Good fat or bad fat?
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The concept of “bad cholesterol” versus “good cholesterol” has received considerable attention in both the scientific and public media. This discussion has now recently extended to adipose tissue, with the concept of “bad fat” versus “good fat.” The major storage depot of fatty acids in the body is contained within white adipose tissue, and the dramatic rise in the incidence of obesity in our society has resulted in marked increases in the amount of white adipose tissue fatty acid stores in the population. Since obesity is a risk factor for a number of diseases, including heart disease and diabetes, these white adipose tissue stores have been labeled the “bad fat.” However, a second type of adipose tissue exists, termed brown adipose tissue, that primarily burns fat as opposed to storing fat. This “good fat” has a high mitochondrial content (giving this fat its brown color), which can readily oxidize fatty acids. A high expression of uncoupling proteins in the mitochondria of this brown adipose tissue results in the production of heat rather than energy stores.

It has long been known that brown adipose tissue in many mammalian species living in cold climates is important in thermogenesis, by burning fat to produce heat. Until recently, however, it was thought that brown adipose tissue was not present in humans in any consequential degree, except during the neonatal period. However, with the development of more sophisticated imaging techniques, brown adipose tissue has been identified in the subcapular and cervical regions of adult humans. Importantly, it has also been shown that the amount of brown adipose tissue is decreased in obesity, insulin resistance, and diabetes. This raises the possibility that increasing brown adipose tissue may be an approach to treating obesity and diabetes by increasing whole-body metabolic rates. Articles in this issue of Heart and Metabolism review the topical area of brown adipose tissue metabolism, as well as the possible therapeutic potential of increasing brown adipose tissue stores in the body. The concept of “beiging” white adipose tissue to increase metabolic rates is also addressed. The potential of increasing stores of “good fat” provides an exciting new approach to combating obesity, diabetes, and heart disease.

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Brown adipose tissue and the genetics of obesity

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Abstract
Obesity is associated with a number of metabolic diseases and various cancers. At the biological level, obesity is caused by a shift in the body’s energy balance toward energy abundance. The main function of one type of adipocyte called brown adipocytes, found in brown adipose tissue (BAT), is to dissipate energy as heat—generated through action of uncoupling protein 1 (UCP1)—in response to certain physiological stimuli, a process called adaptive thermogenesis. A second type of UCP1-positive thermogenic adipocyte appears in the subcutaneous white adipose tissue (WAT) in response to chronic exposure to cold or to agonists for β3-adrenergic receptor (β3-AR) and peroxisome proliferator–activated receptor γ (PPARγ); these are called beige/brite adipocytes. Brown and beige/brite cells originate from different developmental lineages and the energy expenditure ability of these cells gave us great hope that BAT can be manipulated to treat obesity. Here, I review the progress made thus far in understanding the developmental origins of brown and beige cells and the molecular regulation of BAT-mediated thermogenesis, studied in various knockout and transgenic mouse models. ■ Heart Metab. 2016;69:4-8

Keywords: beige cells; brown fat; genetic mouse models; oxidative metabolism; PGC1α; thermogenesis; UCP1

Obesity is a significant risk factor for a number of diseases, such as chronic heart disease, hypertension, type 2 diabetes, fatty liver disease, and various cancers.1 For the past few decades, obesity has steadily increased, not only in the well-developed Western nations, but also throughout the world. According to the World Health Organization (WHO), around 2.1 billion people are obese or overweight. Some of the important contributing factors of obesity include cheap energy-rich diets, sugary drinks, technology-dependent lifestyles, and lack of physical activity. At the biological level, these factors shift the body’s energy balance toward energy abundance, which ultimately causes obesity. If energy (food) consumption is chronically higher than expenditure, the extra energy is stockpiled in the white adipose (fat) tissue (WAT) as triglycerides. Conversely, if energy consumption is lower than expenditure, triglycerides in the WAT are broken down for use by other organs. Thus, WAT functions as the body’s energy storage and supply center.

Brown adipose tissue

Another type of adipose tissue, called brown adipose tissue (BAT), also exists in rodents and in humans.
The main function of BAT is to dissipate energy as heat in response to cold temperatures and probably to diet. This process is called adaptive thermogenesis; small mammals living in cold conditions produce heat by efficiently employing BAT. BAT is rich in mitochondria and has a high cellular respiration, but a very low capacity for ATP synthesis because of low levels of ATP synthase and expression of a unique protein called uncoupling protein 1 (UCP1). BAT generates heat through UCP1, which is located in the inner mitochondrial membrane. UCP1 uncouples oxidative phosphorylation from ATP production and releases chemical energy as heat, thereby increasing the body’s energy expenditure. Recent studies demonstrated a second type of UCP1-positive thermogenic adipocyte in the subcutaneous WAT; adipocytes of this type are called beige/brite (brown in white) cells. These cells appear only in response to exposure to chronic cold and agonists for \( \beta_3 \)-adrenergic receptor (\( \beta_3 \)-AR) or peroxisome proliferator–activated receptor \( \gamma \) (PPAR\( \gamma \)) and display several morphological and biochemical characteristics similar to brown adipocytes. The energy dissipation ability of BAT and beige/brite cells gave us the idea of exploiting BAT to treat an energy-abundant obese condition. However, in order to execute this idea it is essential to understand the development of brown and beige adipocytes and the molecular regulation of BAT-mediated thermogenesis. In this regard, extensive research in various genetic mouse models has provided in-depth insight into genes that play critical roles in BAT origin and differentiation and in BAT-associated thermogenesis.

Abbreviations

- 3-AR: \( \beta_3 \)-adrenergic receptor
- BAT: brown adipose tissue
- cAMP: cyclic adenosine monophosphate
- Cidea: cell death–inducing DNA-fragmentation factor
- FOXC2: forkhead box C2
- LXR\( \alpha \): liver-X-receptor \( \alpha \)
- NRF1: nuclear respiratory factor 1
- PGC1\( \alpha \): peroxisome proliferator activated–receptor gamma coactivator 1 \( \alpha \)
- PKA: protein kinase A
- PRDM16: PR domain containing 16
- Rip140: receptor-interacting protein 140
- SRC1: steroid receptor co-activator 1
- Twist1: Twist family basic helix-loop-helix transcription factor 1
- UCP1: uncoupling protein 1
- WAT: white adipose tissue

Developmental origins of brown and beige/brite cells

Brown adipocytes (interscapular and perirenal BAT) and beige/brite cells originate from different developmental lineages. The progenitor cells of the embryonic mesoderm that transiently express the myogenic factor 5/paired box 7 genes (Myf5/Pax7\(^{-} \)) give rise to skeletal muscle cells or brown adipocytes. In these cells, early B-cell factor 2 (EBF2) and PPAR\( \gamma \) cooperatively induce the expression of the gene named PR domain containing 16 (Prdm16), which determines the brown-adipocyte-cell fate. The PRDM16 protein regulates the transcriptional activity of various thermogenic genes, such as PPAR\( \alpha \), PPAR\( \gamma \), and PPAR\( \gamma \) coactivator 1\( \alpha \) (PGC1\( \alpha \)), thus functioning as a central regulator of brown-adipocyte-cell fate. Genetic ablation of Prdm16 disrupts cell fate determination between brown adipocytes and myocytes. Prdm16-deficient brown-adipocyte precursors display elevated expression of skeletal-muscle-cell genes such as myogenin. Myogenin-knockout mice totally lack differentiated skeletal muscle, but have an enlarged interscapular BAT. Conversely, forced expression of Prdm16 stimulates beige-cell formation in subcutaneous WAT, and transgenic mice overexpressing PRDM16 display increased energy expenditure and reduced weight gain in response to a high-fat diet. As an upstream regulator of PRDM16, EBF2 determines brown-adipocyte differentiation, and induced expression of Ebf2 reprograms white preadipocytes into brown adipocytes. Consequently, brown adipose cells and tissues from Ebf2-knockout mice show a loss of brown-specific characteristics and thermogenic capacity.

In response to various stimuli, such as cold and \( \beta_3 \)-AR or PPAR\( \gamma \) agonists, pools of UCP1-expressing beige/brite cells arise in WAT, mainly in inguinal WAT. Beige cells come from a Myf5-negative cell lineage, and recent studies indicate that they arise from platelet-derived growth-factor-receptor \( \alpha \) (Pdgfra)-positive progenitors in response to \( \beta_3 \)-AR agonists. Beige and brown cells share a number of thermogenic genes such as Pgc1a, Ucp1, Cidea (cell death–inducing DNA-fragmentation factor, \( \alpha \)-subunit–like effector A), and Prdm16; however, beige cells also express several unique genes, such as CD137, Tmem26 (transmembrane protein 26), and Tbx1 (T-box tran-
scription factor TBX1), that apparently reflect their distinct developmental origins.11 Interestingly, human brown adipocytes isolated from different regions of the body express beige-cell markers, suggesting that these brown adipocytes have a molecular signature similar to that of beige cells.12

**BAT-mediated thermogenesis and obesity**

The relationship between BAT thermogenesis and obesity is evident in a transgenic mouse model in which Ucp1 promoter–directed expression of diphtheria toxin caused obesity.13 Furthermore, Ucp1-knockout mice exhibit an obese phenotype under thermoneutral conditions, and cold intolerance and impaired heat production under ambient temperatures, suggesting that activation of UCP1-mediated thermogenesis in BAT can have antiobesity effects.14 Conversely, induced expression of Ucp1 in vivo maintains mitochondria in an active uncoupled state in BAT; however, constitutively enhanced expression of Ucp1 is cytotoxic and causes BAT atrophy.15 The question arises as to what activates Ucp1. In response to certain physiological stimuli, such as cold or free fatty acids, norepinephrine released from sympathetic nerves act on β-adrenergic receptors of the brown adipocytes. Activation of β-adrenergic/cAMP pathway also increases PGC1α protein stability through p38 mitogen-activated protein kinase (MAPK). PGC1α regulates thermogenesis by directly inducing the expression of Ucp1. In addition, PGC1α also regulates a number of other factors, such as PPARα, PPARβ, PPARγ, nuclear respiratory factors 1 and 2 (NRF1 and NRF2), thyroid hormone receptor, liver X receptor (LXR), and forkhead box O1 (FOXO1), thus functioning as the central regulator of various pathways involved in mitochondrial biogenesis and thermogenesis. As a result, deletion of PGC1α in mice results in defective cold-induced thermogenesis due to impaired induction of thermogenesis genes.17 Conversely, forced expression of PGC1α in white adipocytes stimulates various genes involved in mitochondrial biogenesis and thermogenesis, including Ucp1, suggesting a central role for PGC1α in mitochondrial thermogenesis.

**Positive and negative regulators of BAT thermogenesis**

Because of their critical role in thermogenesis, PGC1α and UCP1 expression and activity are tightly controlled by inducers (positive regulators) or inhibitors (negative regulators). Some of the important factors that positively regulate PGC1α and UCP1 are forkhead box C2 (FOXC2), SRC1, CREB, sirtuin-3 (SIRT3), and p38 MAPK; however, receptor-interacting protein 140 (RIP140), LXRα, Cidea, retinoblastoma protein (pRB), SRC2, Twist1, transient receptor potential cation channel subfamily V member 4 (TRPV4), and inhibitor of differentiation 1 (Id1) negatively regulate PGC1α and/or UCP1 (Figure 1).

**Fig. 1 Regulators of BAT thermogenesis.** Cartoon showing some of the important negative and positive regulators of PGC1α/UCP1-mediated thermogenesis. Detailed mechanisms are described in the text.

**Abbreviations:** Cidea, cell-death–inducing DNA-fragmentation factor, α-subunit–like effector A; CREB, cAMP-response-element–binding protein; FOXC2, forkhead box C2; Id1, inhibitor of differentiation 1; LXRα, liver-X-receptor α; p38, p38 mitogen-activated protein kinase; p107, pocket protein p107; PGC1α, peroxisome proliferator–activated receptor γ coactivator 1α; pRB, retinoblastoma protein; RIP140, receptor-interacting protein 140; SHP, short heterodimer partner; SIRT3, sirtuin-3; SRC, steroid receptor coactivator; TRPV4, transient receptor potential cation channel subfamily V member 4; Twist1, Twist-family basic helix-loop-helix transcription factor 1; UCP1, uncoupling protein 1; VDR, vitamin D receptor.

**Positive regulators**

FOXC2 belongs to the family of forkhead/winged-helix transcription factors. Transgenic expression of FOXC2 in adipose tissues increases expression of Ucp1 and other mitochondrial genes and induces
browning in WAT. FOXC2 induces browning by sensitizing cells to the β-adrenergic PKA/cAMP pathway. As a result, FOXC2-transgenic mice are resistant to diet-induced obesity. WAT browning due to loss of pRB is regulated through FOXC2, for which expression is elevated in pRB−/− cells. pRB regulates thermogenesis by directly binding to the PGCoα promoter and suppressing transcription. In addition, pRB also functions as a PPARα co-repressor, and loss of pRB results in increased PPARγ activity. Adipose-specific deletion of pRB in mice leads to browning of WAT, activation of BAT, and protection from diet-induced obesity. Mice deficient for another pocket protein, p107, also display brown-like adipocytes within WAT. In p107-deficient adipocyte precursors, pRB levels are reduced, indicating that p107 function could be facilitated through regulation of pRB. The members of the SRC family, SRC1 (nuclear receptor coactivator 1 [NcoA1]), SRC2 (transcriptional mediators/intermediary factor 2 [TIF2]), and SRC3 (p/CIP), have divergent functions in the regulation of thermogenesis. SRC1 positively regulates thermogenesis by mediating its effects via PGCoα. SRC1 augments co-activation of PPARγ by PGCoα, and genetic ablation of SRC1 leads to impaired thermogenesis due to reduced expression of UCP1. SRC1−/− mice are prone to obesity because of reduced energy expenditure.

**Negative regulators**

In contrast to SRC1, SRC2 inhibits the interaction between PPARγ and PGCoα, leading to reduced activity of PGCoα. SRC2−/− mice are protected against obesity and show increased thermogenesis and improved energy expenditure. RIP140 is a transcriptional corepressor that shares a number of downstream target gene promoters with PGCoα. RIP140 binds to PGCoα and inhibits its transcriptional activity on the target gene promoters. Genetic deletion of RIP140 leads to the formation of brown-like adipocytes within WAT, with increased expression of UCP1; RIP140−/− mice are lean and are resistant to diet-induced obesity. Conversely, induced expression of RIP140 in adipocytes impairs expression of mitochondrial and thermogenic genes. Similarly, LXRα suppresses Ucp1 gene expression by interfering with the transactivation of the Ucp1 promoter by dismissing PPARγ from the Ucp1 enhancer. LXRα achieves PPARγ discharge from the Ucp1 enhancer by recruiting RIP140 as a corepressor to the LXRα binding site. In the absence of RIP140, LXRα fails to dismiss PPARγ from the Ucp1 promoter. Mice lacking LXR have a lean phenotype, with increased expression of UCP1 in BAT and WAT. Twist1 is a basic helix-loop-helix (bHLH) transcription factor that directly binds to PGCoα and suppresses its transcriptional activity. Adipocyte-specific overexpression of Twist1 in a transgenic mouse model results in reduced mitochondrial density and suppression of UCP1 in BAT, leading to diet-induced obesity. Conversely, Twist1-knockout mice are resistant to diet-induced obesity owing to higher mitochondrial density and enhanced expression of UCP1.

Cidea, which is a member of the CIDE apoptotic family directly interacts with UCP1 and suppresses its uncoupling function, thus reducing energy expenditure. Cidea−/− mice are resistant to obesity and display increased energy expenditure. Similarly, the TRPV4 receptor, which belongs to a family of ion channels, negatively regulates the expression of both PGCoα and UCP1. TRPV4−/− mice display higher energy expenditure and increased UCP1 expression in adipose tissues. In addition, the translational inhibitor 4E-BP1 suppresses the translation of PGCoα mRNA. Mice deficient for 4E-BP1 (Eif4ebp1−/−) have increased translation of PGCoα, and enhanced expression of UCP1 results in an increased metabolic rate and a reduced WAT mass.

**Conclusions**

The primary function of BAT is to generate heat in response to cold, thus protecting animals from hypothermia. The idea of utilizing BAT to increase energy expenditure and reduce body fat has generated tremendous interest in understanding BAT biology. As a result, much effort has been invested and significant progress has been made in understanding the developmental lineages of brown and beige/brite adipocytes, in cell-fate determination, and the molecular regulation of BAT thermogenesis. In this regard, genetic mouse models have played a pivotal role in identifying several critical transcription factors and their specific functions in these processes. On the basis of this knowledge, the next step toward increasing energy expenditure with an eye toward treating obesity should focus on expanding BAT mass and activating BAT thermogenesis in adult humans.
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Integrated control of brown adipose tissue

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Abstract
Brown adipose tissue (BAT) has evolved as a unique thermogenic organ that allows placental mammals to withstand cold environmental temperatures through the dissipation of metabolic energy in the form of heat. Although traditionally believed to be lost shortly after birth, metabolically active BAT depots have recently been identified in a large percentage of human adults. Besides classical brown cells, a distinct type of thermogenic adipocytes named beige or brite (brown in white) cells are recruited in white adipose tissue depots under specific stimuli. Given the well-known energy-dissipating properties of thermogenic adipose tissue and its function of metabolic sink for glucose and lipids, this tissue has attracted considerable research interest as a possible target for treating obesity and metabolic disease. The complex network of interorgan connections that regulate BAT and brite tissue mass and function is a major hurdle for the development of therapeutic strategies against metabolic disorders. This review provides an overview of the current knowledge on the regulation of BAT and brite adipose tissue function. The possibility of targeting these tissues to treat obesity and other metabolic disorders is also discussed. ■ Heart Metab. 2016;69:9-14

Keywords: adiposity; beige cells; diabetes mellitus; energy metabolism; inflammation; mitochondrial respiration; positron emission tomography (PET); sympathomimetics; weight loss

Adipose tissue: much more than a fat reservoir

Adipose tissue is a complex organ that significantly affects the physiology and pathophysiology of an organism. Originally viewed as a lipid droplet–containing inert variant of connective tissue, adipose tissue is now recognized to be an integral component in nutrient homeostasis, energy metabolism, and modulation of inflammatory pathways. Adipose tissue also has protective and cushioning functions and is a source of thermal insulation and thermogenesis. In addition, the distribution of adipose tissue depots has an important role in defining secondary sexual characteristics in several species, including humans.

Traditionally, two forms of adipose tissue are distinguished based on their particular morphological, physiological, and biochemical characteristics: white adipose tissue (WAT) and brown adipose tissue (BAT). WAT is the largest store of energy in humans. Morphologically, white adipocytes are characterized by the presence of a single large lipid
droplet that makes up the majority of the cell volume, with the cytoplasm and nucleus found at the cell periphery. These cells possess few mitochondria. Besides being an energy-storing tissue, WAT and its resident macrophages also act as an endocrine organ by releasing an array of signaling molecules, collectively termed adipokines. Adiponectin, leptin, interleukin (IL) 6, tumor necrosis factor α (TNF-α), resistin, and omentin 1 are among the best known adipokines. Alterations in WAT plasticity and expandability in the context of overnutrition are key factors in the pathogenesis of obesity-associated metabolic dysregulations. Indeed, the imbalance between proinflammatory and anti-inflammatory adipokines is involved in the development of insulin resistance and diabetes through a state of chronic low-grade inflammation.

BAT differs markedly from WAT. Brown adipocytes contain multiple small lipid droplets, conferring a multilocular appearance, and are enriched in mitochondria. BAT is densely innervated by the sympathetic nervous system (SNS) and is highly vascularized (up to five capillaries per cell). The main function of BAT is nonshivering thermogenesis (NST), which serves to maintain body temperature in small mammals and hibernating species and allows human newborns to cope with the thermal shock of delivery. Indeed, brown adipocytes actively oxidize fatty acids and glucose to sustain heat generation through uncoupling mitochondrial substrate oxidation from adenosine triphosphate (ATP) production (Figure 1). A role for BAT beyond thermogenesis has also been postulated. Studies in experimental rodents have shown that brown adipocytes may release a number of signaling molecules with autocrine and paracrine actions (eg, triiodothyronine, prostaglandins, angiotensinogen, IL-1α, IL-6, insulin-like growth factor 1, fibroblast growth factors [FGF] 2 and 21). However, the actual existence of BAT-derived endocrine factors and their relevance to human health are yet to be established.

A distinct type of brown adipocyte has recently been identified, termed the beige or brite (brown in white) adipocyte. Beige cells share characteristics both with white (ie, low basal expression of uncoupling protein 1 [UCP1]) and brown adipocytes (ie, presence of multilocular fat droplets and high mitochondrial density); however, their gene-expression pattern is distinct from either WAT or BAT. In response to stress stimuli (eg, chronic cold exposure, exercise, and severe adrenergic stress), pharmacological treatments (eg, long-term treatment with peroxisome proliferator-activated receptor γ [PPARγ] agonists), and pathological conditions (eg, cancer cachexia), brite adipocytes appear at WAT anatomical sites through differentiation of precursor cells that are distinct from BAT and closer to the white-cell li-
About 140 million years ago, the emergence of BAT-mediated NST in placental mammals offered these animals a remarkable evolutionary advantage by enabling them to maintain body temperature in the cold. Briefly, cutaneous thermal receptors activated by exposure to cold environmental temperatures send signals to thermoregulatory areas of the hypothalamus. This evokes efferent SNS signaling and subsequent stimulation of \( \beta_3 \)-adrenoceptors in BAT, followed by intracellular lipolysis, activation of the mitochondrial electron transport chain, and uncoupling of respiration (Figure 2).\(^6\) UCP1, a mitochondrial carrier protein inserted into the inner mitochondrial membrane, is the main actor in BAT-mediated NST.\(^7\) Indeed, UCP1 promotes a state of metabolic inefficiency by inducing maximum mitochondrial respiration through dissipation of the proton-motive force in the form of heat (Figure 1).\(^7\) Cold adaptation is achieved through different mechanisms in wild-type and UCP1-ablated mice. Indeed, UCP1-null mice may still develop cold tolerance after adaptation; however, contrary to their NST-competent littermates, they can rely only on shivering for heat production.\(^7\)

Although BAT depots have long been assumed to regress shortly after birth, recent studies using \( ^{18} \)F-fluorodeoxyglucose positron emission tomography/computed tomography (\( ^{18} \)FDG-PET/CT) have revealed that human adults possess BAT located in cervical-supraclavicular (the most common location), perirenal/adrenal and paravertebral regions, and around the major arteries.\(^8\) Indeed, BAT depots are highly prevalent in adult humans.\(^1\) The abundance of BAT is greater in women than in men, and it is inversely related to outdoor temperature, age, \( \beta \)-blocker use, body mass index, percent body fat, and plasma glucose levels.\(^1,8\)

Interestingly, recent studies employing three-dimensional magnetic resonance imaging in combination with histologi-
cal and biochemical analyses have shown that, while BAT in the cervical region consists of classic brown adipocytes, BAT depots found in other anatomical sites possess a molecular signature consistent with beige cells.\textsuperscript{9} In addition, gene-expression profiling of BAT samples obtained from the supraclavicular region revealed that molecular markers of both brown and beige adipocytes coexist in human adults.\textsuperscript{10} This finding suggests that human BAT might indeed consist of both brown and recruitable beige adipocytes.\textsuperscript{1}

As previously mentioned, BAT prevalence and activity are lower in overweight and obese persons than in lean individuals. On the other hand, weight loss via bariatric surgery increases BAT activity in morbid obese patients.\textsuperscript{11} Weight loss also leads to the appearance of functional BAT depots in obese persons previously void of such tissue.\textsuperscript{12} BAT activity is also inversely associated with diabetes and fasting glucose levels.\textsuperscript{13} Interestingly, prolonged (5-8 hours) cold-induced BAT activation has been shown to improve resting energy expenditure, whole-body glucose disposal, plasma glucose oxidation, and insulin sensitivity in middle-aged, overweight men with 18FDG-PET/CT-detectable BAT.\textsuperscript{14} Collectively, these observations raise the possibility that BAT dysfunction might contribute to the development of obesity and insulin resistance.

As a whole, evidence accumulated so far strongly suggests a pivotal role for BAT in energy balance and metabolic homeostasis. This tissue may therefore be a promising target for interventions against obesity and other metabolic disorders.

**Integrating central and peripheral signals: BAT choreography**

The regulation of BAT mass and function is achieved via a complex profusion of signals and feedback systems involving multiple organs and tissues. Almost all organs may produce, on exposure to either physiological or pathological stimuli, specific signals that positively or negatively modulate BAT activity.

According to the classical view, sympathetic neural-adipose connections induce the BAT thermogenic program and browning of WAT in response to cold exposure.\textsuperscript{1} A cold environment may also activate a thermogenic circuit consisting of eosinophils, cytokines, and alternatively activated (type 2/M2) macrophages.\textsuperscript{15} Once activated by eosinophil-derived ILs 4 and 13, M2 macrophages are recruited to subcutaneous WAT and release catecholamines to activate the WAT-browning process and drive thermogenesis.\textsuperscript{15}

Several neuropeptides and hormones—including leptin, thyroid hormones, estradiol, brain-derived neurotrophic factor (BDNF), irisin, FGF21, bone morphogenetic protein (BMP) 7 and 8B, glucagon-like peptide 1, nesfatin-1, and cannabinoids—modulate BAT function by acting both centrally (on different hypothalamic nuclei) and peripherally to adapt thermogenesis and energy homeostasis to various stimuli (Figure 2).\textsuperscript{16}

Exercise has been shown to confer some of its beneficial effects through the induction of WAT browning. Exercise-derived myokines and metabolites (eg, irisin, IL-6, β-aminoisobutyric acid [BAIBA], lactate, meteorin-like peptide, and FGF21) stimulate beige adipocyte development and increase energy expenditure.\textsuperscript{17}

Conversely, under obesity conditions, WAT browning is impaired by multiple factors, including increased activation of signaling pathways that inhibit beige adipocyte development (eg, transforming growth factor β and TNF-α) and concomitant reduced SNS activity.\textsuperscript{18}

A number of other signaling molecules are involved in the formation and activation of brown adipocytes. Identifying their peripheral and central targets would be useful for fine-tuning whole-body energy expenditure and improving metabolic control.

**Targeting BAT to fight metabolic disorders**

Excess body weight and obesity result from a prolonged imbalance between energy intake and energy expenditure. It follows that, in obese persons, fat mass reduction can be achieved either by decreasing food consumption or by increasing energy expenditure to obtain a sustained negative energy balance. Unfortunately, this is not easily attained, because of the sedentary lifestyle and the unrestricted availability of calorie-dense, inexpensive food that characterize modern societies. Indeed, with the exception of bariatric surgery, most anti-obesity interventions tackling energy intake result in moderate, often temporary, improvements. Thus far, pharmacological agents that have been proposed to increase energy expenditure
have been either ineffective or toxic. Finally, physical activity—the most physiological way of burning energy—is not easy to sustain in the long term.

Recent years have seen the emergence of the possibility to achieve weight loss through harnessing the thermogenic properties of BAT.\textsuperscript{19} Whereas chronic cold exposure is obviously not a strategy worth pursuing, an alternative might be the administration of certain sympathomimetic agents (Figure 2). For instance, the highly specific β\textsubscript{3}-adrenoceptor agonist CL-316,243 promotes thermogenesis, induces BAT hypertrophy and the appearance of brown adipocytes in WAT, and reduces obesity in rats on a high-fat diet.\textsuperscript{20} CL-316,243 administration was shown to increase insulin action and fat oxidation in lean men, though the effects were markedly diminished after 8 weeks of treatment.\textsuperscript{21} The compound did not affect resting energy expenditure, body weight, or body composition at any time over the course of treatment. In contrast, acute administration of the β\textsubscript{1}-adrenoceptor agonist L-796568 increases lipolysis and energy expenditure in overweight men.\textsuperscript{22} Similar to observations for CL-316,243, L-796568 effects are lost with long-term administration of the compound, likely as a result of β\textsubscript{1}-adrenoceptor downregulation.\textsuperscript{23}

The stimulation of the thyroid hormone receptor β (THRβ) might be an alternative strategy to increase BAT-mediated thermogenesis (Figure 2). For instance, the selective THRβ agonist GC-24 improves metabolic control in rats fed a hypercaloric diet.\textsuperscript{24} Furthermore, treatment with GC-1, another selective THRβ agonist, increases energy expenditure and prevents fat mass accumulation in rats.\textsuperscript{25} Notably, administration of chenodeoxycholic acid has been shown to activate type 2 iodothyronine deiodinase (DIO2) in BAT of healthy women, resulting in increased whole-body energy expenditure and mitochondrial uncoupling in brown adipocytes.\textsuperscript{26}

A very appealing strategy may be the transformation of the more abundant WAT into beige fat. In this regard, a number of compounds may be explored, including BMP4 and BMP7, FGF19 and FGF21, natriuretic peptides, BAIBA, capsinoids, and inhibitors of phosphodiesterase 5 (eg, sildenafil) (Figure 2).\textsuperscript{27} Further studies are needed to establish the effectiveness and safety profiles of such compounds in order to avoid undesired side effects such as those encountered with other BAT activators (eg, cardiovascular toxicity).

Conclusion

The escalating prevalence of obesity carries a number of detrimental consequences, including an increased risk of type 2 diabetes, dyslipidemia, cardiovascular disease, and several cancers. With the exception of bariatric surgery, none of the available interventions achieve substantial weight loss over the long term. The recent discovery of active BAT and recruitable beige cells in most human adults, together with the role played by these tissues in energy balance and insulin sensitivity, has sparked considerable interest into the possibility of targeting BAT to treat obesity and its negative correlates. Whereas a number of factors and pathways that enhance BAT and beige fat recruitment and function in rodents are known, the mechanisms of activation of these tissues in humans are only partly characterized. This is reflected in the conflicting results from interventions targeting BAT in rodents and humans. An increased understanding of the function and regulation of BAT is therefore instrumental for devising pharmacological and nutritional interventions that harness the thermogenic properties of this tissue to treat obesity and other metabolic disorders. ■

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Assessing brown adipose tissue in humans

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Abstract
In obesity, impaired energy expenditure is associated with adaptive thermogenesis. The current standard methodology for detecting brown adipose tissue (BAT), a thermogenic tissue important in nonshivering thermogenesis, uses fluorodeoxyglucose positron emission tomography (FDG-PET) and requires cold stimulation. Knowing that BAT is highly innervated and regulated by the sympathetic nervous system, we tested a new mechanistically driven approach that images the norepinephrine recycling component, the norepinephrine transporter (NET), using \((S,S)-[11C]O\)-methylreboxetine (a highly selective NET ligand). Our preliminary ex vivo and in vivo imaging studies in rodents and human subjects have shown that \((S,S)-[11C]O\)-methylreboxetine can efficiently label BAT at both room temperature and mild cold conditions, whereas reliable labeling of BAT with \([18F]FDG\) occurs only under mild cold conditions. Thus, \((S,S)-[11C]O\)-methylreboxetine imaging offers a unique opportunity to investigate the role of BAT in humans under nonstimulated basal conditions. By targeting NET, a primary regulatory system component of BAT, we have established a basis for future mechanistic studies of BAT function/dysfunction in obesity and diabetes, as well as for therapeutic approaches for these disorders. ■ Heart Metab. 2016;69:15-20

Keywords: brown adipose tissue; \([11C]MRB\); obesity; norepinephrine transporter; positron emission tomography (PET); sympathetic nervous system

According to the statistical data from the National Health and Nutrition Examination Survey, 2009-2010,\(^1,2\) more than 2 in 3 adults are overweight or obese, and more than 1 in 3 adults are obese. As a result, the obesity-associated comorbidities, such as type 2 diabetes mellitus, cardiovascular disease (including coronary heart disease, stroke, and heart failure), Alzheimer disease, and cancer, as well as many other adverse health conditions, can be anticipated to increase dramatically. The cost of obesity to society is tremendous, due to obesity-associated illnesses and losses in productivity at work.\(^3\) It is imperative to develop strategies that will affect energy balance, particularly during periods of weight loss or weight loss maintenance. The recently confirmed existence of brown adipose tissue (BAT) in biopsy samples from adult humans\(^4,5\) has renewed interest in this tissue, now thought possible to be involved in conditions where energy balance is disrupted. For example, in human obesity and insulin resistance, adaptive thermogenesis is often impaired.\(^6-9\) BAT is one of the primary tissues responsible for adaptive nonshivering thermogenesis in mammals.\(^10\) Obese animal models demonstrate impaired sympathetic nervous system (SNS) activation and postsynaptic processing in BAT.\(^11,12\) However, the relative importance of BAT...
activity for whole-body energy expenditure in an adult human is still unknown.4,11 Before we can investigate the function/dysfunction of BAT in human energy balance, we need new methodological approaches that can target specific attributes of BAT in humans.

FDG-PET for BAT detection

BAT in adult humans was serendipitously revealed by fluorodeoxyglucose (FDG)-positron emission tomography (PET) with the radiolabeled tracer \(^{18}\text{F}\)FDG.5,14-16 However, FDG uptake is not specific to BAT, and glucose is not the primary substrate used for BAT heat production. Nevertheless, to detect BAT, \(^{18}\text{F}\)FDG has an advantage over other radiolabeled compounds in that \(^{18}\text{F}\)FDG-labeling of BAT is markedly increased under mild cold conditions. However, good resolution is unachievable at room temperature.17 Indeed, in human studies, imaging performed at room temperature showed no appreciable detection of BAT, whereas imaging in those same individuals after mild cold exposure in a climate chamber did detect BAT.18,19 Therefore, the recent FDG-PET–based observations of BAT in humans may bias our basic understanding of BAT as a function of sex, obesity, and aging: individual differences in BAT activation due to different thermal responses probably complicate comparisons between obese subjects and those of normal weight. It is therefore important to develop new and more specific methods to address both the plasticity of BAT activation and the basal characteristics that do not require stimulation. Such methods could also be combined with those that highlight BAT-stimulated activity.

NET-PET for BAT detection

Lessons learned from steps taken to reduce undesirable \(^{18}\text{F}\)FDG-labeling during clinical scans demonstrate that repeat scans after \(\beta\)-adrenergic blockade eliminate the interfering BAT signal.20 These data suggest that the SNS strongly activates BAT in humans and that the noradrenergic system may be involved in regulating BAT activity. This is not surprising; noradrenaline (NE) is the principle neurotransmitter in the SNS, and the interaction of NE, insulin, and BAT has been previously shown. Studies in animal models point to disruption of NE sensitivity in BAT activation related at least in part to insulin resistance.12,21 Direct effects of insulin on peripheral SNS have been examined in the mesentery and show that insulin may diminish NE overflow by increasing reuptake by the NE transporter (NET),22 thus lowering the NE concentration available for postsynaptic activation. Recently, insulin has been reported to regulate NET function both centrally and peripherally, and acute insulin treatment has been associated with a significantly decreased surface expression of NET.23

In the central nervous system, data also support noradrenergic dysfunction in obesity (eg, inhibition of either \(\alpha_1\)- or \(\beta_2\)-noradrenergic receptors in the ventromedial/paraventricular hypothalamus reduces endogenous levels of NE), which decreases food intake, whereas activation of \(\alpha_2\)-noradrenergic receptors of the lateral hypothalamus increases levels of NE and increases food intake.24-26 In humans, intake of high-caloric diets increases noradrenergic turnover in peripheral tissues, raising resting plasma NE levels, which may underlie a higher excretion of NE and higher rates of hypertension in obese people than in lean.27,28 These findings suggest an important role for the NE recycling system, the NET, in eating behaviors, obesity, and obesity-related health conditions.28

Here, we discuss our investigations, both preclinical and clinical, into whether BAT can be visualized with NET-PET imaging and whether NET binding can be measured, by using the highly selective NET ligand \((S,S)\)-\(^{11}\text{C}\)-methylreboxetine ((\(^{11}\text{C}\)MRB), under both basal and activated conditions in humans. For comparison, the gold standard method, \(^{18}\text{F}\)FDG-BAT imaging, was also used. The radiotracer \(^{11}\text{C}\)MRB has previously been used centrally for brain imaging studies in humans and nonhuman primates,29-32 as well as peripherally for NET imaging studies in nonhuman primates.33 The use of the \(^{11}\text{C}\)MRB ligand offers specific advantages, as studies have shown that NET function is regulated both centrally and peripherally by insulin.23 Unlike other potential NET ligands, such...
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as \(^{18}\)F-fluorodopamine\(^{34,35}\) and metaiodobenzylguanidine (MIBG; a single-photon emission computed tomography [SPECT] ligand).\(^{16}\) \(^{[1]}\)C\]MRB can cross the blood-brain barrier, allowing for simultaneous central and peripheral imaging. Thus, \(^{[1]}\)C\]MRB may be useful to further elucidate mechanisms of BAT action and the role of NET in energy balance in obesity and insulin resistance by simultaneously correlating the functions of the central nervous system and the peripheral SNS.

Preclinical NET-BAT imaging study in rodents

We first conducted BAT imaging studies, both ex vivo and in vivo, in rats to determine \(^{[1]}\)C\]MRB uptake in BAT in the basal state (at room temperature); imaging of basal state BAT is unreliable with \(^{[18]}\)F\]FDG.\(^{36}\) In an ex vivo study, we administered intravenous \(^{[18]}\)F\]FDG or \(^{[1]}\)C\]MRB to awake male Sprague-Dawley rats after they were exposed to cold (4 °C for 4h, \(n=9\)) or room temperature conditions (\(n=9\)) and then sacrificed the rats at 20, 40, and 60 minutes after injection. Analysis of BAT samples showed that uptake of \(^{[1]}\)C\]MRB (% injected dose) was 3 times higher than that of \(^{[18]}\)F\]FDG at room temperature (\(P=0.0088\)). \(^{[18]}\)F\]FDG uptake after cold exposure was 10 times higher than in the room temperature control (1.6±0.3% injected dose per gram of tissue [ID/g] [cold] vs 0.2±0.05% ID/g [room temperature]; \(P=0.0009\)), whereas no significant thermal effect was observed with \(^{[1]}\)C\]MRB uptake (0.87±0.18% ID/g [cold] vs 0.63±0.09% ID/g [room temperature]; \(P=0.082\)). In addition to uptake in the brain and heart, BAT exhibited specific \(^{[1]}\)C\]MRB uptake that was significantly reduced to near baseline levels (\(P=0.0013\)) by pretreatment with unlabeled MRB or nisoxetine (a selective NET inhibitor). These ex vivo results were concordant with the in vivo PET imaging of anesthetized rats, which clearly demonstrated intense \(^{[1]}\)C\]MRB uptake in the interscapular BAT both at room temperature and after cold exposure; in contrast, \(^{[18]}\)F\]FDG uptake in BAT was detected only after cold exposure. Furthermore, \(^{[1]}\)C\]MRB uptake in BAT was completely abolished by pretreatment with unlabeled MRB, demonstrating the specific and saturable binding of the ligand to BAT (Figure 1).\(^{36}\) High-performance liquid chromatography analysis revealed that 94%-99% of total radioactivity in BAT represented unchanged \(^{[1]}\)C\]MRB (ie, the parent tracer and not a metabolite), further supporting the NET-PET strategy for imaging BAT in humans under basal conditions.

Clinical NET-BAT imaging study in humans

In humans, we conducted \(^{[1]}\)C\]MRB PET-computed tomography (CT) imaging (mCT PET/CT scanner, Siemens/CTI, USA) and \(^{[18]}\)F\]FDG PET-CT imaging of cervical supraclavicular BAT under room temperature and cold-stimulated conditions.\(^{37}\) Our study included 10 healthy white subjects (5 men, aged 24.6±2.6 years, body mass index [BMI] 21.6±2.7 kg/m\(^2\); 5 women, aged 25.4±2.1 years, BMI 22.1±1.0 kg/m\(^2\)). Subjects wore a climate vest that was loaded with cold packs (RPCM Cool Vest, Glacier Tek, USA; enthalpy 15°C) during cold-condition scans and with room temperature packs during room temperature scans in order to avoid potential biases from changes in attenuation between the two conditions. For \(^{[18]}\)F\] FDG studies, subjects wore the climate vest for 30 minutes before intravenous \(^{[18]}\)F\]FDG administration, during the 60-minute period between the injection and scan, and during the 25- to 30-minute scan. For \(^{[1]}\)C\]MRB PET-CT scans, subjects wore the vest for 30 minutes before intravenous \(^{[1]}\)C\]MRB administration and for the duration of the 120- to 125-minute dynamic scan. PET-CT scanning began on administration of \(^{[1]}\)C\]MRB. All scans were performed under fasting conditions.

For analysis of tracer uptake, BAT was defined as tissue showing a \(^{[18]}\)F\]FDG standardized uptake value

(SUV) greater than 1.5 and Hounsfield units ranging from -200 to -50, as used by others. We measured uptake of [11C]MRB as the distribution volume ratio (DVR), using the occipital cortex as the reference region; the tracer binding was well-characterized in our previous brain studies in humans, and uptake of [11C]MRB metabolites is minimal in rodent BAT. We used muscle as a peripheral reference and calculated the ratio of BAT-DVR to muscle-DVR (BAT/muscle). Total body fat and lean body mass were assessed via bioelectrical impedance analysis.

Consistent with previous studies, we found that [18F]FDG uptake in BAT was difficult to detect at room temperature, but easy to detect after cold stimulation (P=0.01). In contrast, BAT [11C]MRB uptake (also normalized for muscle) was equally evident both under room temperature and cold conditions (BAT-DVR: 1.0±0.3 [room temperature] vs 1.1±0.3 [cold], P=0.31; BAT/muscle-DVR: 2.3±0.7 [room temperature] vs 2.5±0.5 [cold], P=0.61) (Figure 2). Important-ly, core body temperature correlated positively with [11C]MRB BAT uptake in response to cold correlated negatively with percent body fat (r= -0.74, P=0.04) and positively with lean mass (r=0.71, P=0.05), which showed a gender difference, most likely reflecting the difference in body composition and body temperature between men and women. There were no relationships between change in [18F]FDG uptake in response to cold and body composition, BMI, body temperature, heart rate, or blood pressure.

Conclusion

Our studies, preclinical in rodents and clinical in humans, provide the first evidence that [11C]MRB PET imaging can be used to visualize BAT by targeting the NET element of the SNS innervation of such tissue. Furthermore, this imaging modality can be used under both cold-stimulated and basal conditions. Thus, [11C]MRB PET imaging offers an alternative and complimentary approach to standard [18F]FDG imaging, which—although well validated for assessing BAT after cold exposure—is unreliable when assessing basal BAT. Our proof-of-concept study does have some limitations, including a small sample size and a relatively homogeneous subject population, which was young, relatively lean, and white. Additional studies will be needed to determine whether individuals with differences in body mass, ethnicity, gender, or age will have different patterns of SNS innervation of BAT as assessed by [11C]MRB imaging.

BAT is the principal thermogenic tissue responsible for adaptive thermogenesis, including cold-induced (CIT) and diet-induced thermogenesis (DIT). By far, most of the imaging studies have focused on CIT. The results from the limited number of CIT studies using [18F]FDG have shown the difficulties in determining the contribution of BAT to CIT, relative to other tissues, such as skeletal muscle. Whether [11C]MRB imaging would provide a better outcome in determining the contribution of BAT to DIT remains to be seen.
The idea of using the thermogenic properties of BAT to combat diabetes and obesity is drawing increasing amounts of interest in clinical circles. However, understanding the mechanisms behind BAT's role in these metabolic diseases is more complex than simply determining what factors activate existing BAT. We must also discover the factors that regulate not only the amount of BAT in the body, but also the effectiveness of BAT with regard to thermogenesis. Understanding the thermogenic potential of human BAT requires that we assess BAT both after acute stimulation and in its basal state (without cold stimulation). Here, we show that \[^{11}C\]MRB can be used for this purpose, as its uptake can be visualized under both these conditions. Furthermore, because it directly assesses the degree of tonic sympathetic activity in BAT, imaging with \[^{11}C\]MRB offers unique advantages for further investigation into other factors, besides cold stimulation, that may be involved in regulating human BAT.

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Brown adipose tissue as a potential therapeutic target in obesity therapy

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Abstract
Strategies to curb the increase in obesity are warranted in order to effectively prevent its associated metabolic complications. Activation of the thermogenic capacity of brown adipose tissue (BAT) in combination with a stable energy intake is a promising approach to effectively reduce body weight. Recent scientific advances have demonstrated that BAT can be found in adult humans and that human BAT is metabolically activated by exposure to a cold environment. In rodents, inducible forms of brown or beige adipocytes can be stimulated pharmacologically, and it is thereby possible to increase BAT mass. In humans, prolonged catecholamine stimulation, as observed in patients with pheochromocytoma and severe burns, can be associated with vast increases in brown adipocyte cell number. These findings indicate that in humans, stimulation of BAT activity and recruitment of uncoupling protein 1 (UCP1)-positive cells can be achieved by adrenergic stimulation. In addition, factors like interleukin 6, irisin, and thyroid hormones may have similar capabilities to stimulate BAT. BAT therefore is an intriguing target for the control of whole-body energy balance, adiposity, and obesity. Heart Metab. 2016;69:21-25

Keywords: activators of thermogenesis; brown adipose tissue; obesity

Obesity develops when energy intake exceeds energy expenditure. Now used increasingly for diagnostic purposes, fluorodeoxyglucose positron emission tomography (FDG-PET) has revealed that active brown adipose tissue (BAT) exists in normal adult humans. The thermogenic capacity of brown adipocytes enables brown adipose tissue (BAT) to effectively increase substrate oxidation, and it has been estimated that in humans as little as 40 to 50 g of BAT, if maximally stimulated, can account for up to 20% of daily energy expenditure. Several studies have demonstrated an inverse relationship between BAT activity and body fat content, and together these data suggest that BAT is a regulatory site of thermogenesis and body fat metabolism in humans. This has made BAT an attractive target for the development of new therapeutic approaches for the treatment of obesity and its associated metabolic complications. However, we must first understand how BAT activity and mass are regulated in humans if we are to fully assess the therapeutic potential of BAT; important questions remain. This review highlights our current understanding.

Brown, beige, and brite cells

Brown adipocytes have traditionally been defined as lipid-containing cells with multivacuolar appearance and a high content of mitochondria expressing uncoupling protein 1 (UCP1). BAT contains large amounts of brown adipocytes and is highly vascularized and innervated. The study of BAT has recently revealed...
that in addition to brown adipocytes in BAT, distinct types of thermogenic fat cells can be found in white adipose tissue depots. These cells have been termed “beige” or “brite” adipocytes, and although they share thermogenic properties with classical brown adipocytes, they do not originate from the same progenitor cell. Lineage-tracing studies show beige cells originate from myogenic factor 5 (Myf5)-negative progenitor cells, much like white adipocytes, whereas classic brown adipocytes derive from Myf5-positive progenitor cells, similarly to skeletal myocytes. In rodents, beige adipocytes are not confined to classical BAT locations—such as interscapular, cervical, and periaortic areas—but can be found scattered in white adipose tissue and between muscle bundles in skeletal muscle. Specific cell-surface markers can be used to identify the different UCP1-expressing types of adipocytes, and using such markers, it has been shown that most brown adipocytes in human BAT are Myf5-negative–beige adipocytes. Interestingly, overfeeding in mice triggers diet-induced thermogenesis, and this physiological mechanism permits excessive calorific intake to be dissipated as heat, allowing animals to eat without gaining weight. UCP1-knockout mice lack this mechanism, and they are prone to diet-induced obesity. In rodents, beige adipocytes are not confined to classical BAT locations—such as interscapular, cervical, and periaortic areas—but can be found scattered in white adipose tissue and between muscle bundles in skeletal muscle. Specific cell-surface markers can be used to identify the different UCP1-expressing types of adipocytes, and using such markers, it has been shown that most brown adipocytes in human BAT are Myf5-negative–beige adipocytes. Interestingly, overfeeding in mice triggers diet-induced thermogenesis, and this is associated with an increase in UCP activity. This physiological mechanism permits excessive calorific intake to be dissipated as heat, allowing animals to eat without gaining weight. UCP1-knockout mice lack this mechanism, and they are prone to diet-induced obesity. Specific strains of mice, such as SvEv, are resistant to diet-induced obesity—whereas other strains, such as C57B6, are prone to obesity—and this resistance is associated with a greater number of UCP1-positive beige adipocytes in the SvEv strain than in C57B6. Higher numbers of beige adipocytes in SvEv mice than in C57B6 mice are especially found intermixed with white adipose tissue adjacent to the thigh muscle. The differences in beige adipocyte numbers between SvEv and C57B6 strains appear to be genetically determined. In humans, BAT activity is negatively correlated with body mass index, body fat content, and visceral fat, and the amount of BAT that can be detected by FDG-PET decreases with age. However, when interventions that activate BAT are repeated for a prolonged period, BAT activity can be detected even in previously BAT-negative subjects. This demonstrates that BAT can be recruited in humans.

Nonpharmacological regulation of BAT

Temperature

Nonpharmacological activation of BAT in humans can be achieved by reducing temperature. Acute (1-2 hours) mild cold exposure (18°C-19°C) is sufficient to activate BAT in humans. In fact, it is often necessary to scan patients at thermoneutrality when performing [18F]FDG-PET imaging in order to reduce distracting signals from areas with BAT. Studies of prolonged cold exposure for a period between 10 days and 4 months have shown significant increases in BAT mass and activity. The increases in BAT mass and activity are predominantly within classical locations, such as cervical and supraclavicular areas. None of these studies reported any significant reduction in total body weight. However, a study with a 6-week intervention period found a significant 5% reduction in body fat mass. Evaluation of body composition in another study after 4 months of repeated cold exposures did not find a similar effect, but this study only included 5 subjects. Simply lowering ambient temperature or introducing a variable indoor environment with frequent cold exposures may therefore be practical, easy, and effective antiobesity regimens. However, care should be taken to prevent an increase in energy intake, because an increased desire to eat has been observed after cold acclimation. Large scale studies are warranted to test whether cold exposure is indeed an efficient strategy to treat obesity in humans.

Exercise

Regular physical exercise prevents many of the metabolic complications associated with obesity, and exercise interventions can be effective in achieving weight loss in obesity. Physical exercise is itself thermogenic, and regular physical activity can decrease the need for thermogenic activity in BAT. Indeed, most animal studies investigating the effect of exercise on BAT activity in classical depots, such as the interscapular area, have shown reduced activity. Nevertheless, some reports indicate that physical ex-
exercise may have a positive effect on ectopic UCP1 expression, and this may be under hormonal regulation. Irisin, a circulating protein that is released by cleavage from its precursor protein, fibronectin type III domain-containing 5, has been described to promote the appearance or recruitment of beige cells in white adipose depots. It is speculated that increased irisin expression with exercise and the recruitment of BAT have evolved as a consequence of muscle contraction during shivering. However, the existence of this protein in humans and its role there is still a matter of debate.

**Diet**

BAT has also been found to be activated by food ingredients, like capsaicin. Capsaicin—an active component of chili peppers—and intake of red peppers can increase energy expenditure. Changing diet to include more “hot foods” may therefore be a simple way to activate thermogenesis in BAT. The underlying mechanism has been suggested to involve capsaicin-induced activation of transient receptor potential (TRP) channels. TRP channels can be activated by cold stimuli and may transmit activation of BAT by cold exposure. However, propranolol can block the increase in energy expenditure induced by intake of red pepper, indicating that adrenergic stimulation may also be involved in capsaicin-induced thermogenesis.

**Pharmacological regulation of BAT**

**Adrenergic stimulation**

Among the pharmacological factors that influence the brown adipocyte is norepinephrine, which is both the most important and also the most well-studied. BAT can be pharmacologically activated by $\beta_3$-adrenergic-receptor–agonist treatment. However, despite the use of somewhat specific $\beta_3$-agonists, other adrenergic receptors may also be involved. In rodents, norepinephrine stimulation of brown preadipocytes and mature adipocytes leads to recruitment in BAT. This mechanism may translate to humans; increases in BAT mass and activity have been observed in patients with pheochromocytoma and paraganglioma. Such patients are subjected to chronic catecholamine stimulation and severe adrenergic stress, as seen in severely burned patients, and also develop ectopic BAT within their white adipose tissue depots. The degree to which BAT can be recruited in humans is clearly relevant for obesity therapies. This can perhaps best be illustrated by a recent case report from our hospital. In a male patient, a catecholamine-secreting paraganglioma was diagnosed and was associated with massive induction of BAT mass in the visceral adipose tissue. The presence of UCP1 expression was clearly detected in tissue biopsies and the metabolic activity of his BAT was documented by FDG-PET (Figure 1). This patient had a resting energy expenditure at presentation of 15 188 kJ, which is more than double the expected value. We observed a massive decrease in BAT activity measured by FDG-PET after initiation of treatment with the $\alpha$-adrenergic-receptor–blocker phenoxybenzamide. In rodents, the presence of $\alpha$-adrenergic receptors in BAT have been documented, and stimulation of these receptors can activate BAT. More investigation of the effects of...
α-adrenergic stimulation of BAT activity in humans, therefore, is attractive. This is further supported by failed attempts to induce BAT activity by sympathomimetics in humans.27

**Thyroid-hormone treatment**

Treatment with thyroid hormones has been shown to induce UCP1 expression in brown adipocytes in animals.7 The underlying mechanism is suggested to be the binding to thyroid hormone-responsive elements in the UCP1 promoter. However, it is not clear whether the effects of thyroid hormones observed in animals translate into humans. Treatment with thyroid hormone reduces weight in patients with hypothyroidism, but there is no consistent evidence that thyroid-hormone treatment induces weight loss in obese euthyroid individuals.7 In addition, a recent study of BAT activity in hyperthyroid patients using [18F]FDG uptake under hyperthyroid and euthyroid conditions showed no positive effect of thyroid hormone on BAT activity.28 Activating BAT through thyroid-hormone treatment is, therefore, not appealing at present. However, development of novel agonists of thyroid hormone-responsive elements in the UCP1 promoter could be a future treatment strategy.

**Other strategies**

Another promising strategy to treat obesity by targeting BAT is simply to implant BAT into obese subjects. Transplantation of BAT into mice improves glucose tolerance and causes weight loss in these mice.29,30 The transplantation of BAT also ameliorated the harmful effects of a high-fat diet.29,30 The beneficial effects on whole-body metabolism are blunted when BAT is transplanted from interleukin 6–knockout mice.30 This finding indicates that some of the beneficial effects of increased BAT mass can be, at least in part, hormonally mediated and that BAT may have endocrine functions. It is well-established that white adipose tissue secretes bioactive peptides, termed “adipokines,” which act locally and distally in an autocrine, paracrine, and endocrine manner. These adipokines regulate multiple functions, such as appetite, energy balance, and insulin sensitivity. Thus, the degree to which BAT secretes adipokines is an interesting topic for future research and may have promising perspectives for new obesity therapies.

**Conclusion**

Our growing understanding of the factors that regulate BAT activity and recruitment continues to support the perspective of targeting BAT in obesity treatment. Humans are capable of recruiting large amounts of ectopic BAT, which can significantly increase energy expenditure. Strategies to activate BAT involve both pharmacological and nonpharmacological interventions (Figure 2). However, if the increased energy expenditure is met by a matching increase in energy intake, the intervention against obesity will be futile.15 This is exemplified by our case report showing massive infiltration of BAT in a patient with paraganglioma.25 This patient was able to maintain a normal body weight despite a massive increase in resting energy expenditure, and he presented with a body mass index of 25.6 kg/m². After removal of the tumor, his resting energy expenditure normalized; however, 3 months later his body weight increased from 82.6 to 90.8 kg and his body fat, from 27.1% to 30.9%.25 Therefore, strategies to activate and/or recruit BAT in humans cannot necessarily stand alone, but are more likely to be effective if they are combined with interventions that reduce energy intake.

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Benefits of trimetazidine (TMZ) in obese patients

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Abstract
Obesity is an increasingly prevalent metabolic disorder worldwide, constituting a major risk factor for an array of chronic diseases, including chronic ischemic heart disease (IHD) and stable angina, two leading causes of death and disability. It is commonly known today that obesity is linked to heart damage, as its development results in accelerated fatty acid oxidation (FAO) and uncoupling of glycolysis from glucose oxidation. Subsequent cardiac steatosis favors oxidative stress and apoptosis, followed by mitochondrial damage, further compromising ATP production and contributing to contractile dysfunction and decreased cardiac efficiency. The anti-ischemic agent trimetazidine (TMZ), a selective 3-ketoacyl-coenzyme A thiolaese inhibitor, is a commonly known modulator of intracellular metabolism observed in a number of basic and clinical studies to directly provide more energy to ischemic cardiomyocytes by directly inhibiting, in an ischemic myocardium, the energy-consuming FAO in favor of glucose oxidation. In obese subjects likely to experience glucose intolerance and cardiac hypertrophy as a consequence of alterations in cardiac energy metabolism, elevated rates of FAO, and decreased glucose-oxidation rates, TMZ could be particularly beneficial. ■ Heart Metab. 2016;69:27-30

Keywords: ischemic heart disease; obesity; trimetazidine

Introduction
Obesity is an increasingly prevalent metabolic disorder affecting both the Western populations and the developing world. According to the World Health Organization’s key facts, in 2014, more than 1.9 billion adults (39%) globally were overweight, and among these, over 600 million (13%) were obese.1 Overweight and obese individuals have an increased prevalence of comorbidities compared with normal weight individuals. Excess weight and obesity are known to be major risk factors for the onset of a variety of chronic diseases—responsible for 60% of deaths worldwide1—among which cardiovascular diseases with ischemic heart disease (IHD) account for the greater part.2,5

Obesity, heart damage, and ischemic heart disease
It is commonly known today that obesity is linked to heart damage.6,7 Obesity onset and development result in an increased availability of fatty acid (FA), a concomitant over-reliance on FA as an energy source, accelerated FA oxidation (FAO), and an uncoupling of glycolysis from glucose oxidation. Consequently, cardiomyocyte FA uptake often exceeds mi-
tochondrial oxidative capacity, and cardiac steatosis ensues, leading to a build-up of lipotoxic intermediates, such as ceramide and acylcarnitine. Collectively, these events favor oxidative stress and apoptosis and subsequent mitochondrial damage, further compromising adenosine triphosphate (ATP) production and contributing to contractile dysfunction and to a decrease in cardiac efficiency.\(^8,9\)

Alterations in mitochondrial energy metabolism are common in many forms of heart disease,\(^10\) including IHD and diabetic cardiomyopathies, and precede the development of glucose intolerance and cardiac hypertrophy.\(^11\) In IHD, energy production is impaired due to a limitation in oxygen supply; residual mitochondrial FAO dominates over mitochondrial glucose oxidation. In diabetes, the ratio of cardiac FAO to glucose oxidation also increases, although primarily due to an increase in FAO and an inhibition in glucose oxidation.\(^8\) In many studies, hearts from animals or humans with diabetes mellitus or obesity were characterized by elevated FAO rates and a marked decrease in glucose oxidation rates,\(^11-16\) resulting in mitochondrial FAO dominating as a source of energy in the diabetic or obese heart.

A number of studies have also shown an accumulation of myocardial triacylglycerol (TG) in diabetes and obesity, although TG itself is not believed to contribute to myocardial insulin resistance.\(^17\) The mechanisms involved in the accumulation of lipid intermediates (eg, diacylglycerol, long-chain fatty acyl-CoA esters, and ceramide) are still to be elucidated. Two scenarios are proposed to explain the increased storage of intramyocardial TG and the accompanying cardiac contractile dysfunction accumulation: (i) impaired FAO and (ii) oversupply of FA.\(^17\) As explained by Zhang and Ren,\(^17\) “myocardial TG accumulation may either protect the heart by ‘storing away’ the detrimental lipid intermediates…, or elicit severe lipotoxicity thereby compromising cardiac function; meanwhile, the insulin-resistant heart in obesity and type 2 diabetes is unable to fully use glucose, forcing the heart to rely on FA for energy demand and thus prompting a vicious cycle of increased cardiomyocyte FA uptake, oxidation, and TG accumulation, all of which are hallmarks of lipotoxic cardiomyopathy.”\(^7\)

The systemic inflammation, generalized enlargement of fat deposits, and uncontrolled release of FA into the circulation associated with type 2 diabetes and obesity support the occurrence of cardiac adiposity, characterized by an increase in intramyocardial TG content and in the volume of fat surrounding the heart and vessels.\(^18\) Whereas initially these events may be defense mechanisms, helping to distribute energy, when excessive they can lead to myocardial damage and heart disease.\(^18\)

FAO inhibition has emerged as a novel approach for the treatment of IHD, subsequent to accumulating evidence that modulating cardiac energy metabolism by increasing glucose oxidation directly, or indirectly by FAO inhibition, can improve cardiac function of the ischemic heart.\(^5\) The potential for FAO inhibition to treat cardiac disease has been called upon in many basic and clinical studies, some of them using FAO inhibitors such as TMZ to achieve this metabolic effect.\(^19-21\) TMZ directly provides energy to the ischemic cardiomyocytes by directly inhibiting, in an ischemic myocardium, the energy-consuming free-FA oxidation in favor of glucose oxidation.

### Preclinical data

A recent study of Yao et al\(^22\) studied TMZ’s influence on sarcoplasmic reticulum calcium-transporting ATPase isoform 2a (SERCA2a) in diet-induced obese rats and palmitic acid–treated cardiomyocytes. SERCA2a has an important role in maintaining the calcium (Ca\(^{2+}\)) balance and contractile function of cardiomyocytes. In obese and type 2 diabetic models, reduced activity and expression of SERCA2a were observed to play a significant role in cardiac dysfunction.\(^22\) Yao’s study found that TMZ partially restored SERCA2a protein in diet-induced obese rats and palmitic acid–treated cardiomyocytes.\(^22\)

The same TMZ mechanism of action is involved in the cardiomyocyte protection from hypoxia-induced ischemia. A study by Wei et al\(^23\) was designed to test the hypothesis that treatment with TMZ would improve intracellular Ca\(^{2+}\) (Ca\(^{2+}\)) handling in hypoxic myocardial injury. The investigators found that in TMZ-treated cardiomyocytes, the amplitude of Ca\(^{2+}\)
Results suggest that TMZ ameliorates Cai oscillations and sarcoplasmic reticulum Ca\textsuperscript{2+} load were recovered; the diastolic Cai\textsuperscript{2+} concentration was decreased; and the activities of ryanodine receptor 2 (RyR2), Na\textsuperscript{+}/Ca\textsuperscript{2+} exchanger (NCX), and SERCA2a were increased. Hypertrophy was reduced in TMZ-treated hypoxic cardiomyocytes, and TMZ treatment enhanced the “metabolic shift” from lipid oxidation to glucose oxidation in the cardiomyocytes. These results suggest that TMZ ameliorates Ca\textsuperscript{2+} homeostasis through a switch from lipid to glucose metabolism, thereby producing the cardioprotective effect and the reduction in hypoxic cardiomyocyte damage.\textsuperscript{23}

In a murine model, a recent study aimed to determine whether pharmacologic inhibition of 3-ketoacyl-coenzyme A thiolase (3-KAT), which catalyzes the final step of FAO, could improve obesity-induced cardiomyopathy. The investigators observed that a 3-week treatment with TMZ prevented obesity-induced reduction in both systolic and diastolic function, concluding that targeting cardiac FAO may be a novel therapeutic approach to alleviate the growing burden of obesity-related cardiomyopathy.\textsuperscript{24}

**Clinical data and clinical implications**

Bucci et al\textsuperscript{25} conducted a study to dissect the contributions of plasma and intracellularly bound FA to myocardial FAO in obese individuals, and to investigate whether the hypothesized action of TMZ to shift myocardial metabolism from the utilization of FA to that of glucose occurs in humans. In these obese subjects, one important finding of the study was that myocardial TG represented a major source of FAs that underwent oxidation, and it was demonstrated that myocardial intracellular TG oxidation significantly provides FA-derived energy for mechanical work. In this study, data showed an important effect of TMZ in reducing the oxidation of TG-derived myocardial FAs from endogenous sources, improving myocardial efficiency.\textsuperscript{25}

In a previous study, the same authors studied regional FA metabolism in skeletal muscle and adipose tissue in humans and investigated the long-term effects of TMZ on glucose and FA metabolism.\textsuperscript{26} TMZ was observed to significantly increase skeletal muscle FA esterification and mildly upregulate glucose phosphorylation. The authors suggest that human obesity is characterized by a defect in tissue-FA storage capability, which is accompanied by an (potentially compensatory) elevation in skeletal muscle FAO; TMZ diverted FA from oxidative to nonoxidative pathways and provoked an initial activation of glucose metabolism in skeletal muscle.

More recently, a study by Shehata\textsuperscript{27} evaluated the effect of periprocedural administration of TMZ on the incidence of percutaneous coronary intervention (PCI)-induced myocardial injury and contrast-induced nephropathy in overweight (body mass index, 27-28 kg/m\textsuperscript{2}) diabetic patients with mild-to-moderate renal dysfunction. TMZ intake before elective PCI in these patients was associated with decreased incidence of contrast-induced nephropathy and myocardial injury.\textsuperscript{27}

**Conclusions**

In obese subjects likely to experience glucose intolerance and cardiac hypertrophy as a consequence of alterations in cardiac energy metabolism, elevated rates of FAO, and decreased glucose oxidation rates, the use of agents such as TMZ could be particularly beneficial. The specific properties of this modulator to reduce the oxidation of TG-derived myocardial FAs and to increase the glucose oxidation, therefore improving myocardial efficiency, possibly represents the additional value of this agent in preventing or minimizing the consequences of obesity, particularly in patients with IHD.

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Benefits of TMZ in obesity


A 72-year-old woman was admitted to the emergency department due to shortness of breath (New York Heart Association [NYHA] functional class III), chest oppression on exertion (Canadian Cardiovascular Society functional class II [CCSII]), and fatigue. She reported that all symptoms had become exacerbated within the past 6 months. The patient reported no previous cardiovascular disease, and the only risk factor she had was a body mass index (BMI) of 30.

Her blood pressure was in the upper limit range (140/90 mm Hg), and persistent resting tachycardia was documented (daytime heart rate [HR] range of 90-150 beats per minute [bpm]). Blood analysis excluded anemia and thyroid disorder as the main cause of her symptoms, while showing an elevated level of B-type natriuretic peptide (BNP; 250 pg/mL). Blood gas analysis revealed the presence of mild hypoxia (Po₂, 68 mm Hg; So₂, 90%) with normal pH, which was correlated to the presence of sleep apnea disorder. Normal chest X-ray and spirometry excluded the presence of a chronic lung disorder. Electrocardiography showed diffuse repolarization abnormalities, with the following features recorded: left atrium enlargement; stage III diastolic dysfunction with increased left ventricular (LV) filling pressure; and concentric remodeling of the left ventricle (normal chamber size with mild increase in wall thickness), which exhibited preserved global and regional systolic function (ejection fraction [EF], 60%). The right atrium was mildly enlarged and the right ventricle, normal; a mild increase in estimated systolic pulmonary arterial pressure was recorded. No valve abnormalities or signs of pericardial disease were documented. At this stage of the evaluation, based on the patient’s signs and symptoms, a diagnosis of heart failure (HF) with preserved EF (HFpEF) was made.

To further investigate if concomitant ischemic heart disease (IHD) was present (the patient complained of chest oppression on exertion), the patient underwent dobutamine stress echocardiography. The stress test ruled out IHD (no ischemic electro-
cardiogram changes, no symptoms, no wall motion abnormalities), therefore medications to treat HF- pEF, including angiotensin-converting enzyme (ACE) inhibitors (initially perindopril), β-blockers (initially nebivolol), and diuretics (initially indapamide), were started. On discharge, noninvasive ventilation with continuous positive airway pressure (CPAP) at night (for sleep apnea), measurements of body weight loss, and daily aerobic activity were also recommended.

One month later, the patient was evaluated during an ambulatory visit. She reported that her symptoms had improved a little, but there were no significant changes in NYHA functional class. She had normal blood pressure (130/80 mm Hg) with a resting heart rate of 80 bpm. Blood gas analysis indicated little improvement ($P_{O_2}$, 79 mm Hg; $S_{O_2}$, 93%; and normal pH). Electrocardiography indicated no relevant changes, with persistence of stage III diastolic dysfunction. Although somewhat reduced, the BNP level was still elevated, measuring 200 pg/mL. The ACE inhibitor perindopril was switched to enalapril, the β-blocker nebivolol was further uptitrated to 10 mg/day, and a more potent diuretic (furosemide 25 mg/day) was initiated, replacing indapamide. Recommendations for weight loss were strengthened.

Three months later, the patient was hospitalized for recurrent HF symptoms, with further limitation of daily activity and orthopnea. Electrocardiography on admission recorded tachycardia/atrial fibrillation, but spontaneous cardioversion was observed later on in the day. Holter monitoring documented several self-limiting episodes of atrial fibrillation, therefore anticoagulation and antiarrhythmic therapy with amiodarone, in combination with β-blocker therapy (now shifted to carvedilol 6.25 mg twice a day), was introduced. Diuretic therapy was further augmented. Despite the recommendations for lifestyle modifications, the patient had gained more weight. There were no changes on echocardiography, which still showed left ventricle EF to be 60% as well as persistent stage III diastolic dysfunction. Hemodynamic parameters at discharge were as follows: blood pressure (BP), 125/80 mm Hg; HR, 75 bpm; $S_{O_2}$, 94%; BMI, 31; and BNP level, 180 pg/mL.

The patient was then evaluated four weeks later, and again at three months and six months. Despite increasing diuretic doses and uptitration of the β-blocker, no further improvement was observed. Her NYHA functional class oscillated between class III and class II and three other episodes of hospitalizations for worsening HF occurred in the same year.

HF therapy was augmented by further introducing ivabradine (5 mg twice a day) in order to potentiate the HR-reducing efficacy of the β-blocker without affecting blood pressure. On top of the β-blocker (carvedilol 25 mg/day), ACE inhibitor (enalapril 10 mg/day), diuretic (furosemide 100 mg/day), and oral anticoagulant (dabigatran 150 mg twice a day), antiarrhythmic prophylaxis with amiodarone 200 mg was deemed necessary in order to maintain the atrial contribution to cardiac output. Moreover, spironolactone 25 mg/day—as a potassium-sparing drug—was added to the daily medication regimen. All medications were maximally uptitrated, with final doses reported in Table I.

However, despite the pharmacological and nonpharmacological (nocturnal noninvasive ventilation for sleep apnea) strategies adopted, the NYHA functional class remained high (class III), and quality of life, poor. The patient was hospitalized several times without real clinical benefit. She continued to gain body weight despite adoption of all the recommendations, and was therefore referred for bariatric surgery. Unfortunately, sudden cardiac death occurred before bariatric surgery could be performed.
Discussion

Obese patients are recognized to be at increased risk for developing HF. Indeed, the prevalence of HF changes according to BMI, with a 5% increase in HF prevalence in men and a 7% increase in HF prevalence in women for every 1 kg/m² increase in BMI. As compared with subjects with a normal BMI, obese subjects had double the risk of HF, with women being at higher risk.1 Functional and structural modifications have been recognized in this population and in experimental models of obesity. Clearly, obesity is associated with eccentric LV hypertrophy (LVH) and systolic and diastolic abnormalities, along with a propensity for more ventricular arrhythmias and sudden cardiac death.2-5 Indication of a specific cardiac pattern in these patients suggests the existence of an obesity-related cardiomyopathy,6 although this is still debated. Obese patients are more prone to develop signs and symptoms of HF despite preservation of cardiac systolic function.7 Several mechanisms have been implicated in the development of these modifications, including lipid accumulation in the heart (lipotoxicity theory), a chronic inflammatory state, volume overload, and neurohormonal activation.8 Moreover, often, patients with obesity suffer from sleep apnea disorders, further aggravating the effect of obesity on cardiac metabolism and general hemodynamics.9 Interestingly, once HF has developed, it seems that obesity confers a beneficial influence on prognosis in what has been termed the “obesity paradox.”10 There is little evidence regarding the impact of weight loss in obese HF and whether or not this is beneficial. Only a few studies have investigated the cardiovascular effects of both dietary weight loss and bariatric surgery, with few specifically including HF patients or animal models.11 Accordingly, the existence of the obesity paradox raises the question of whether or not obesity should be treated.12,13 Inconsistent results have also been obtained in clinical trials investigating patients with HFpEF. Moreover, mortality rates and rates of rehospitalization are not significantly different between HFpEF and HF with reduced LV function. This may be partially attributed to the multiple mechanisms responsible for HF development. Therefore, therapeutic pharmacological and nonpharmacological strategies should be tailored to patient needs.

REFERENCES

Mitochondria have a key role in regulating energy metabolism in most cells. They are best known for their pivotal contribution to energy production (in the form of adenosine triphosphate [ATP]) through the process of oxidative phosphorylation. This occurs via the production of reduced cofactors from oxidizable substrates (such as fatty acids, carbohydrates, proteins, and ketones) that supply electrons for the mitochondrial electron transport chain (ETC). Electron transfer through the ETC results in the conversion of O₂ to H₂O, and the pumping of protons out of the mitochondrial matrix into the intermembrane space (Figure 1). This creates a proton motive force, the energy from which is used to synthesize ATP as protons flow back into the mitochondrial matrix. Any disruption of the proton gradient across the inner mitochondrial membrane will end up in a decrease in ATP production. Instead, dissipation of the mitochondrial proton gradient will produce heat.

Uncoupling proteins (UCPs) are a family of mitochondrial anion carrier proteins that are differentially expressed in many tissues. The first UCP identified, UCP1, is predominantly expressed in brown adipose tissue (BAT). As a UCP, it dissipates the mitochondrial proton gradient, and therefore has a thermogenic role (Figure 1). Additional UCPs subsequently identified include UCP2 and UCP3. UCP3 is expressed mostly in skeletal muscle and heart, whereas UCP2 is expressed in a variety of tissues, including the brain. While UCP2 and UCP3 may also play a role in thermogenesis, these UCPs have also been implicated in
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Uncoupling proteins as mediators of mitochondrial metabolic rates

Abbreviations

BAT: brown adipose tissue; ETC: electron transport chain; GSIS: glucose-stimulated insulin secretion; ROS: reactive oxygen species; UCP: uncoupling protein

regulating fatty acid oxidation, reactive oxygen species (ROS) production, inflammation, neurodegenerative function, cell proliferation, and metabolic processes.2-4

Role of UCP1 in thermogenesis

BAT has a key role in regulating thermogenesis. UCP1 is highly expressed in BAT, which has a high capacity for mitochondrial respiration. Whereas mitochondrial respiration rates are much higher in BAT compared with white adipose tissue, actual ATP synthesis rates are not. This is likely due to the dissipation of the inner mitochondrial protein gradient by UCP1, resulting in the production of heat as opposed to ATP. While a role for BAT in regulating thermogenesis in rodents and human newborns has been recognized for decades, only recently has it been demonstrated that BAT in human adults may also play an important role in energy expenditure and thermogenesis.5 Using techniques involving positron emission tomography combined with computed tomography, the presence of BAT in the subscapular and cervical regions of adult humans was recently identified.6 The adipocytes in this BAT are enriched with UCP1, and cold exposure has been shown to increase BAT content.7 Of importance, an inverse relationship between BAT content and obesity, insulin resistance, and diabetes has been shown.8 This suggests that increasing BAT and UCP1 content may be an approach to treating obesity, insulin resistance, and diabetes by increasing energy expenditure and thermogenesis.

BAT and UCP1 expression can be modified by a number of different mechanisms. Cold exposure is one approach to increase BAT and UCP1 expression,6 which is inversely related to the body weight index. Cold exposure is also associated with an increase in β3-adrenergic receptor activation of UCP1 transcription and translation,9 thereby promoting thermogenesis. Thyroid hormones also promote UCP1 transcription and thermogenesis. Various peptides such as irisin, fibroblast growth factor 21 (FGF21), and bone morphogenetic protein 7/8 (BMP7/8) have also been proposed to promote the “beiging” of white adipose tissue, resulting in increased UCP1 expression.10 Combined, this is thought to increase energy expenditure,
increase insulin sensitivity, increase glucose and fatty acid uptake, and thereby modulate obesity and diabetes severity.

**Role of UCP2 and UCP3 in regulating energy metabolism in skeletal muscle and heart**

The close homology of UCP2 and UCP3 to UCP1 resulted in these UCPs also being initially implicated as being primarily involved in thermogenesis. UCP2 and UCP3 have a wider tissue distribution than UCP1, with UCP3 being primarily expressed in skeletal muscle. While these UCPs may have a role in thermogenesis, other functions are also evident. UCP3 has a key role in regulating mitochondrial fatty acid oxidation in heart and skeletal muscle, and as well as in preventing mitochondrial ROS production. By reducing mitochondrial membrane potential, UCP3 may decrease ROS production by the ETC. Deletion of UCP3 has also shown to decrease skeletal muscle fatty acid oxidation, possibly contributing to an increased accumulation and storage of fatty acids. UCP3 may also be a fatty acid anion carrier, resulting in transport of fatty acids out of the matrix of the mitochondria. Combined, these actions of UCP3 (and possibly UCP2) suggest a protective role in preventing obesity and diabetes.

**Role of UCP2 in other tissues**

UCP2 has a wider tissue expression than UCP1 and UCP3, including the expression of UCP2 in the central nervous system, macrophages, and pancreatic β cells. As such, UCP2 may have an important role in the regulation of food intake, energy expenditure, and glucose homeostasis. In the arcuate nucleus of the hypothalamus, UCP2 is highly expressed, and has an important role in neuronal control of food intake. Activation of UCP2, such as by the orexigenic hormone ghrelin, promotes food intake and decreases energy expenditure. These actions of UCP2 are thought to be mediated by a decrease in ROS production. Hypothalamic UCP2 also decreases glucose sensing in the hypothalamus, and increased UCP2 expression leads to a promotion of appetite and obesity. In the pancreas, UCP2 is also involved in glucose-stimulated insulin secretion (GSIS). Increased UCP2 expression is associated with a decrease in GSIS.

**Modifying UCP2 as an approach to modifying energy metabolism**

Stimulating UCP1 and UCP3 in BAT has the potential to increase thermogenesis and treat obesity and its associated diseases. The use of β₃-adrenergic agonists to increase UCPs and BAT is one such approach, although this approach needs further study. While thyroid hormone treatment can increase UCP1 and BAT, such treatment is associated with serious adverse side effects, such as tachycardia. The use of irisin to increase BAT and UCP1 has also been proposed, although the role of irisin in controlling BAT and UCP1 levels in humans has recently been questioned. Other peptides such as BMP7/8, meteorin-like protein (Metrn1), and FGF21, as well as small-molecule activators of UCPs are also being tested, although the potential of these approaches to upregulate UCPs and BAT has yet to be established.

**Conclusions**

UCPs have a critical role in regulating energetics through their action of decreasing mitochondrial coupling and efficiency. In addition to their role in regulating thermogenesis, UCPs have potential roles in regulating mitochondrial oxidative metabolism, as well as central nervous system control of food intake and peripheral control of glucose metabolism. As a result, targeting UCPs is a potentially promising approach to treating obesity and diabetes-related disorders.

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Uncoupling proteins as mediators of mitochondrial metabolic rates


low-density lipoprotein cholesterol (LDL-C) level is the most potent modifiable risk factor for cardiovascular disease. Indeed, LDL-C levels can be lowered with statin treatment, and this effect translates into a reduced incidence of cardiovascular events. However, not all patients on statin therapy achieve guideline-recommended LDL-C levels; thus, an additional and/or alternative lipid-lowering therapy may sometimes be required. Proprotein convertase subtilisin/kexin type 9 (PCSK9) inhibitors represent an emerging class of nonstatin therapy. PCSK9 is a circulating serine protease that binds to the LDL receptor (LDL-R) in the liver or in the systemic circulation and enhances intracellular LDL-R degradation. This effect reduces the ability of the cells to take up and degrade additional LDL-C and results in hypercholesterolemia. Following mutations involving the LDL-R and apolipoprotein B genes, a gain-of-function mutation in the PCSK9 gene is the third most common form of autosomal-dominant familial hypercholesterolemia (FH). Conversely, a loss-of-function mutation in the PCSK9 gene is associated with reduced LDL-C levels and rates of cardiovascular events. Therefore, the PCSK9 gene and its downstream products are promising therapeutic targets. Since 2003, a number of strategies—including monoclonal antibodies, small interfering ribonucleic acid (siRNA), and antisense oligonucleotides (ASOs)—have been developed and have already been tested in phase 1-3 clinical trials.

Monoclonal antibodies (eg, evolocumab, alirocumab, and bococizumab) bind to the region of PCSK9 that is required for interaction with the LDL-R, thus inhibiting the interaction between PCSK9 and LDL-R and consequent LDL-R degradation. Single-stranded siRNAs bind to the messenger RNA and inhibit PCSK9 function, thereby reducing circulating PCSK9 and LDL-C levels. Similarly, ASOs bind to and inhibit messenger RNA, causing a decrease in PCSK9 and LDL-C concentrations. More recently, a vaccine that targets circulating PCSK9 has also been developed and appears to significantly reduce total cholesterol, free cholesterol, phospholipids, and triglycerides in animal models, although this will need further clinical evaluation.

Taken together, available evidence suggests that PCSK9 inhibitors can reduce LDL-C levels by 50% to 70%. This effect appears to be dose-dependent and not affected by baseline LDL-C concentrations, age, gender, or ongoing statin treatment. In addition, PCSK9 inhibitors can reduce lipoprotein(a) levels by up to 30%, with a more modest effect on serum triglyceride and high-density lipoprotein cholesterol (HDL-C) levels. Hence, PCSK9 inhibition is associated with a lipid profile that is at least as good as the one obtained with statin treatment. Furthermore, these drugs appear to have a very favorable safety profile, with no significant adverse side effects. In line with these considerations, the US Food and Drug Administration (FDA) recently approved the use of two monoclonal antibodies, alirocumab (http://www.fda.gov/NewsEvents/Newsroom/PressAnnouncements/ucm455883.htm) and evolocumab (http://www.fda.gov/NewsEvents/Newsroom/PressAnnouncements/ucm460082.htm). Nonetheless, route of administration (by injection, received monthly or once every two weeks), exorbitant costs, and inadequate out-

**Will PCSK9 inhibitors stand up to statins till the end?**

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comes data could represent significant limitations for PCSK9-inhibitor therapy. Moreover, there is a final, but very important, question that remains to be answered: Will laboratory results showing cholesterol reduction translate into clinical benefits? In fact, except for ezetimibe, nonstatin therapies have not been shown to confer additional cardiovascular benefits when added to statins. Although a linear relationship between LDL-C and cardiovascular event rates was initially proposed for statins, the clinical utility of “surrogate end points,” such as LDL-C levels, has not been proven. Indeed, further evidence has suggested that, besides lowering LDL-C levels, statins have a series of positive effects on plaque stabilization and endothelial homeostasis (eg, anti-inflammatory, antioxidant, antiproliferative, and immunomodulatory effects), globally called “pleiotropic effects,” that could explain all of the beneficial clinical outcomes.

PCSK9 inhibitors are also expected to significantly improve cardiovascular outcomes. These results are eagerly awaited since they could newly challenge our understanding of the relationship between cholesterol levels and cardiovascular outcomes. However, results from the first trial testing for clinical benefits—the FOURIER trial (Further cardiovascular OUTcomes Research with PCSK9 Inhibition in subjects with Elevated Risk; https://clinicaltrials.gov/ct2/show/NCT01764633)—will not be available until 2018. Until then, PCSK9 inhibitors can be considered for use in patients who are intolerant to statins or who do not achieve target levels despite statin therapy, both in those with FH and those with nonfamilial forms of hypercholesterolemia.

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Adipokines
Adipokines is a term that is used to collectively describe a variety of signaling molecules that are released/secreted from the adipose tissue. Examples of adipokines include adiponectin and cytokines, such as tumor necrosis factor α (TNF-α).

Irisin
Irisin is a myokine (protein secreted from the skeletal muscle) encoded by the gene FNDC5. Its secretion from skeletal muscle is stimulated by exercise, where it acts in mice to promote browning of white adipose tissue, increased energy expenditure, weight loss, and the lowering of blood glucose levels. However, the relevance of these findings in humans has been questioned.

Myogenic factor
Myogenic factors are protein transcription factors that regulate myogenesis, which is the formation of muscular tissue. Myogenic factors include the proteins myogenin, herculin, myogenic factor 5, and MyoD.

Peroxisome proliferator–activated receptor γ coactivator 1α (PGC1α)
PGC1α is a transcriptional coactivator that plays a key role in the regulation of cellular energy metabolism. Activation of PGC1α increases mitochondrial biogenesis. In muscle, PGC1α activation results in a muscle that is more oxidative and less glycolytic.

Proprotein convertase subtilisin/kexin type 9 (PCSK9)
PCSK9 is a protein that binds to the low-density lipoprotein (LDL) cholesterol receptor in the liver. PCSK9 binding to the LDL receptor results in breakdown of the LDL receptor, thereby increasing blood cholesterol levels. Thus, inhibition of PCSK9 to increase liver LDL-receptor expression and subsequent lowering of blood cholesterol levels has been pursued for the treatment of high cholesterol. Some approved agents include alirocumab and evolocumab.

Steatosis
Steatosis is the accumulation of lipids (usually neutral lipids, such as triacylglycerol [TAG] and cholesterol) in a cell in higher than normal amounts. For example, increased TAG levels in the heart is referred to as cardiac steatosis and in the liver is referred to as hepatic steatosis.

Thermogenesis
Thermogenesis is the process by which an organism produces heat. This occurs either via shivering thermogenesis, where heat is produced from the conversion of the chemical energy of adenosine triphosphate (ATP) into kinetic energy, and some of this produced energy is lost as heat, or via nonshivering thermogenesis, which occurs primarily in brown adipose tissue and involves the actions of uncoupling protein 1 (UCP1). UCP1 is present in the inner mitochondrial membrane of cells and dissipates the proton gradient across this membrane. As a result of this action, mitochondrial respiration produces heat instead of ATP.

Uncoupling protein
Uncoupling proteins (UCPs) are proteins that are present in the inner mitochondrial membrane of cells; UCPs dissipate the proton gradient across this membrane. As a result of this action, mitochondrial respiration produces heat instead of adenosine triphosphate (ATP). Heart and skeletal muscle contain two isoforms of UCPs—UCP2 and UCP3. The exact function of these UCPs is not clear, but they may be involved in decreasing reactive oxygen species production by the mitochondria or transporting excess fatty acids out of the mitochondria. The expression of UCPs in the mitochondria is increased in muscle exposed to high fats.
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