01379170
	PET/CT imaging and measurement of whole body EE	Patients with hyperthyroidism	Hyperthyroidism: ↑ BAT activity	64
	Methimazole treatment and PET/CT scan	Patients with untreated hyperthyroidism	Not yet published; will investigate the effects of hyperthyroidism on BAT activity	NCT01376648
β_3-adrenergic receptor agonist	β_3-adrenergic receptor agonist treatment and cold exposure (13–16°C) (PET scanning, and resting metabolic rate measurement)	Healthy men, BMI 18–40	Not yet published; will investigate the effects of β_3-adrenergic receptor agonist on BAT activation and compare it to cold exposure	NCT01783470

(*Continued*)

Table 1 (*Continued*)

	Intervention/analysis	Patients/probands	Results	NCT number and/or reference(s)[a]
β-adrenergic receptor agonist	Treatment with β-adrenergic receptor agonist (ephedrine or isoproterenol) followed by PET/CT scan	Healthy men	No BAT activation after ephedrine and isoproterenol treatment	123, 154
Norepinephrine transporter	NA	Healthy subjects, BMI < 24	Not yet published; will investigate a novel approach to image BAT using the norepinephrine transporter	NCT02038595
Glucagon	Glucagon administration (thermal imaging, indirect calorimetry, and PET scanning)	Healthy subjects	Not yet published; will investigate the effects of glucagon administration on BAT activity	NCT01935791
Capsinoids	Capsinoid ingestion (thermographic imaging and indirect calorimetry)	Healthy men, BMI 18–23	Not yet published; will investigate the effects of capsinoid ingestion on BAT activity	NCT01961674
	Capsinoid ingestion for 6 weeks (cold exposure, PET/CT imaging, measurement of EE)	Healthy men	Capsinoids: ↑ EE, ↑ CIT	27
	Capsinoid ingestion for 12 weeks (indirect calorimetry and measurement of body composition)	Obese subjects	Capsinoids: ↑ loss of abdominal adipose tissue	129
Natriuretic peptides	Infusion of recombinant human BNP, i.e., nesiritide	Lean and obese subjects	Not yet published; will investigate effects of nesiritide on gene expression profile in adipose tissue	NCT01977859
L-arginine	L-arginine supplementation in addition to hypocaloric diet	Obese T2DM patients	↓ body weight beyond that achieved with a hypocaloric diet	135
FGF21	LY2405319 treatment for 28 days	Obese T2DM patients	Insignificant ↓ body weight, ↓ apolipoproteins and lipids (significant in high-dose groups treated with 10 and 20 mg LY2405319)	137
Melanocortin	Detection of mutations within MCR4 and correlation with metabolic data	Probands with severe childhood obesity	MCR4 deficiency: obesity, ↑ food intake	59

Abbreviations: BAT, brown adipose tissue; BMI, body mass index; BNP, brain natriuretic peptide; CIT, cold-induced thermogenesis; CT; computer tomography; EE, energy expenditure; FDG, 2-deoxy-2-(^{18}F)fluoro-D-glucose; MCR4, melanocortin receptor 4; NA, not applicable; PET, positron emission tomography; T2DM, type 2 diabetes mellitus; VNS, vagus nerve stimulation.

[a]National Clinical Trial (NCT) numbers from **http://www.clinicaltrials.gov/**.

profound morphological changes (see 19 and references therein) and an increase in BAT mass (BAT recruitment) (40).

Melanocortin (MC) is a hormone system in the central nervous system; the MC receptor type 4 (MCR4) is especially important for BAT activity. The MCR4 pathway is involved in the regulation of SNS outflow, thereby controlling BAT (56). Deletion of MCR4 in mice led to abrogation of UCP1 induction (57). Moreover, dysfunction of the MC signaling cascade resulted in obesity in mice and humans (58, 59).

The thyroid hormone triiodothyronine (T3) is a well-established inducer of mitochondrial biogenesis (60) and UCP1 expression (61). T3 is also an important component of cocktails used to differentiate brown preadipocytes (62). The thyroid and adrenergic signaling pathways are linked; hypothyroidism reduces adrenergic effects in BAT (see 63 and references therein), whereas hyperthyroidism increases BAT activity (64). The type 2 iodothyronine deiodinase (D2) plays a critical role in the local conversion of the prohormone thyroxine into T3 in BAT (see 65 and references therein), and D2-null mice have an impaired response to cold (63). D2 is also induced by bile acids. Administration of bile acids increases EE in murine BAT, thereby preventing obesity and insulin resistance (66).

Other hormones regulate classical and inducible BAs and are potential drug targets. Several members of the transforming growth factor-β superfamily, particularly bone morphogenetic proteins (BMPs), play significant roles in adipogenesis (67). BMP2 and BMP4 promote commitment of pluripotent stem cells to the adipocyte lineage and recruit inducible BAs within WAT depots (68). BMP7 is crucial to brown adipogenesis (69) and reduces body weight by increasing EE and reducing appetite and food intake (70). BMP8b, which is induced by nutritional and thermogenic factors, increases BAT thermogenesis via both central (SNS activation) and peripheral actions (71).

Irisin, a myokine secreted by skeletal muscle after cleavage from fibronectin-type III domain-containing protein 5 (FNDC5), was first identified in mice and humans after exercise (72). Because it enhances inducible BAT and protects against DIO in mice, irisin may mediate the positive effect of exercise on metabolic health, at least in part. However, the physiological relevance of irisin in humans is controversial (73). On the one hand, some studies did not find evidence for an increase in irisin after exercise training (74, 75). On the other hand, a recent study showed that shivering increases irisin levels in cold-exposed humans and that FNDC5 stimulates the thermogenic activity in human adipocytes (76). This study also showed a functional link between irisin and fibroblast growth factor 21 (FGF21): Both hormones have additive effects on inducible BAs. FGF21 is involved in the control of glucose metabolism and ketogenesis. It is mainly expressed in the liver but is also found in BAT (see 77 and references therein), and thermogenic activation induces FGF21 release (78).

Another member of the FGF family, FGF19, appears to regulate BAT, as its administration or transgenic overexpression in mice increases BAT mass and EE (79, 80). Prostaglandins also contribute to central BAT activation and BA induction in WAT (43, 81).

Melanocortin (MC): group of peptide hormones produced in the brain; acts via G protein–coupled receptors such as MCR4

Bone morphogenetic proteins (BMPs): hormones of the TGFβ-superfamily that are involved in differentiation of classical BAs and recruitment of inducible BAs

Irisin: hormone (myokine) secreted by skeletal muscle after exercise; potentially induces BAs in WAT

Fibroblast growth factor (FGF): fibroblast growth hormones such as FGF21 and FGF19 play a role in BAT regulation

Cyclic adenosine monophosphate (cAMP): second messenger generated by adenylyl cyclases; activates lipolysis and mediates BAT thermogenesis after NE stimulation

Second Messengers

Second messengers are important for transmitting hormonal signals from receptors at the cell membrane into the cytosol of adipocytes (**Figure 2**). Important second messengers in adipocytes are cyclic adenosine monophosphate (cAMP) and cGMP and their respective signaling networks.

cAMP production by adenylyl cyclases is activated by G protein–coupled receptors via Gαs. In adipocytes of all colors and origins, cAMP is a key messenger that activates cAMP-dependent protein kinase (PKA). Hormone-sensitive lipase and perilipin A are important targets of PKA (11).

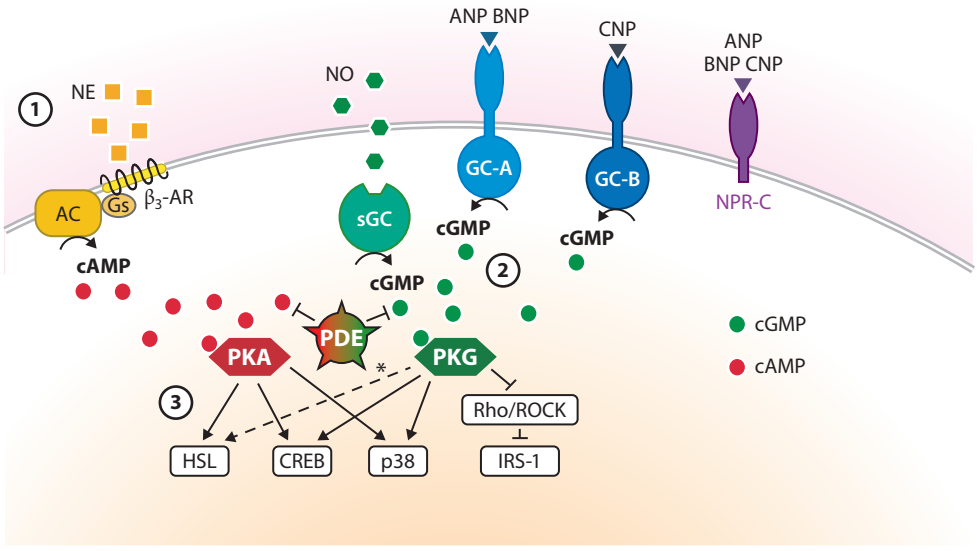

Figure 2

Second messenger signaling in adipocytes. The pathways driven by the second messengers cAMP (pathway shown in red/yellow) and cGMP (pathway shown in green/blue) are central to the activation and recruitment of adipocytes. ① NE released by the SNS binds to β_3-AR, which activates AC in a G protein–coupled manner to produce cAMP. PKA is the major effector molecule of the cAMP pathway. ② cGMP is produced by sGC, GC-A, and GC-B, which are activated by NO, BNP and ANP, and CNP, respectively. NPR-C binds natriuretic peptides but lacks cGMP-generating properties. PKG is the major effector molecule of cGMP. ③ Downstream targets (*white boxes*) include HSL, transcription factors, or MAPKs. PDEs break down cAMP and cGMP. The dashed line and asterisk indicate species-specific PKG-dependent activation, which is observed in humans but not in rodents. Abbreviations: β_3-AR, β_3-adrenoceptor; AC, adenylate cyclases; ANP, atrial natriuretic peptide; BNP, brain natriuretic peptide; cAMP, cyclic adenosine monophosphate; cGMP, cyclic guanosine monophosphate; CNP, C-type natriuretic peptide; CREB, cAMP response element–binding protein; Gs, stimulatory G protein; GC-A and GC-B, particulate guanylyl cyclases; HSL, hormone sensitive lipase; IRS-1, insulin receptor substrate 1; MAPK, mitogen-activated protein kinase; NE, norepinephrine; NO, nitric oxide; NPR-C, natriuretic peptide receptor C; p38, p38-MAPK; PDE, phosphodiesterase; PKA, cAMP-dependent protein kinase; PKG, cGMP-dependent protein kinase; Rho, small GTPase Rho; ROCK, Rho-associated kinase; sGC, soluble guanylyl cyclase; SNS, sympathetic nervous system.

PKA-mediated phosphorylation induces translocation of the lipase to the surface of lipid droplets and enhances its catalytic activity (82). In addition to this major cAMP/PKA pathway, a PKA-independent cascade can activate lipolysis at higher agonist concentrations (83). The released free fatty acids are then shuttled into mitochondria, where they activate UCP1. The cAMP/PKA pathway also activates UCP1 expression through a complex process involving multiple signaling modules, including p38–mitogen-activated protein kinase, activating transcription factor 2, cAMP response element–binding protein, and PPARγ coactivator–1α (PGC-1α) (84). Moreover, cAMP/PKA-mediated activation of the Src and extracellular signal-regulated kinase 1 and 2 (ERK1/2) pathways inhibits apoptosis (85).

BAT research has focused mainly on cAMP, but cGMP also plays an important role (86–88). It is generated by two types of guanylyl cyclases (GCs): soluble GC (sGC) and particulate GCs (pGCs). sGC is activated by nitric oxide (NO) (89), which is synthesized by NO synthases (NOSs) from the

amino acid L-arginine (L-Arg). The three isoforms of NOS are inducible NOS, endothelial NOS (eNOS), and neuronal NOS. eNOS is abundantly expressed in vessels of the highly vascularized BAT and in BAs (90, 91). Mice deficient in eNOS displayed reduced mitochondrial biogenesis and impaired BAT-dependent thermogenesis (92). pGCs, by contrast, are activated by binding of atrial, brain, and C-type NPs (ANP, BNP, and CNP, respectively) (see 86 and references therein). The two pGCs that synthesize cGMP are NP receptors A and B (GC-A and -B). NP receptor C (NPR-C) has no GC activity but acts as a clearance receptor (93) (**Figure 2**).

microRNAs (miRNAs): small noncoding RNA molecules that regulate gene expression and are involved in BAT regulation

The effects of NPs and NO in BAs are mediated by cGMP (91, 92). cGMP signals through three major downstream mechanisms (88): cGMP-dependent protein kinases (PKGs), cGMP-regulated ion channels, and PDEs (94–96). PDEs can be activated by cyclic nucleotides and hydrolyze cGMP, cAMP, or both (97) (**Figure 2**). Two PKGs, PKGI and PKGII, have been identified in mammals (94–96). PKGI enhances insulin signaling through inhibition of the small G protein Rho and is essential for normal adipogenic as well as thermogenic differentiation of BAs (91). PKGI-deficient mice had severely disturbed development of BAT and reduced BAT-dependent thermogenesis (91). In contrast, mice with transgenic overexpression of PKGI displayed increased expression of UCP1 and enhanced mitochondrial biogenesis and were protected from DIO (98). A major downstream target of PKGI is the vasodilator-stimulated phosphoprotein (VASP). Unexpectedly, VASP ablation leads to increased cGMP signaling in adipocytes and enhanced browning of iWAT, suggesting the existence of a negative feedback loop that regulates cGMP levels in BAs (99). These studies demonstrate the pivotal role cGMP signaling plays in the development of BAT as well as its function as a central mediator in the recruitment of brite cells.

TRANSCRIPTION FACTORS

BAs and WAs share many features, and adipogenesis is quite similar in both cell types (50, 100). After differentiation is induced, the CCAAT/enhancer-binding protein (C/EBP) family of transcription factors plays important roles in adipogenesis (100). C/EBPβ activates transcription of C/EBPα and PPARγ, the master transcription factor of adipogenesis (101). C/EBPα is required for differentiation of WAT but not BAT (102). In contrast, the crucial role played by C/EBPβ in BAT (see 103 and references therein) may result from differences in the temporal dynamics of C/EBPβ expression (104) and from its interaction with coregulatory protein PR domain containing 16 (PRDM16) (105). However, ablation of PRDM16 leaves BAT functionally intact (44). Another important regulator is the transcriptional coactivator PGC-1α, which promotes mitochondrial biogenesis and is essential for BAT thermogenesis but not BA differentiation (106).

microRNAs

microRNAs (miRNAs) are a class of small noncoding RNAs that regulate gene expression in plants and animals (107, 108). They are encoded in the genome and are processed to hairpin RNAs approximately 22 nucleotides long.

Initial profiling studies revealed that WAs and BAs express different miRNA patterns, with classical myogenic miRNAs being expressed in BAs but not WAs (109). Recently, miRNAs that regulate BA differentiation and function have been identified (110–112). Two important examples are miR133, which targets PRDM16 and is downregulated after cold exposure (112), and miR155, which is highly expressed in brown preadipocytes but declines after induction of differentiation (110). miR155 targets C/EBPβ, which in turn regulates miR155 expression; thus, miR155 and C/EBPβ form a bistable feedback loop that integrates pro- and antiadipogenic cues. Such bistable loops have been identified in other developmental processes and lead to a robust commitment to one of two possible states (see 110 and references therein).

miRNAs also regulate inducible BAT. Depletion of miR155 increased C/EBPβ expression in WAs, and miR155-deficient mice exhibited increased browning of iWAT (110). miR196, which is induced after cold exposure in WAT, is also linked to C/EBPβ, albeit in a different manner: miR196 suppresses the white fat gene *HOXC8*, which represses C/EBPβ; miR196 thus releases C/EBPβ from suppression by *HOXC8* and induces browning (113).

This picture is far from complete: In vitro experiments indicated that miR193b-365 plays an important role in BA differentiation (111); however, BAT function was not affected by ablation of the *miR-193b-365-1* locus in mice (114).

In summary, miRNAs and other noncoding RNAs, such as long noncoding RNAs (108), apparently constitute an additional layer of BAT regulation. This may be similar to what occurs in cancer, in which certain miRNAs have positive or negative effects on tumor growth (oncogenic and antioncogenic miRNAs, respectively) (107). A potential approach to increase BAT mass might be to increase proadipogenic miRNAs or reduce antiadipogenic miRNAs. Furthermore, miRNAs may be potential biomarkers for BAT mass and function.

BAT DIAGNOSTICS

The initial identification of functional BAT in adult humans was based on FDG-PET/CT, which is most often used clinically to detect metabolically active metastases. Although FDG-PET/CT has been the modality of choice to monitor BAT activity in humans, it has several drawbacks: It only detects active BAT. Moreover, because of the risks associated with exposure to ionizing radiation, it can be used only to a limited extent for repetitive studies. Thus, novel approaches are needed to analyze the abundance and function of BAT in humans.

MR-based imaging using an array of relaxometry-based (T1, T2) and chemical shift–based approaches (115) can differentiate fat tissue from lean tissue as well as identify BAT (49). MR-based imaging has great promise for characterizing human BAT morphology and metabolic activity, but imaging human BAT with MR is challenging, and further development of MR-based strategies is needed (115). MR-based imaging can also be combined with nanotechnology to analyze BAT function (17). Other imaging technologies, such as near-infrared fluorescence imaging coupled to organ homing peptides, have been used in rodents to detect BAT depots (116).

In addition to imaging approaches, BAT biomarkers could monitor BAT activity in basic research and clinical medicine. Biomarkers are molecules that indicate a certain biological state, such as the metabolic activity of BAs. Unfortunately, no BAT biomarkers that can easily be accessed by blood analysis are presently available. In addition to different omics (e.g., metabolomics and secretomics) approaches, miRNAs are of major interest as potential BAT biomarkers. Although little is known about the origin and function of circulating miRNAs, the diagnostic power of miRNAs has been clearly established in the field of oncology, as circulating miRNAs can be used to detect tumors and assess disease progression and therapeutic outcome (117). Because miRNAs are differentially expressed in BAT, they may prove to be useful BAT biomarkers.

THERAPEUTIC APPROACHES: BAT-CENTERED THERAPIES

Obesity treatments center mainly on reducing energy intake through a healthy, low-calorie diet and increasing EE through exercise. These behavior-centered approaches have met only limited success in abating the worldwide obesity pandemic. Other antiobesity treatments such as bariatric surgery (e.g., gastric banding and gastric bypass surgery) can result in long-term weight loss, but they are major surgical procedures that can pose serious risks. Therefore, there is an urgent need for safe and effective antiobesity pharmacotherapy. Despite the known relationship between

fat mass and obesity comorbidities, only a few approved drugs directly target WAT (118). No pharmacological therapies are available that selectively target BAT.

Pharmacological BAT-Centered Therapies

To rebalance energy homeostasis in obese patients, pharmacological therapies aim to either decrease energy intake or increase EE. Approved drugs address energy input and function mainly by reducing appetite or calorie intake. Only one antiobesity drug is currently approved by the FDA for long-term use: orlistat, a lipase inhibitor that reduces intestinal absorption of fat. Moveover, the FDA approved combined use of phentermine (a sympathomimetic amine) and topiramate (trade name Qsmia®, an anticonvulsant that lowers weight through unknown mechanisms) in 2012 as a means to lower appetite and as an adjunct to a reduced-calorie diet and exercise for chronic weight management in overweight adults with at least one weight-related comorbidity such as hypertension or T2DM (**http://www.fda.gov**).

An alternative approach to treat obesity would be to increase EE. Given its large capacity to dissipate energy, BAT is a potentially promising target to accomplish this. There are several possible therapeutic targets that regulate BAT (see the section titled Signaling Pathways that Regulate BAT). Although potential drug candidates are available, data on their effects in human BAT are limited.

There are two general strategies for future BAT-centered therapies: (*a*) activating BAs (activators) and (*b*) increasing BAT mass (recruiters). The latter is also the major focus of cell- and gene-based therapies.

Activating BAs. Activated BAT has beneficial effects on metabolic health and counteracts hyperlipidemia, obesity, and diabetes, at least in mice. Given the physiological role of BAT in cold-induced thermogenesis, cold exposure is a major activator of BAT in lab animals and humans. Repetitive exposure to cold (daily 2-hour exposure to 17°C for 6 weeks) increased BAT activity in humans and significantly decreased body fat mass (27). However, cold-induced activation causes discomfort and may have unwanted side effects, such as altered lipid profiles and accelerated development of atherosclerotic plaques (119). These potential side effects could be especially problematic in patients with comorbidities of obesity and may enhance the risk of cardiovascular events. Importantly, decreased ambient temperature has been linked with an increased incidence of and mortality by cardiovascular disorders such as myocardial infarction and stroke (120, 121).

An alternative approach to cold-induced BAT activation is to stimulate BAs through endogenous signaling pathways. Obese rats, dogs, and mice showed antiobesity responses when treated with a β_3-adrenergic agonist (11). β_3-adrenoceptors induce lipolysis and increase BA markers in human subcutaneous and visceral adipocytes (122), but β_3-agonists have not been successful in humans (see 25 and references therein), perhaps because human WAT and inducible BAs express β_3-adrenoceptors only at low levels (19).

Therefore, stimulation of adrenoceptors other than β_3 may be a means to reduce body weight. However, nonspecific stimulation of β-adrenoceptors throughout the body has important side effects, especially in the cardiovascular system, where it can cause arrhythmias and hypertension. PET studies in humans revealed that administration of the nonspecific β-adrenergic agonist isoproterenol at concentrations that induced EE without adverse effects did not result in significant activation of BAT in 9 of 10 subjects (123), presumably because a low concentration of the drug was used. Thus, pharmacological strategies are needed that will activate BAT and EE in obese patients independently of β-adrenoceptors.

Endogenous hormones such as NPs (see above) are potential candidates for alternative BAT activators. In mice, administration or transgenic overexpression of BNP (98) increased expression of

UCP1, PGC-1α, and mitochondrial markers in WAT and BAT (34). NPs can also activate lipolysis, although there are important species differences: NPs stimulate lipolysis in human adipocytes in vitro and in vivo (124, 125) but not in rodents, perhaps because rodent adipocytes express a relatively high level of the clearance receptor NPR-C. NP action on human BAs has so far been tested only in human multipotent adipose-derived stem cells (hMADS), a brown-like human cell line (126). In this cell line, ANP increases lipolysis and oxygen consumption rates (34). Because of the lack of human in vivo data, it is not presently clear whether NPs can be used to activate human BAT to fight obesity. Furthermore, potential side effects have yet to be ruled out (see below).

Capsinoids: nonpungent substances from chili peppers that activate TRP channels

In addition to direct activation of BAs, indirectly or centrally acting drugs could be used to activate BAT. Following observations of their potential positive effects on EE in mice, MCR4 agonists have been tested in a primate model of obesity and in humans. This treatment reduced body weight in monkeys; however, cardiovascular side effects (increased blood pressure and heart rate) were observed in humans (127, 128).

Increasing BAT mass. Pharmacological activation of BAT can work only if sufficient amounts of BAT are present, but only ~0.05% of the human body mass is BAT (18). β-adrenergic stimulation causes proliferation of brown preadipocytes (see 24 and references therein). However, as noted above, chronic use of β-adrenergic agonists can cause severe cardiovascular side effects.

Because TRP channels on sensory neurons play an important role in cold-induced stimulation of the SNS, stimulation of TRP channels by substances such as capsinoids may enhance BAT recruitment. Promising results were obtained after prolonged treatment (6–12 weeks) with capsinoids, which reduced body fat and increased EE, indicating that capsinoids can recruit human BAT (27, 129). Given the risk of exposure to ionizing radiation, subjects did not undergo repeated FDG-PET/CT measurements. Nevertheless, the data provided evidence for increased BAT activation after drug treatment, even in subjects with low or undetectable BAT activity (27, 129).

Promising results have been achieved in animal models and in vitro with cGMP-based approaches that seek to increase inducible BAT. Administration of the PDE5 inhibitor sildenafil induced browning in mice after only seven days of treatment (35). PDE5 is the major PDE that hydrolyzes cGMP in human adipocytes (130). Moreover, chronic (12-week) administration of sildenafil to mice on HFD decreased weight gain, reduced fat mass, and increased EE (131). As PDE5 inhibitors are well tolerated and widely used for the treatment of erectile dysfunction and pulmonary hypertension, this class of drugs has potential as antiobesity agents that increase the abundance of inducible BAs. Further studies are needed to clarify the dose and duration of treatment required to achieve this effect in humans. Just as cGMP enhances browning of WAT, increasing cGMP via BNP/GC-B caused browning effects and increased EE in mice, similar to PDE inhibitors (34). Unfortunately, side effects that include worsening of renal function and increased mortality have been identified in meta-analyses of clinical trials of patients with acute decompensated heart failure (ADHF) treated with recombinant BNP (nesiritide) (132). Such side effects have not been observed in ADHF patients who received different BNP dosing or in patients with other cardiac diseases (133). Hence, BNP may be beneficial in obesity, but unwanted side effects associated with comorbidities and different doses of BNP must be ruled out.

Stimulation of cGMP signaling could also be achieved by supplementation with the NO precursor L-Arg. In rats, dietary supplementation with L-Arg reduced weight gain in a model of DIO (134). Intake of L-Arg also has antiobesogenic effects in humans: In a 21-day clinical trial, L-Arg intake significantly reduced body weight beyond the reduction achieved with a hypocaloric diet and exercise (135).

Another approach to enhance BAT recruitment could be to use endogenous hormones (described above), such as members of the BMP and FGF families. Incubation with BMP4 or BMP7 increased expression of brown or thermogenic markers in isolated human adipocytes (136). Recombinant BMP7 is already used in humans to treat bone diseases; therefore, using BMP7 to boost BA activity is an attractive concept.

T2DM patients treated with the FGF21 analog LY2405319 (LY) for 28 days had a less atherogenic profile of serum apolipoproteins and lipids and had reduced fasting insulin levels (137). Adverse effects, including global allergic reactions and antibodies against LY, were detected in the majority of subjects receiving 10 or 20 mg LY. Body weight was reduced, albeit not significantly compared to placebo. Prolonged LY treatment may decrease body weight by enhancing inducible BAT, as observed in animal models (138–140). FGF19 is another endogenous hormone that could be useful as a BAT recruiter. In humans, FGF19 contributes to the remission of T2DM after gastric bypass surgery (141). Hence, these members of the FGF family may have beneficial effects in obesity, but the effects of FGFs on BAT in humans remain unreported. Again, potential side effects of long-term use must be addressed, as FGF19 may be involved in hepatocellular and prostate cancer (142, 143).

Cell- and Gene-Based Approaches

The rationale behind BAT transplantation and gene therapy strategies is that increasing BA number should increase EE and counteract metabolic syndrome. Several groups found that transplantation of engineered preadipocytes or brown fat pads yielded functional BAT tissue in mice. Transplantation of fibroblasts expressing PRDM16 and C/EBPβ into nude mice resulted in the formation of adipose tissue that contained multilocular cells, expressed BAT-specific genes, and was labeled by FDG in PET/CT scans (105). Furthermore, transplantation of BAs derived from human embryonic stem cells or induced pluripotent stem cells into mice established adipose tissue that exhibited features of BAT and lowered fasting blood glucose levels for at least 3 weeks (144).

Whole BAT can also be used for transplantation: Transplanted BAT was revascularized within 8 weeks after transplantation (145) and improved the metabolic profile in recipient HFD-fed mice, decreasing weight gain, improving glucose clearance, and increasing EE (145, 146). Unexpectedly, BAT transplantation enhanced the whole-body metabolic response with increased insulin-stimulated glucose uptake into other organs (e.g., WAT, heart muscle, and endogenous BAT), indicating that transplanted BAT had paracrine or endocrine effects or both on other organs (147).

These transplantation experiments show that transplanted BAT counteracts HFD-induced obesity in mice, but important unresolved issues remain. Efficacy may depend on the site of BAT implantation (146–148), and decay of transplanted BAs may occur (146).

Gene therapy approaches have focused mainly on the delivery of genes that enhance browning. One simple but far-reaching idea is to generate transgenic mice that express UCP1 in WAT; body weight and WAT mass were reduced significantly in such mice (149). To further extend these findings, adenoviral vectors were used for ectopic expression of UCP1 in WAT (150). Although the levels of UCP1 protein achieved in WAT were far below (<10%) those found in BAT, a markedly positive effect on the metabolic phenotype was observed in mice on HFDs (150). Vectors derived from lentiviruses, which permanently integrate into the host genome (151), have also been used to transfer genes in BAs and WAs in vitro and in vivo (35, 91, 152).

SUMMARY POINTS

1. BAT has high metabolic activity and could help to rebalance energy homeostasis in obese patients.
2. Recent studies clearly demonstrated the presence of metabolically active BAT in adult humans; however, debate remains about the true color and nature of BAT.
3. Major progress has been made in understanding the origins and regulation of BAT.
4. Human BA cell lines and models are needed to validate the potential drug targets identified in animal models and identify new targets.
5. New diagnostic tools for human BAT, such as MR imaging and biomarkers, are urgently needed to translate basic BAT research into clinical studies.

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