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REVIEW

Induction of thermogenic adipocytes: molecular targets and thermogenic small molecules

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Adipose tissue is a central metabolic organ that controls energy homeostasis of the whole body. White adipose tissue (WAT) stores excess energy in the form of triglycerides, whereas brown adipose tissue (BAT) dissipates energy in the form of heat through mitochondrial uncoupling protein 1 (Ucp1). A newly identified adipose tissue called 'beige fat' (BAT-like) is produced through a process called WAT browning. This tissue mainly resides in WAT depots and displays intermediate characteristics of both WAT and BAT. Since the recent discovery of BAT in the human body, along with the identification of molecular targets for BAT activation, stimulating energy expenditure has been considered as a great strategy to treat human obesity and metabolic diseases. Here we summarize recent findings regarding molecular targets and thermogenic small molecules that can stimulate BAT and increase energy expenditure, with an emphasis on possible therapeutic applications in humans.
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INTRODUCTION

Obesity is caused by an imbalance between energy intake and energy expenditure.[1] The excess energy is stored as triglycerides in adipocytes. The prevalence of obesity and its related metabolic diseases is increasing worldwide. However, current approaches to combat obesity are limited due to their adverse side effects. For example, Orlistat, a well-known medication that blocks fat digestion, causes multiple side effects, including diarrhea, body aches, headache and nausea.[2–4] Phentermine is another widely used drug that suppresses appetite. However, phentermine targets the central nervous system and can cause severe mental changes and sensory deficits.[5] The recent discovery of brown adipose tissue (BAT) in adults has brought about a new interest in alternative therapies that activate BAT to treat obesity and associated metabolic diseases.[6–8] These studies have thus fueled the development of therapeutic strategies that increase energy expenditure.

The major site of energy storage is the white adipose tissue (WAT). BAT generates heat by oxidation of stored energy with the help of uncoupling protein 1 (Ucp1). Cold exposure and adrenergic activation can simulate Ucp1 expression and activity.[8–10] Beige adipocyte (BAT-like) is another subtype within white adipose tissue. The cells that make up this tissue are called brite (brown in white) or brown-like adipocytes. Similar to brown adipocytes, beige adipocytes also express Ucp1, which allows protons to cross the inner mitochondrial membrane, resulting in increased oxygen consumption and heat generation.[8,11] Heat generated through the activation of brown and beige adipocytes can protect mammals against cold exposure. This process greatly affects energy homeostasis and whole body metabolism.

The activation and/or induction of thermogenic adipocytes can lead to significant body weight reduction and improved metabolic parameters in animal studies. Thus, a promising therapeutic strategy to increase the energy expenditure is to use chemical agents to stimulate the induction of beige adipocytes or the activation of brown adipocytes (Figure 1 and Table 1). Several compounds, including berberine, butein, salsalates, fucoxanthin and peroxisome proliferator-activated receptor γ (Pparγ) agonists, have been identified as they exhibit great potential to activate/induce BAT or beige fat.[12–16] This review summarizes findings regarding the molecular mechanisms responsible for the induction of thermogenic adipocytes (Figure 1). This review also summarizes thermogenic small molecules (Table 1) that are derived from plants (natural thermogenic compounds), artificially synthesized small molecules (synthetic thermogenic compounds) or endogenous small molecules (endogenous thermogenic compounds). Finally, the possible therapeutic applications of these thermogenic compounds in human diseases are discussed.

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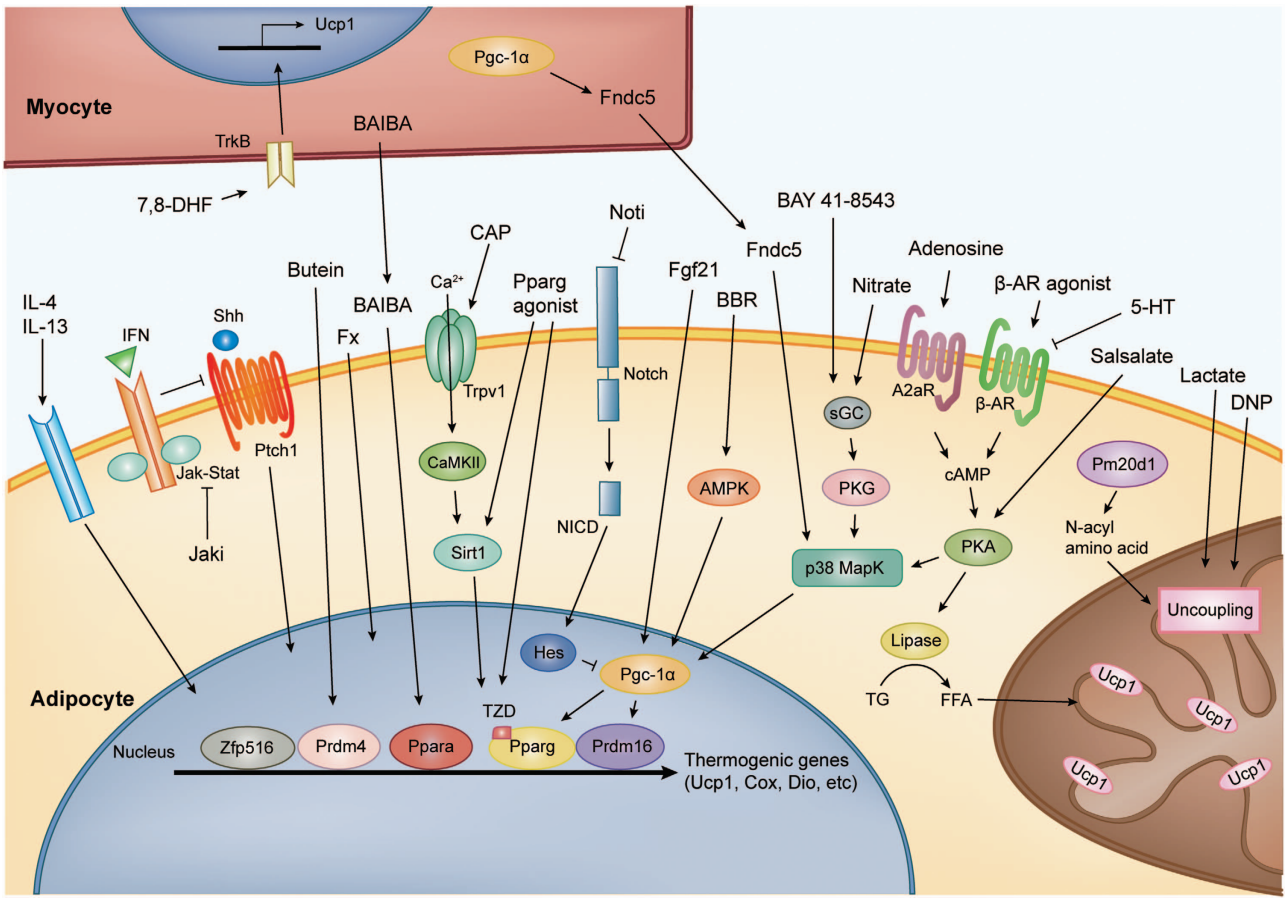

Figure 1 Molecular mechanism of thermogenic induction by small molecules. Activation of cell surface receptors, such as Trpv1, β3-AR, Ptch1 and A2aR, in adipocytes and TrkB in muscles involves cellular signaling cascades (PKA, PKG, Sirt1, AMPK and p38 MAPK), transcriptional regulators (Prdm family, Pgc-1α, Ppar family and Zfp516) and cytokines (IL-4 and IL-13) to induce Ucp1 expression. This process also stimulates brown adipocytes followed by Ucp1-mediated heat production. Natural thermogenic small molecules, such as berberine, butein, capsaicin and fucoxanthin, activate thermogenic transcriptional factors through their cell surface receptors or by modulating cellular signaling cascades in adipocytes. 7,8,DHF stimulates TrkB and induces sustained AMPK activity in muscles. Synthetic thermogenic compounds Ppar agonists, Jak inhibitors, Notch inhibitors, salsalate, β-AR agonists, BAY 41–8543 and DNP can also increase thermogenesis. Thermogenic small molecules, including serotonin, lactate, BAIBA, nitrate, and adenosine, are endogenously produced upon certain stimuli to increase thermogenic responses. BAIBA and lactate secreted from myocytes upon exercise can act upon white adipocytes and stimulate thermogenic conversion. A2aR, adenosine A2a receptor; AMPK, AMP-activated protein kinase; β-AR, β-adrenergic receptor; BAIBA, β-aminoisobutyric acid.; BBR, berberine; CaMKII, Ca^{2+}/calmodulin-dependent protein kinase II; CAP, capsaicin; 7,8 DHF, 7,8 dihydroxyflavone; DNP, dinitrophenol; Fx, fucoxanthin; Hes, hairy and enhancer of split; IFN, interferon; Jaki, Jak inhibitor; p38 MAPK, p38 mitogen-activated protein kinase; Pgc-1α, peroxisome proliferator-activated receptor gamma coactivator-1α; PKA, protein kinase A; PKG, protein kinase G; Pm20d1, peptidase M20 domain-containing 1; Ppar, peroxisome proliferator-activated receptor; Prdm, PR domain-containing proteins; Ptch1, Patched-1; sGC, soluble guanylate cyclase; Shh, Sonic hedgehog; Sirt1, Sirtuin 1; TrkB, tropomyosin-related kinase receptor B; Trpv1, transient receptor potential cation channel subfamily V member 1; TZD, thiazolidinedione; Ucp1, uncoupling protein 1; Zfp516, zinc finger protein 516; Noti, Notch inhibitor.

MOLECULAR TARGETS FOR INDUCING THERMOGENIC ADIPOCYTES

Uncouplers

Uncoupling protein 1. Ucp1 is a multipass transmembrane protein that is highly expressed in the mitochondria of thermogenic adipocytes, brown adipocytes and beige adipocytes. Ucp1 modulates the proton gradient between the mitochondrial matrix and the intermembrane space and generates heat by uncoupling the respiratory chain with a low rate of ATP production.[17,18] Because the proton gradient is essential for ATP synthesis, the activity of Ucp1 is tightly regulated. Normally, Ucp1 is activated in response to cold exposure. Cold exposure stimulates the secretion of noradrenaline from the sympathetic nervous system, leading to the activation of adrenergic receptors and the stimulation of the thermogenic response in adipocytes.[9,19] Leptin and other factors that stimulate free fatty acid release can activate BAT or beige fat through direct binding to fatty acids with Ucp1.[20–22] Cold exposure also increases Ucp1-dependent thermogenesis by increasing the levels of reactive oxygen species in the mitochondria and sulfenylation of Ucp1.[23] Ucp1 transcription is also regulated

Table 1 Thermogenic small molecules and their biological actions

Compound name	*Mechanism*	*Biological action*	*Reference*
Natural compound			
Berberine	AMPK activation	Classical BAT activation/browning of WAT	12
Butein	Prdm4 induction	Browning of WAT	13
Capsaicin	TrpV1 activation	Classical BAT activation/browning of WAT	64
7,8-Dihydroxyflavone	Muscular TrkB activation	Ucp1 induction in skeletal muscle	68
Fucoxanthin	Unknown	Ucp1 induction in WAT	15,72
Synthetic compound			
Pparγ agonist	Prdm16 stabilization	Browning of WAT	16,74–76
Jak inhibitor	Jak/Stat pathway inhibition	Unknown	77
Notch inhibitor	Notch pathway inhibition	Browning of WAT	80
Salsalate	Pka pathway	Classical BAT activation	14
β3-AR agonists	β-adrenergic receptor activation	Classical BAT activation/browning of WAT	46,85–91
BAY 41–8543	cGMP-dependent pathway	Classical BAT activation/browning of WAT	94
Dinitrophenol	Uncoupler	Heat production	95
Endogenous small molecule			
Serotonin	5-HTR activation, β-AR inactivation	Inhibition of classical BAT activation/browning of WAT	99,100
Lactate	Redox state modification	Browning of WAT	102
β-Aminoisobutyric acid	Pparα-mediated	Improving glucose tolerance and increasing energy expenditure	103
Nitrate	cGMP-dependent pathway	Classical BAT activation/browning of WAT	105
Adenosine	A2a receptor activation	Classical BAT activation/browning of WAT	107–109,131

Abbreviations: BAT, brown adipose tissue; cGMP, cyclic GMP; WAT, white adipose tissue.

by multiple transcription factors, including Pparγ, PR domain-containing 16 (Prdm16) and peroxisome proliferator-activated receptor gamma coactivator-1 alpha (Pgc-1α).[24–29] Thus, to generate functionally active thermogenic adipocytes, regulation of Ucp1 transcription and activity should be carefully considered.

Peptidase M20 domain-containing 1. Most studies related to the thermogenic action of adipocytes have focused on the expression and activity of Ucp1 due to limited knowledge regarding thermogenic enzymes. Nonetheless, questions have been steadily raised for the presence of Ucp1-independent thermogenic processes. Until recently, the underlying mechanism of Ucp1-independent thermogenic processes has been unclear.[30,31] Peptidase M20 domain-containing 1 (Pm20d1) is a bi-directional *N*-acyl amino acid producing enzyme (from fatty acids and amino acids) that has been recently identified as a new thermogenic molecule that functions independent of Ucp1. *N*-Acyl amino acids processed from Pm20d1 can directly bind to the mitochondria and function as endogenous uncouplers, leading to increased mitochondrial respiration to replenish the ATP pool. Mice injected with Pm20d1-containing adenovirus have been found to be protected from diet-induced obesity via an increase in energy expenditure.[32] Moreover, injection of *N*-acyl amino acid itself can also improve glucose metabolism and increase energy expenditure.[32]

Transcriptional regulators

PR domain-containing protein. PR domain-containing protein 16 (Prdm16) was first identified as a powerful transcriptional regulator of brown adipogenesis via modulation of the muscle-to-brown fat switch.[26] Forced expression of Prdm16 in white adipocyte precursor cells can lead to the induction of brown adipocyte-selective genes, resulting in higher metabolic rates.[25,26,33] Transgenic mice expressing Prdm16 in WAT depots are protected from obesity due to improved glucose metabolism.[34] Consistently, adipocyte-specific knock-out of Prdm16 exacerbates obesity and glucose homeostasis.[35] Mechanistic studies have further revealed that Prdm16 interacts with multiple transcriptional regulators, including Pparγ, euchromatic histone-lysine *N*-methyltransferase 1 (Ehmt1), CCAAT/enhancer binding protein beta (Cebpβ) and transducing-like enhancer of split 3 (Tle3).[26,36–38] Another transcriptional Ucp1 regulator, Prdm4, has also been recently identified. Loss of Prdm4 can increase white adipocyte differentiation while suppressing the expression of thermogenic genes in beige and brown adipocytes. Mice lacking Prdm4 have shown increased weight gain and insulin resistance on a high-fat diet.[13] Therefore, targeting pathways to increase Prdm16/Prdm4 expression may lead to new therapeutic avenues for obesity and diabetes.

Peroxisome proliferator-activated receptor gamma coactivator-1α. Peroxisome proliferator-activated receptor gamma coactivator-1α (Pgc-1α) was identified as a binding partner of Pparγ in brown adipocytes.[27] Pgc-1α plays a critical role in

cold-mediated WAT browning downstream of β3-adrenergic receptor signaling pathways. β3-Adrenergic receptor agonist or cold exposure can activate MAPK and cAMP signaling to modulate the activity and expression of Pgc-1α. The ectopic expression of Pgc-1α in white adipocytes induces a brown adipocyte-selective gene program and increases cellular respiration.[28,29] Mechanistic studies have revealed that Pgc-1α binds with multiple transcription factors to regulate brown adipocyte-specific gene programs. Interaction of Pgc-1α with Prdm16 and mediator of RNA polymerase II transcription subunit 1 (Med1) can increase the expression of Ucp1.[39,40] Pgc-1α also binds with interferon regulatory factor 4 (Irf4) to control transcription of the *Ucp1* mRNA.[41]

Forkhead box protein c2. Forkhead box protein c2 (Foxc2) is a well-known transcription factor that regulates adipocyte differentiation and metabolism.[42–44] Foxc2 transgenic mice have increased beige fat formation and BAT activity, subsequently increasing the rates of oxygen consumption and energy expenditure. Foxc2-activated thermogenic adipocytes are mediated by an elevated level of β-adrenergic receptor-PKA signaling, leading to higher Ucp1 expression.[45]

Zinc finger protein 516. Cold-inducible zinc finger protein 516 (Zfp516) was identified by its interaction with the proximal region of the Ucp1 promoter.[46,47] Prdm16 and Zfp516 complexes can also induce the expression of Ucp1 and Pgc-1α. Knockout of Zfp516 has caused embryonic lethality, with a significant reduction in BAT mass. Conversely, adipocyte-specific expression of Zfp516 can activate thermogenic adipocytes in WAT depots and prevent diet-induced obesity by increasing energy expenditure.[46]

Hormones

Irisin. Although intrinsic transcription factors regulating thermogenic adipocytes have been widely studied, extrinsic factors are relatively less elucidated. One important hormone that induces beige fat from WAT is irisin.[48,49] Transgenic mice expressing Pgc-1α in muscles have increased beige fat and are protected from diet-induced obesity. Subsequent studies have shown that induction of thermogenic adipocytes by muscle Pgc-1α is mediated by muscle-secreted irisin. Injection of irisin (also called fibronectin type III domain-containing protein 5)-expressing adenovirus can induce beige fat formation with increased thermogenic gene program expression, improve glucose metabolism, and increase energy expenditure.[48] Circulating levels of irisin appear to be correlated with acute exercise. However, its physiological roles in humans remain to be determined.[50]

Fibroblast growth factor 21. Fibroblast growth factor 21 (Fgf21) has been studied as a critical metabolic regulator in multiple organs, including adipose tissues, the liver and the pancreas.[51] Fgf21-knockout mice have abnormal gene expression patterns and body temperatures when exposed to low temperatures. Therapeutic doses of Fgf21 can lower glucose levels shortly after being administered in both mice and humans. The impaired expression of a thermogenic gene program is also associated with a reduced level of Pgc-1α protein in adipocytes.[52]

Cytokines

Type II cytokines, including IL-4 and IL-13, play beneficial roles in adipose tissue remodeling. Cold exposure activates eosinophils to secrete IL-4 and IL-13, resulting in alternative activation of macrophages in adipose tissues. These activated macrophages in turn produce catecholamines, resulting in the induction of WAT browning and of the thermogenic capacity of BAT.[53] It has also been demonstrated that IL-4 and IL-13 are critically involved in the commitment of beige adipocytes from the progenitors in WAT depots, thereby revealing dual roles of type II cytokines in the induction of thermogenic adipocytes.[54]

NATURAL THERMOGENIC COMPOUNDS

Berberine

Berberine is a natural quaternary ammonium salt found in medicinal herbal plants including barberries (*Berberis* spp.), Oregon grape holly (*Mahonia aquifolium*), goldenseal (*Hydrastis canadensis*) and Chinese goldthread (*Coptis chinensis*).[55–57] These medicinal plants are mainly used to treat diarrhea and metabolic diseases. Previous studies have shown that berberine-treated db/db mice can be protected from obesity by increasing energy expenditure via the activation of BAT and browning of inguinal but not epididymal WAT. Mechanistic studies have shown that the AMPK/Pgc-1α pathway is responsible for the induction of Ucp1 expression and brown adipocyte-selective gene program expression.[12]

Butein

Butein, a naturally occurring chalcone, is isolated from the medicinal Chinese lacquer tree (*Toxicodendron vernicifluum*).[58] *Toxicodendron vernicifluum* has been shown to possess anti-inflammatory, anti-obesity and anti-cancer activities.[59–61] Butein also has the ability to induce *Ucp1* mRNA expression. Although the *in vivo* efficacy of butein in obese or diabetic mice has not yet been demonstrated, butein has been utilized to identify Prdm4 as a key driver of the brown and beige fat gene program. Prdm4 knockdown and overexpression studies have further revealed that Prdm4 can stimulate Ucp1 expression and increase energy expenditure.[13]

Capsaicin

Capsaicin is one of the bio-active compounds found in chili peppers.[62] This compound produces a burning sensation in humans. Capsaicin has been shown to be able to activate transient receptor potential cation channel subfamily V member 1 (TrpV1), resulting in anti-obese effects in mice.[63,64] Capsaicin treatment can increase BAT activity and *Ucp1* mRNA expression in inguinal WAT, leading to weight loss.[65] Similar to the action of capsaicin, the capsinoids capsiate, dihydrocapsiate and nordihydrocapsiate can also activate TrpV1 and subsequently increase energy metabolism; however, they do

not cause pungency.[66] Thus, further investigation of capsinoids may also provide alternative treatment for metabolism-related diseases.

7,8-Dihydroxyflavone

7,8-Dihydroxyflavone (7,8-DHF) is a naturally occurring flavone found in primula tree (*Godmania aesculifolia*) leaves.[67] This flavone has been shown to have beneficial effects on central nervous system-related diseases.[68,69] In metabolic diseases, 7,8-DHF-treated female, but not male, mice have been shown to be resistant to obesity induced by a high-fat diet. This resistance can be explained by increased expression of Ucp1 in muscles accompanied by increased energy expenditure without appetite suppression.[70] However, the mechanism for its differential effects in the different sexes is currently unclear. Regardless, it is known that 7,8-DHF treatment increases Ucp1 expression and AMPK activity via TrkB (tropomyosin-related kinase receptor B) in skeletal muscles. The anti-obese effect of 7,8-DHF is blunted in TrkB-knockout mice, further indicating that TrkB plays a crucial role in 7,8-DHF-mediated anti-obese effects.[70]

Fucoxanthin

Fucoxanthin is a highly enriched carotenoid found in edible seaweeds.[71] Fucoxanthin has been shown to prevent obesity, metabolic disease and cancer.[72,73] Fucoxanthin treatment in mice can reduce whole body weight and abdominal fat. These improvements in metabolic parameters are also related to the induction of Ucp1 protein levels in white adipose tissue.[15,74]

SYNTHETIC THERMOGENIC COMPOUNDS

Ppar γ agonists

Pparγ, a nuclear receptor, belongs to the class of transcription factors with a characteristic ligand binding domain. Pparγ is regarded as the master regulator of adipogenesis.[6,75] Treatment with Pparγ agonists can stimulate the formation of beige adipocytes. However, its underlying mechanism remains unclear.[76–78] A recent study has indicated that Pparγ agonists, particularly rosiglitazone, can induce beige adipocyte formation through stabilizing the Prdm16 protein. The induction of beige adipocyte by rosiglitazone is blunted in Prdm16-knockdown cells, further indicating a role for Prdm16 in rosiglitazone-mediated WAT browning.[16]

JAK inhibitors

In a recent study, Moisan *et al.*[79] screened small-molecule inducers of human beige fat from white adipocytes and identified JAK inhibitors—tofacitinib and R406—as key molecules. JAK inhibitors can reduce lipid-droplet size, increase cellular oxygen consumption and increase Ucp1 expression in human adipocytes. These JAK inhibitor effects could be due to the repression and activation of the interferon and hedgehog signaling pathways, respectively.[79]

Notch inhibitor

Notch signaling is a critical pathway in the central nervous system. This pathway also plays an important role in metabolic regulation.[80,81] Adipocyte-specific deletion of notch1 or Rbpj (a downstream activator of notch signaling) in mice can induce beige adipocytes, resulting in an increase in whole body energy expenditure. Treatment with the chemical notch inhibitor DAPT has shown similar upregulation of brown fat-specific genes, including Ucp1, in cultured and primary adipocytes. Injection of another notch inhibitor, DBZ, in leptin-deficient mice resulted in a reduction in body weight gain and improvement in metabolic parameters.[82]

Salsalate

Salsalate is a powerful anti-inflammatory drug originating from salicylates. This drug has been traditionally used to reduce pain and inflammation. Its action can be explained by reducing inflammatory chemical signals such as TNF-α and IL-6.[83] The beneficial effects of salsalate in metabolic disease have been observed in salsalate-treated patients.[84–86] A recent study has shown that salsalate-treated mice have increased BAT activity and are resistant to diet-induced obesity. Salsalate has also resulted in a reduction in body weight in preestablished obese mice, further suggesting its therapeutic potential in obesity. Mechanistically, salsalate appears to modulate PKA activity in brown adipocytes.[14]

β3-Adrenergic receptor agonists

β3-Adrenergic receptor (AR) has a critical role in BAT activation and WAT browning through PKA-mediated signaling.[87,88] Cold-exposed β3-AR knockout mice have shown reduced Ucp1 expression compared to wild-type counterparts.[89] Mice with triple knockout of all beta receptors (β1, β2 and β3) are more prone to diet-induced obesity and metabolic diseases due to defects in thermogenic activities.[90] In accordance with this finding, two well-known β3-AR agonists, isoproterenol and CL316.243, have been shown to cause marked increases in Ucp1 expression at the mRNA and protein levels in both brown and white adipocytes.[91,92] Furthermore, these agonists have been shown to activate BAT and induce WAT browning in mice.[46,93]

BAY 41–8543 (soluble guanyl cyclase)

Cyclic GMP has also been shown to be connected to metabolic processes in BAT via its roles in regulating mitochondrial activity.[94,95] Cyclic GMP-dependent pathways can be targeted as a new therapeutic approach to treat obesity and metabolic diseases. Treatment with BAY 41–8543 can sustain soluble guanylyl cyclase, reduce body fat mass and improve glucose metabolism in diet-induced obese mice. Accordingly, increased thermogenic adipocytes and higher levels of energy expenditure are observed in mice treated with BAY 41–8543. The thermogenic activity of BAY 41–8543 is mediated by the activation of lipid uptake in BATs and increased differentiation of brown adipocytes.[96]

Dinitrophenol

Dinitrophenol (DNP) is a proton ionophore that enables protons to cross mitochondrial membranes.[97] This process allows protons to leak out of the mitochondria without coupling the proton gradient to ATP synthesis. In the past, DNP was widely used as a dieting aid. However, it has been discontinued due to numerous side effects, including death in several patients.[98] Acute exposure to DNP increases the metabolic rate; it also causes nausea, vomiting and headache.[99] Furthermore, long-term treatment with DNP causes severe side effects, including cataracts, lesions and cardiovascular failure among others.[98]

ENDOGENOUS THERMOGENIC SMALL MOLECULES

Serotonin

Serotonin is a monoamine neurotransmitter. The role of serotonin and its related proteins in metabolism has also been widely investigated.[100] Recent studies performed by two independent groups have reported that serotonin can negatively act on WAT browning. Whole body or adipose tissue-specific deletion of Tph1, the enzyme that produces serotonin from its precursor tryptophan has protected mice from high-fat diet-induced obesity. Such an effect is due to an increased energy expenditure. Consistently, mice injected with chemical inhibitors of Tph1 are also resistant to diet-induced obesity.[101,102]

Lactate

Lactate is a well-known cellular metabolite produced in muscles during anaerobic glycolysis and high-intense activity.[103] Lactate has been traditionally thought as a cellular waste product. However, recent studies have revealed a new function of lactate in the browning effect. Carrière *et al.* presented evidence showing that cold exposure can increase circulating lactate levels and induce monocarboxylate transporter (Mct1) (lactate importer) gene expression in BAT and subcutaneous WAT. Exposure to lactate in adipocytes can stimulate mitochondrial activity, fatty acid oxidation and Ucp1 expression. These metabolic effects of lactate are negated in the presence of Mct1 inhibitors, showing that lactate transport is important in lactate-mediated WAT browning.[104]

β-Aminoisobutyric acid

Exercise has been considered the best treatment for obesity and metabolic diseases. Robert *et al.*[105] suggested that the modulation of circulating hormones and small molecules by increasing muscle activity (exercise) is closely associated with adipocytes. These authors identified β-aminoisobutyric acid (BAIBA) as a key small-molecule myokine responsible for muscle-mediated WAT browning. BAIBA treatment in human cells can increase thermogenic gene expression, lipid oxidation and oxygen consumption rates. Consistently, BAIBA treatment in mice has decreased weight gain and improved glucose tolerance through a Pparα-mediated mechanism.[105] Thus, BAIBA can add benefit to exercise against metabolic diseases.

Nitrate

Inorganic nitrate is a cellular metabolite produced from NO oxidation.[106] In the past, nitrate was considered a non-bioactive molecule; however, it has been recently reported that nitrate has anti-obesity effects via thermogenic adipocyte induction.[107] Nitrate treatment can induce brown adipocyte-selective genes in primary adipocytes, and dietary nitrate supplementation can increase beige adipocyte formation in WAT depots. The effect of nitrate on WAT browning is dependent on the nitrate–nitrite–NO pathway and cyclic GMP signaling.[107]

Adenosine

Adenosine is an abundant ribonucleoside in the human body. This ribonucleoside plays critical roles in energy transfer and signal transduction.[108] Adenosine regulates BAT lipolysis and respiration in hamster and mouse models.[109,110] Adenosine treatment can activate thermogenic gene programs in both human and mouse brown adipocytes. Loss of adenosine receptor or treatment with adenosine antagonists has been shown to impair BAT-dependent thermogenesis, whereas activation of adenosine receptor prevents diet-induced obesity by inducing WAT browning and increasing energy expenditure.[111]

UNRESOLVED ISSUES FOR THERMOGENIC SMALL MOLECULES

Modern technologies in cell, molecular biology and genetic model systems have greatly advanced our understanding of the molecular mechanisms of cell biology, including brown adipocytes and WAT browning. In addition, recent progress in the identification of chemical regulators has further suggested that BAT should be considered promising therapeutic targets for weight management and metabolic diseases. Although recent studies have indicated that modulating energy expenditure by BAT or beige fat is highly effective in treating metabolic diseases, the significance of BAT in human physiology and unsolved issues for future therapeutic applications remain to be clarified.

Therapeutic applications in humans

Can brown fat in humans help increase energy expenditure beyond its role in maintaining body temperature? One of the main concerns is whether induction of browning in humans is a legitimate strategy against obesity and metabolic diseases. It is clear that diet-, cold- and exercise-induced WAT browning and BAT activation in mice can prevent obesity and its associated metabolic diseases. However, this strategy has not yet been deemed attractive against human metabolic diseases. Indeed, the proportion of brown fat mass in humans is nearly 1/10 of that in mice. Systemic administration of catecholamines is negatively associated with human obesity; it is ineffective for human thermogenesis, with potential sympathomimetic effects.[112] By contrast, 3 h of cold exposure in humans can increase energy expenditure by 1.8-fold. This result is thought to be largely mediated by increased BAT activity.[113]

The amount of BAT in human is inversely correlated with BMI, suggesting that the activation of BAT can play a significant role in counteracting human metabolic diseases.[114] Nevertheless, further studies are needed to determine whether WAT browning can be a valuable therapeutic strategy in humans.

Potential negative outcomes by BAT stimulation

Are there any unwanted side effects of browning (or increased BAT activity) in humans? WAT browning or increased BAT activity can protect animals from weight gain, and it can increase insulin resistance by enhancing energy expenditure and thermogenesis. As seen in DNP cases, increased uncoupling can also affect body temperature, free radical levels, injury risk and cellular metabolic rates.[98,99] However, further research is needed to determine the potential harmful effects of sustained BAT activity. Alternatively, to prevent any possible negative effects, temporal control of BAT activation could be used as an essential therapeutic intervention against metabolic diseases. In addition, targeted delivery to adipocytes (discussed below) may be required to circumvent psychological and other effects on non-adipose tissues.

Tissue-specific control of BAT activity

Because currently available anti-obesity medications are often limited by their psychological or cardiovascular side effects, specific targeting of adipose tissue is needed to remove any potential side effects. Most small molecules, including berberine, butein and β3-AR agonists, have shown effects on the neuronal system, thus suggesting that they might have unwanted actions on the cardiovascular system or neuronal tissues. By increasing the concentration of the drugs in specific tissues, but not in others, targeted drug delivery can avoid the interaction of thermogenic small molecules with healthy tissues, thereby overcoming the downfalls of conventional methods of drug delivery. Indeed, a recent discovery by the Langer group has shown that nanoparticle drug delivery methods targeting adipose tissues can be effective for obesity and insulin resistance without drug accumulation in other tissues.[115] Involvement of different types of precursor cells during the induction of beige fat in subcutaneous WAT and inguinal WAT may also provide potential therapeutic approach to modulate WAT depot-specific induction of thermogenesis.[116]

In addition, targeting CNS or mimicking outflow of PNS to activate brown fat or WAT browning can have therapeutic potential. Autonomic hypothalamic innervation and peripheral temperature-sensitive neurons are involved in BAT activation and energy expenditure.[117,118] Menthol-activated Trpm8 and capsaicin-activated Trpv1 or Trpv4 have been shown to be effective for BAT activation and weight management.[119–121] The melanocortin system also plays a significant role in the sympathetic outflow, BAT activation, and energy expenditure.[122] Mirabegron, a β3-AR agonist, can activate BAT thermogenesis, induce glucose uptake, and increase energy expenditure in humans, although some concerns, such as increased heart rate and blood pressure, still exist.[10] In human studies, the GLP1 analog liraglutide and dipeptidyl peptidase-4 inhibitors have been used as GLP-1 activators and have been shown to be able to activate BAT, increase WAT browning, and lower body fat.[123,124] Therefore, a better understanding of neuronal control of BAT activity and selective targeting to specific neurons can also provide new optimal strategies without causing harmful effects in humans.

BAT activity in other diseases

Cancer cachexia is an atrophy of muscle and adipose tissue in cancer patients. This condition can be easily observed in cancer patients and is one of the main causes of decreased survival rates and survival periods in cancer patients.[125] Cancer patients have higher energy expenditure rates, indicating that brown or beige fat might have been induced.[126] Using microarray analysis in cancer clones, PTHrP has been identified as a crucial thermogenic factor secreted by cancer cells. Neutralization of PTHrP in cancer-bearing mice has abrogated the WAT browning effect by cancer.[127] Another possible explanation of WAT browning by cancer cells is chronic inflammation.[128] Cancer-induced chronic inflammation and IL-6 production have been reported to be responsible for WAT browning in cancer-bearing mice. Similar to PTHrP, anti-inflammatory treatments can also reduce the thermogenic activity of adipose tissues. As such, current approaches for diminishing symptoms of cancer cachexia rely on anti-inflammatory treatments. In the future, more specific targets, such as PTHrP and IL-6, should be investigated to alleviate cancer cachexia. Because numerous genetic factors and small molecules have been reported to have effects on WAT browning, approaches that reduce thermogenic adipocytes should be considered for cancer patients.

MOVING FORWARD

How can we better and effectively activate BAT? With only a handful of thermogenic small molecules being available, a few immediate strategies can be used to achieve better treatment effects. First, chemical modifications can be made to increase solubility, enhance targeted delivery, and improve controlled release. For instance, structure–activity relationship studies by chemical optimization can bring about thermogenic small molecules that are more effective against obesity and metabolic diseases. For example, optimized sirtuin inhibitors based on resveratrol have been developed, and their efficacies have been tested in diabetic animals. Modifications, such as PEGylation, encapsulation with nanoparticles, and various other approaches executed for cancer, could also be applied for diabetes treatment. Targeted delivery to regions, such as hypothalamic sites, brown adipose tissues, and white adipose tissues, can further reduce dosages and side effects in healthy tissues. This approach can also reduce the fluctuation of chemical levels in the circulation. The field of drug delivery has advanced markedly in the past few decades. Collaboration is needed to

increase the pharmacokinetics of thermogenic small molecules in the near future.

Second, combinatorial compounds are currently being used for various diseases. Combinatory treatments with two or more drugs with different mechanisms may thus increase the beneficial metabolic effects. For example, GLP-1 agonist, liraglutide, and melanocortin receptor agonist, RM-493, have been shown to have additive metabolic benefits in diet-induced obese mice.[129] Similarly, combinations of plant-derived polyphenols, such as carotenoids and isoflavones, also offer great potential to facilitate energy metabolism. Third, 'precision or individualized medicine' is emerging as a result of advancement in research technologies and clinical practice. The amounts of BAT, BMI, environments (local temperatures, exercise, and food) and genomes can vary widely among individuals. Therefore, unique approaches can be made for each patient. To achieve this, improved diagnosis of BAT activation in humans (preferably with non-invasive approaches) and identification of biomarkers would be required to dissect the differences needed for personalized medicine.

Finally, aside from focusing on the chemistry of small molecules, small molecules can also be used as tools to identify new molecular targets for therapeutic intervention and thus provide novel insights on the plasticity of adipocytes. Using the same concept, PPARγ, MyoD and Sirt1 have been identified as molecular targets of a thiazolidinedione, suberanilohydroxamic acid and resveratrol, respectively. These molecules have been highlighted as targets for the therapeutic intervention of metabolic diseases and have offered novel insights into the biology of such diseases. Likewise, the identification of Prdm4 by using butein also emphasizes the utility of small molecules in the better understanding BAT physiology. Therefore, identification of better small molecules could provide new insights into thermogenic adipocytes. These molecules can thus be used as alternative therapeutic targets to develop interventions against obesity and metabolic dysregulation.[130]

CONCLUSION

Recent studies of metabolism have focused on understanding the biology of BAT. This adipose tissue utilizes glucose and fatty acids as energy sources to burn calories and generate heat in response to cold exposure.[130] Since the discovery of functional BAT in humans, targeting BAT is a promising therapeutic approach for treating obesity and metabolic diseases. However, further research is needed to reveal the significance of BAT (and WAT browning) in humans and its potential applications in human metabolic diseases.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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