Current Topics

Ion Channels as Therapeutic Targets for the Immune, Inflammatory, and Metabolic Disorders

Review

Role of Thermo-Sensitive Transient Receptor Potential Channels in Brown Adipose Tissue

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Brown and beige adipocytes are a major site of mammalian non-shivering thermogenesis and energy dissipation. Obesity is caused by an imbalance between energy intake and expenditure and has become a worldwide health problem. Therefore modulation of thermogenesis in brown and beige adipocytes could be an important application for body weight control and obesity prevention. Over the last few decades, the involvement of thermo-sensitive transient receptor potential (TRP) channels (including TRPV1, TRPV2, TRPV3, TRPV4, TRPM4, TRPML, TRPC5, and TRPA1) in energy metabolism and adipogenesis in adipocytes has been extensively explored. In this review, we summarize the expression, function, and pathological/physiological contributions of these TRP channels and discuss their potential as future therapeutic targets for preventing and combating human obesity and obesity-related metabolic disorders.

Key words transient receptor potential (TRP) channel; calcium; energy metabolism; differentiation; brown adipocyte; beige adipocyte

1. INTRODUCTION

Transient receptor potential (TRP) ion channels constitute a major class of calcium-permeable channels, most of which are non-selective.1) TRP channels were originally discovered in mutant Drosophila that responded abnormally to light stimulus.1) TRP channels exhibit six trans-membrane (TM) domains (TM1 to TM6) and a pore loop between TM5 and TM6, with both N- and C-termini in the cytosol.2–6) TRP channels are unique cellular sensors characterized by various activation mechanisms, such as those that are activated by thermal and mechanical stimuli.7) The TRP channel superfamily is subdivided into seven subfamilies: TRPV (vanilloid), TRPC (canonical), TRPM (melastatin), TRPML (mucolipin), TRPN (NOMPC), TRPP (polycystin), and TRPA (ankyrin) based on their primary amino acid sequences.8–10) In mammals, there are six TRP subfamilies and 28 channels. TRP channels are expressed in many tissues and exhibit a wide variety of physiological functions, including detection of various physical and chemical stimuli in vision, taste, olfaction, hearing, touch, and thermosensation.11,12) The gene encoding the capsaicin receptor as a noxious heat sensor, which is now called TRPV1, was isolated from a rodent sensory neuron cDNA library in 1997 and considered a breakthrough for research concerning temperature sensing.13) Since then, several TRP channels exhibiting thermosensitive abilities have been identified in mammals, with 11 thermo-sensitive TRP (thermo-TRP) channels reported to date. These channels belong to the TRPV, TRPM, TRPA, and TRPC subfamilies, and their temperature thresholds for activation are in the range of physiological temperatures, which can be discriminated. TRPV1 and TRPV2 are activated by elevated temperatures, whereas TRPM8 and TRPA1 are activated by lower temperatures. TRPV1, TRPV2, TRPV3, TRPV4, TRPM2, TRPM4, and TRPM5 are activated by warm temperatures. In addition, TRPM3 was identified as a sensor for noxious heat and TRPC5 was activated by temperature decreases.14,15) Thermo-TRP channels usually function as “multimodal receptors” that respond to a variety of stimuli: not only physical stimulation such as temperature and mechanical force, but also endogenous and exogenous chemicals including pungents, cations, oxidants, lipids, drugs, and hormones. Activation of these channels may contribute to changes in intracellular Ca2+ concentrations ([Ca2+]i) and control of membrane potentials in many cell types, except for TRPM4 and TRPM5, which are not permeable to divalent cations. While thermo-TRP channels expressed in sensory neurons and skin can act as ambient temperature sensors, these channels are also expressed in many tissues that are not exposed to dynamic temperature changes. This observation suggests that thermo-TRP channels have physiological roles that could be unrelated to sensation of temperature changes. Thermo-TRP channels are also expressed in tissues that influence energy intake and expenditure, including the hypothalamus, peripheral sensory neurons, gastrointestinal tract, liver, and adipose tissue.16–21) The physiological significance of thermo-TRP channels in

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these tissues, especially adipose tissue, has recently been reported.

2. BROWN AND BEIGE ADIPOSE TISSUE AND ENERGY METABOLISM

2.1. Brown and Beige Adipose Tissue Adipose tissue is a highly specialized tissue that plays a key role in energy homeostasis. There are two types of adipose tissue in mammals, termed “white adipose tissue” (WAT) and “brown adipose tissue” (BAT). Recently, a new type of recruitable brownish adipocytes, termed “beige adipocytes” (also known as “brite adipocytes”), was discovered among white adipocytes, especially in subcutaneous WAT. Beige adipocytes are induced by sympathetic nerve activation after cold exposure or by treatment with β3-adrenergic receptor agonists. Beige adipocytes also have many mitochondria and small lipid droplets. Although a proportion of the gene expression profile in beige adipocytes is different from that in brown adipocytes, beige adipocytes have mitochondrial uncoupling protein 1 (UCP1) expression and high ability of thermogenesis.

The functions of WAT and BAT are almost the opposite of one another in terms of their function in energy metabolism. While WAT is a tissue for energy storage as lipid droplets, the main function of BAT is energy expenditure. BAT is a major tissue for mammalian non-shivering thermogenesis via activation of mitochondrial UCP1, a physiological process during which heat production increases in response to body temperature changes.

The release of norepinephrine from sympathetic nerve terminals after sensory nerve activation by cold exposure or vagus nerve activation by intake of pungents is the initial thermogenesis. Followed by UCP1 activation and increase in H+ conductance in the mitochondria. While it is uncertain whether non-shivering thermogenesis in BAT occurs in humans, studies using fluorodeoxyglucose (FDG)-positron emission tomography (PET) in combination with computed tomography (CT) techniques have revealed that BAT is also found in adult humans. A histological study also demonstrated that some adipocytes express UCP1 in human adipose tissue.

Moreover, the gene expression profile in BAT suggests that human BAT might be heterogeneous, containing not only white and brown adipocytes but also beige adipocytes.

Obesity is recognized as a serious global health problem and is believed to result from an imbalance between energy intake and energy expenditure. Considerable evidence has demonstrated that BAT is involved in the long-term regulation of energy metabolism and body fat mass. By measuring oxygen consumption, BAT thermogenesis was found decreased in obese mice. Long-term cold stimulation and/or β3-adrenergic receptor agonist treatment decreased the amount of body fat associated with BAT hyperplasia. Furthermore, it was reported that UCP1 knockout mice became obese following treatment with a high-fat diet (HFD) for longer than 6 months. Several studies have also reported an inverse relationship between BAT activity/amount and obesity-related parameters, such as body fat content and body mass index (BMI) in humans. Thus activation of thermogenesis in BAT could be a means of prevention or attenuation of obesity and the obesity-related metabolic syndrome.

2.2. Mechanism of Thermogenesis in Brown and Beige Adipocytes UCP1 has been found exclusively expressed in the inner membranes of the mitochondria of brown adipocytes, and its activation increases H+ conductance in the mitochondria to dissipate the mitochondrial proton gradient and convert energy into heat. The mechanism of UCP1 activation downstream of β3-adrenergic receptor activation has been investigated in several studies. Some reports have suggested that long-chain fatty acids (LCFAs) produced within brown adipocytes by lipolysis of cytoplasmic lipid droplets on adrenergic stimulation help for H+ transport via UCP1. In contrast, some reports suggest that UCP1 activation does not require LCFAs. Another proposed mechanism suggests that thermogenic reactive oxygen species (ROS) alter the redox status of cysteine thiols in brown adipose tissue to drive increased respiration, and that Cys253 of UCP1 is a key target for sulfonylation. However, thermogenesis signaling pathways downstream of β3-adrenergic receptor activation have not been well clarified.

Some reports suggest that [Ca2+] signaling is involved in the differentiation of adipocytes. For example, increases in [Ca2+] negatively regulate adipocyte differentiation via calcineurin and/or the signaling pathway related to G-protein-coupled receptor (GPCR) in the 3T3-L1 adipocyte cell line. Elevation of [Ca2+] was found markedly to suppress adipocyte differentiation by inhibiting the insulin pathway, triglyceride accumulation, and peroxisome proliferator-activated receptor gamma (PPARγ) expression. However, the physiological and pathological roles of changes in membrane potential and [Ca2+] signaling are not well known, and recent efforts have focused on better understanding the role of changes in [Ca2+], and membrane potential in adipocytes. Changes in [Ca2+], via Gq-coupled receptor activation (possibly the α1 adrenergic receptor) could be involved in the expression of UCP1 and whole-body energy expenditure of BAT and beige adipocytes.

In beige adipocytes, ATP-dependent Ca2+ cycling by sarcoplasmic reticulum Ca2+-ATPase 2b (SERCA2b) and ryanodine receptor 2 can generate heat, which is independent of UCP1. Membrane hyperpolarization by activation of a two-pore domain potassium channel (KCNE3) reduced Ca2+ influx and impaired adrenergic receptor-mediated thermogenesis in brown adipocytes. However, it is not entirely understood how [Ca2+] increases occur and what levels of [Ca2+] increases are occurring in adipocytes.

3. ROLE OF THERMO-SENSITIVE TRP CHANNELS IN ADIPOSE TISSUE

We provide a brief and systematic summary of recent analyses of the involvement of thermo-TRP channels, in particular TRPV1, TRPV2, TRPV4, and TRPM8, in the regulation of thermogenesis in BAT and beige adipocytes in WAT (Table 1 and Fig. 1).

3.1. TRPV1 TRPV1 was the first member of the TRPV subfamily to be identified as a receptor of capsaicin, the pungent ingredient in “hot” chili peppers. TRPV1 has also been reported as a noxious thermo-sensitive channel with a temperature threshold of 43°C.

TRPV1 is expressed in brown adipocytes, and adipocyte cell lines such as the HB2 brown adipocyte cell line and the 3T3-L1 adipocyte cell line. In 3T3-L1 cells, activation of TRPV1 by capsaicin induces calcium influx and prevents...
adipogenesis.\textsuperscript{58} Opposite results were also reported and showed that treatment with capsaicin in the late stage slightly increased expression levels of fatty acid-binding protein 4 (Fabp4), Ppar\(\gamma\)2, and peroxisome proliferator-activated receptor \(\gamma\)coactivator-1\(\alpha\) (Pgc-1\(\alpha\)) in the HB2 brown adipocyte cell line.\textsuperscript{55,59} Pharmacological inhibition of TRPV1 activity also enhanced expression levels of Pgc-1\(\alpha\) mRNA, which is a key transcriptional coregulator of oxidative metabolism and thermogenesis.\textsuperscript{60} In addition, activation of TRPV1 by capsaicin up-regulates expression of thermogenic genes and induces the “browning” phenotype in differentiating 3T3-L1 pre-adipocytes (Fig. 1). In adipocytes, calcium influx may also inhibit differentiation, based on results obtained following application of an ionophore (ionomycin) and a SERCA inhibitor (thapsigargin).\textsuperscript{61} It is possible that TRPV1 plays different roles in the differentiation of white and brown adipocytes. While further in vivo analysis using conditional knockout mice or other genetically modified models is necessary to clarify the functional differences, many experiments have demonstrated that dietary intake of TRPV1 agonists improves

Table 1. The Expression of Thermo-Sensitive TRP Channels in Adipocytes

| Subtype | Temp. threshold | Agonists, activating stimuli | Inhibitors | Expression in adipocytes |
|---------|----------------|------------------------------|------------|--------------------------|
| TRPV1   | >42°C          | capsaicin, proton, shanshool, allicin, camphor, resiniferatoxin, vanillotoxin, 2-APB, propofol, anandamide, arachidonic acid metabolic products (by lipooxygenases), NO, extracellular cation probenecid, 2-APB, cannabidiol, mechanical stimulation | capsazepin, ruthenium red, BCTC, AMG517, AMG628, SB705498 | 3T3-L1 adipocytes, HB2 adipocytes human pre-adipocytes, mouse white adipose tissue |
| TRPV2   | >52°C          | ruthenium red, amiloride, SKF96365, tranilast, La\(^{3+}\) | human pre-adipocytes, mouse brown adipocytes |
| TRPV3   | >32°C          | ruthenium red, DPTHF | 3T3-L1 adipocytes, mouse white adipose tissue |
| TRPV4   | >27–41°C       | ruthenium red, La\(^{3+}\), Gd\(^{3+}\), GSK2193874, HC067047, RN1734 | human pre-adipocytes, 3T3-F442A adipocytes, mouse white adipose tissue |
| TRPA1   | <17°C          | allyl isothiocyanate, camphor, cinnamaldehyde, allicin, acrolein, icilin, tetrahydrocannabinol, menthol, formalin, H\(_2\)O\(_2\), alkalization, treachellular Ca\(^{2+}\), NSAIDs, anesthesia, primary alcohols, etc. | ruthenium red, gentamicin, HC030031, A967079, camphor, caffeine (human), AP18 | 3T3-L1 adipocytes |
| TRPM4   | warm           | intracellular Ca\(^{2+}\) | human adipocyte-derived stem cell |
| TRPM8   | <27°C          | prostaglandin E\(_2\), prostaglandin D\(_2\), thromboxane A\(_2\), thromboxane B\(_2\), thromboxane B\(_3\), prostacyclin, PGE\(_2\), PGD\(_2\), TXA\(_2\), Weibel-Palade body, intracellular Ca\(^{2+}\), cell stretch, mechanical stimulation | human pre-adipocytes, mouse brown adipocytes |
| TRPC5   | cold           | Gq/11-coupled receptors, diacylglycerol, LPC, rosiglitazone, Gd\(^{3+}\), sphingosine-1-phosphate, progesterone and membrane stretch, | La\(^{3+}\), 2-APB, SKF96365, flufenamic acid, chlorpromazine | human pre-adipocytes, 3T3-L1 adipocytes, mouse white adipose tissue |

\(2\text{APB}, 2\)-Aminoethoxydiphenyl borate; LPC, lysophosphatidylcholine; NO, nitrogen oxide; 4\(\alpha\)-PDD, 4\(\alpha\)-Phorbol 12,13-didecanoate.

**Fig. 1. Involvement of Thermo-Sensitive TRP Channels in BAT Differentiation and Thermogenesis**

In pre-adipocytes, TRPV2 negatively regulates differentiation through Ca\(^{2+}\)-calmodulin (CaM)-calcineurin pathway. Ca\(^{2+}\)-calmodulin (CaM)-calcineurin complex impaired expression of peroxisome proliferator-activated receptor gamma (PPAR\(\gamma\)) and CCAAT/enhancer-binding protein \(\alpha\) (C/EBP\(\alpha\)). In differentiated brown and beige adipocytes, activation of \(\beta_3\)-adrenergic receptor via sympathetic nerve activation enhances expression of genes related to thermogenesis such as uncoupling protein 1 (UCP1) and activation of UCP1. UCP1 activation is thought caused by free fatty acids (FFA) degraded from triglyceride (TG). TRPV1, TRPV2, and TRPM8 up-regulate thermogenic gene expression in brown adipocytes, which causes further heat generation. On the other hand, TRPV4 negatively regulates expression of genes related to thermogenesis in beige and/or brown adipocytes, resulting in lowered thermogenesis. (Color figure can be accessed in the online version.)
obesity and energy imbalance. For example, capsaicin supplementation (0.014% in diet) stimulated lipid metabolism in adipose tissue and lowered perirenal adipose tissue weight and serum triglyceride concentrations in HFD-fed rats.62) While functional expression of TRPV1 was decreased in white preadipocytes from mice fed HFD, this reduction was recovered by dietary administration of capsaicin.58,63,64) Because it would be difficult for dietary intake of capsaicin directly to activate TRPV1 expressed in adipose tissue, these phenotypes might be caused via mechanisms other than TRPV1 expression in adipose tissue. Rather, TRPV1 expressed in peripheral sensory neurons derived from the nodose ganglion is more important for controlling energy metabolism (see also references from Saito et al.65) and Uchida et al.66).

Capsinoids (such as capsiate, dihydrocapsiate, and nordihydrocapsiate) are structurally similar to capsaicinoids,67,68) and are reported to activate both TRPV1 and TRPA1.69,70) Capsinoids were also demonstrated to enhance metabolic rate and energy expenditure in WT mice, but not in TRPV1KO mice. In addition, capsiate administration contributes to enhancement of aerobic ATP production and reduction of body fat content,71) and also up-regulates UCP1 and UCP2 expression.72,73) Mice given the TRPV1 agonist monoacylglycerol showed increases in UCP1 expression levels in brown adipocytes and suppressed accumulation of visceral fat in mice fed HFD and sucrose.74) Oral intake of fish oil and fatty acids that can activate TRPV1 also exhibit the ability to enhance energy expenditure in mice.75) A study investigating gastrointestinal administration of TRPV1 agonists and vagus nerve ablation76) suggested that activation of TRPV1 expressed in the vagus nerve might be critical for TRPV1 agonist-induced modulation of energy metabolism.

Studies in humans showed that ingestion of capsaicin enhanced fat oxidation and energy metabolism during aerobic exercise.76) It has also been reported that capsaicin and capsinoids as food ingredients enhance energy metabolism via activation of BAT thermogenesis and subsequently decrease body fat accumulation in humans.77,78) While the anti-obesity effects of TRPV1 and capsaicin have been well studied, the use of capsaicin is limited due to its strong pungency. Therefore the major component of non-pungent CH-19 sweet pepper, namely capsinoids, may offer advantages over capsaicinoids, such as capsaicin, for clinical uses such as weight loss and cancer prevention.67) Indeed, continuous treatment with capsinoids for 4–12 weeks also increased energy expenditure and fat oxidation, with a reduction in abdominal adiposity in human subjects with high BMI.79,80) Another natural product known for its anti-obesity effects in humans is green tea.81,82) Catechins in green tea increase PPARα expression levels in subcutaneous WAT, and activate TRPV1 and TRPA1 expressing sensory neurons in mice,83,84) although it is not known whether catechins directly activate TRPV1. Moreover, anti-obesity products could activate the vagus nerve. Thus activation of TRPV1 expression in the vagus nerve could lead to prevention and attenuation of obesity and other metabolic diseases.

3.2. TRPV2 Among the TRPV channel family, TRPV2 was initially reported activated by noxious heat with an activation temperature threshold >52°C,85) and also by mechanical stimulation and/or cellular swelling.86,87) TRPV2 was subsequently found activated by chemicals such as 2-aminoe-thoxydiphenyl borate (2APB) and lysophospholipids including lysophosphatidylcholine (LPC) and lysophosphatidylinositol (LPI).88,89) SKF96365 is commonly used as a TRPV2-selective antagonist.88,89) It has been reported that TRPV2 is expressed in the central and peripheral nervous systems and plays an important role in axon outgrowth in developing neurons and intestinal movement.90,91) TRPV2 is also expressed in non-neuron cells, such as pancreatic β-cells92) and cardiomyocytes.93)

TRPV2 has been reported expressed in WAT and BAT.56) We previously showed that TRPV2 is more highly expressed in mouse brown adipocytes than TRPV1, TRPV3, TRPV4, and TRPM8.94) Our results demonstrated that primary TRPV2-deficient (TRPV2KO) adipocytes show decreased mRNA levels of multiple genes involved in mitochondrial oxidative metabolism, such as Ucp1 and PPARγ coactivator 1-alpha (Ppargca). Moreover, reduced responses to the β-adrenergic receptor agonist isoproterenol were observed in TRPV2KO adipocytes in vitro. Similar results were observed when intracellular calcium was chelated with BAPTA-AM, suggesting that TRPV2-mediated calcium influx could be involved in thermogenic gene induction on β-adrenergic receptor activation. Indeed, TRPV2KO mice showed cold intolerance and impairment of increases in Ucp1 mRNA and protein levels following cold stimulation at 4°C for 1 d without changes in their locomotion activities or sympathetic nerve activities. In addition, TRPV2KO mice showed impaired adaptive thermogenesis in interscapular BAT (iBAT) following administration of the β3-adrenergic receptor agonist BRL37344, indicating that TRPV2 expressed in BAT modulates non-shivering thermogenesis. Importantly, TRPV2KO mice exhibited significant increases in body weight and metabolism-related tissues on treatment with HFD for 8 weeks,57) supporting the concept that lack of TRPV2 in BAT impaired thermogenesis and energy expenditure. Data demonstrating up-regulation of TRPV2 expression in BAT from HFD-induced obesity and genetically diabetic (db/db) mice suggests that TRPV2-induced enhancement of thermogenesis may be effective against obesity.57)

Although expression of TRPV2 was increased during the differentiation of brown adipocytes, TRPV2 is also expressed in pre-adipocytes.94) In isolated pre-adipocytes from mice iBAT, activation of TRPV2 by its agonist (2APB or LPC) prevented differentiation of brown adipocytes in a dose-dependent manner during the early stages of differentiation.94) Mechanical force, which can activate TRPV2, also inhibited differentiation of brown adipocytes in a strength-dependent manner, and the effect was reversed by SKF96365. TRPV2 agonist-induced prevention of differentiation can be blocked by the calcineurin inhibitors cyclosporine A and FK506, which are reported to recover the [Ca2+]i-induced impairment of differentiation in 3T3-L1 cells.94) Moreover, differentiation of brown adipocytes was facilitated in cells from TRPV2KO mice. These results demonstrate that activation of TRPV2 can prevent differentiation against over-development of brown adipocytes. TRPV2 activation-mediated prevention of differentiation is observed only in the early stage of differentiation (from 0 to 2 d after changing to differentiation medium). As described above, application of a TRPV1 agonist in the late phase of differentiation facilitated differentiation in a brown adipocyte cell line. Increasing in [Ca2+]i, could have a biphasic regulatory role. Indeed, one report showed that [Ca2+]i-
crease in the early stages inhibits the differentiation whereas [Ca^{2+}], increase in the late stage promotes differentiation. \(^{51}\)

How does this intracellular calcium increase occur via TRPV2? Since TRPV2 antagonists (transilast and SKF96365) facilitated the differentiation of brown adipocytes, endogenous ligands and/or stimuli of TRPV2 could be included in cell culture conditions. Although endogenous TRPV2 agonists in BAT are still unknown, some TRPV2 agonists were shown to work both in vitro and in vivo in the experiments described above. Candidate TRPV2-activating stimuli include mechanical stimulation (membrane stretch), lipid metabolites, LPC, LPI, and endocannabinoids.\(^{86,87,89,95}\) Moreover, insulin growth factor-1 (IGF-1) is known to enhance transient translocation of TRPV2 from intracellular compartments to the plasma membrane.\(^{96,97}\) TRPV2 activation might occur by membrane stretch due to incorporation of TRPV2-containing vesicles into the plasma membrane. The synergistic effect of these stimuli on TRPV2 activation might be important for the modulation of BAT function. These findings, together with the involvement of TRPV2 in BAT thermogenesis (described above), suggest that TRPV2 plays developmental stage-dependent roles in BAT (Fig. 1). In WAT, the expression level of TRPV2 was increased in mice fed HFD. In addition, while cold exposure for 3 d enhanced expression levels of UCP1 and Pgc-1α in WAT, these enhancements were impaired in TRPV2KO mice,\(^{57}\) suggesting TRPV2 could also be involved in thermogenesis in beige adipocytes. However, there are no reports regarding the expression and physiological role of TRPV2 in beige adipocytes.

### 3.3. TRPV4

TRPV4 was originally reported by changes in osmolarity or mechanical stimuli.\(^{98-100}\) TRPV4 is also activated by diverse chemical compounds, including a synthetic phorbol ester, 4α-phorbol-12, 13-didecanoate (4α-PDD) and GS1016790A,\(^{101,102}\) as well as moderate warmth (temperature threshold >27°C).\(^{101,103}\) Epoxycosatrienoic acids (EETs) are produced by CYP in the arachidonic acid cascade are thought endogenous agonists for TRPV4.\(^{104}\) TRPV4 is widely expressed in many types of cells and tissues, including sensory neurons, the hypothalamus, trachea, cochlear hair cells, vascular smooth muscle cells, endothelial cells, kidney, and keratinocytes.\(^{98,99,105-107}\)

TRPV4 is reported expressed in mouse BAT and WAT, as well as cultured human adipocytes.\(^{60,108}\) While it has been reported that knockdown of TRPV4 did not affect adipogenesis in 3T3-F442A adipocytes, it did enhance basal and norepinephrine-induced induction of expression of thermogenic genes such as Pparγ2 and Ucp1\(^{60}\) (Fig. 1). In addition, TRPV4 activation caused rapid phosphorylation of extracellular signal-regulated kinase 1/2 (ERK1/2) and c-Jun N terminal kinase 1/2 (JNK1/2), which suppressed expression of thermogenic genes in 3T3-F442A adipocytes.\(^{60}\) TRPV4 knockdown also reduced adipose tissue inflammation by inhibiting a number of pro-inflammatory mediators.\(^{60}\) Higher expression of TRPV4 was detected in WAT than in BAT, and the significant up-regulation of thermogenic gene expression following TRPV4 inhibition by GS10205 lead to development of metabolically active brown fat-like features.\(^{60}\) Taken together, TRPV4 in WAT may negatively regulate the “browning” of white adipocytes or differentiation of beige adipocytes. On the other hand, TRPV4 mRNA expression was increased in WAT of mice fed HFD, and recovered by treadmill running.\(^{109}\) However, this increase in TRPV4 mRNA expression could be due to infiltration of macrophages to some extent, because TRPV4 is also expressed in macrophages, and inflammation in WAT is a trigger for the development of insulin resistance. The role of TRPV4 in human adipocytes has not yet been reported, although a study revealed that polymorphisms of the TRPV4 gene affects BMI and body fat mass in subjects in Taiwan.\(^{110}\) Further analysis is necessary to understand the involvement of TRPV4 in BAT and human adipocytes.

### 3.4. TRPM8

The TRPM subfamily consists of eight different ion channels (TRPM1 to TRPM8).\(^{111}\) Unlike the TRPV subfamily, TRPM subunits do not have N-terminal ankryin repeat motifs, but instead contain entire functional domains in their C-termini. TRPM8 was initially named TRP-p8 because of its homology with members of the TRP family, and was identified as a menthol receptor by Mckemy et al. in 2002.\(^{112}\) TRPM8 is expressed in a subset of sensory neurons, where it acts as a cold sensor (temperature threshold <30°C) and is activated by menthol and icilin.\(^{112-116}\)

TRPM8 is expressed in BAT, and activation of TRPM8 by menthol in brown adipocytes was found to up-regulate UCP1 expression, which required activation of protein kinase A.\(^{117}\) Long-term dietary menthol treatment significantly increased core body temperatures and locomotor activity in WT mice, whereas these effects were absent in both TRPM8KO and UCP1 knockout mice.\(^{117}\) Dietary application of menthol was shown to have no effects on expression levels of PPARγ or bone morphogenetic protein 7 (BMP7), both of which are involved in the induction of differentiation of brown adipocytes. HFD-induced obesity and insulin resistance were also prevented by menthol treatment. These findings suggest that stimulation of TRPM8 enhances BAT thermogenesis, which could offer promising approaches for the prevention and treatment of obesity.\(^{117}\) TRPM8 was also expressed in a human white adipocyte cell line, in which TRPM8 activation by menthol or icilin induced UCP1 expression, mitochondrial activation, and heat production.\(^{118}\) In addition, mRNA and expression levels of TRPM8 protein were significantly increased during adipocyte differentiation, suggesting the importance of TRPM8 for adipocyte thermogenesis.\(^{118}\) On the other hand, it has been reported that menthol treatment on the skin or cold exposure causes a TRPM8-dependent increase in core body temperature, which might be related to an increase in UCP1 expression.\(^{119}\) Intragastric administration of menthol enhanced BAT thermogenesis and browning of WAT in mice.\(^{120-122}\) However, these experiments cannot distinguish between the functions of TRPM8 expressed in BAT and sensory neurons. At least, activation of TRPM8 in sensory neurons by its ligands may mimic cold stimulation-induced thermogenesis.

### 3.5. Other Thermo-Sensitive TRP Channels

In a pharmacological experiment, TRPA1 was shown expressed in the 3T3-L1 adipocyte cell line,\(^{123}\) and indirect activation of TRPA1 by trans-pellitorine reduced lipid accumulation. However, functional expression of TRPA1 in adipocytes has not been well established. Rather, TRPA1 expressed in the vagus nerve may be more important for energy metabolism, similar to TRPV1. TRPA1 is activated by several food components, such as allyl isothiocyanate (AITC), icilin, menthol, and cinnamonaldehyde. It was reported that TRPA1 agonists induce adrenaline secretion and prevent fat accumulation and obesity in rodents.\(^{124}\) In addition, capsaicin activates not only TRPV1
but also TRPA1, suggesting that capsiate-induced modulation of energy metabolism may be due to activation of TRPA1. Further explorations of the involvement of TRPA1 in thermogenesis, adipogenesis, and adipose tissue are warranted.

One report shows that TRPV3 may be involved in adipogenesis of white adipocytes under in vitro and in vivo conditions. A novel compound found in green tea and cocoa, namely (−)-epicatechin, inhibited 3T3-L1 adipocyte differentiation via direct activation of TRPV3. TRPV3 activation suppresses adipogenesis, probably by inhibiting phosphorylation of insulin receptor substrate 1, the downstream phosphoinositide 3-kinase/Akt/forkhead box protein O1 axis and the expression of PPARγ and CCAAT/enhancer-binding protein α (C/EBPα). In addition, chronic treatment with TRPV3 activators prevented adipogenesis and body weight gain in mice fed HFD, and TRPV3 expression was reduced in the visceral adipose tissue of mice fed HFD, as well as obese (ob/ob) and genetically diabetic (ob/ob and db/db) mice. However, TRPV3 expression in brown adipocytes has not been reported.

In contrast to other TRP channels, TRPM4 is a calcium-permeable monovalent cation channel activated by intracellular calcium, and its activity is modulated by temperature increases. TRPM4 is reported expressed in human adipose tissue-derived stem cells and modulates calcium signaling. In particular, histamine-induced intracellular calcium concentration changes through voltage-gated calcium channel were reduced by suppression of TRPM4 expression, suggesting that TRPM4 could be involved in adipogenesis.

Activation of TRPC1 and TRPC5 was induced following differentiation of adipocytes. Generation of adiponectin was negatively regulated in adipocytes containing TRPC1/ TRPC5 channels. Both knockdown of TRPC1/TRPC5 in vitro and conditional knockout of TRPC5 in vivo can increase adiponectin generation in mice. The effect of α-linolenic acid, which was used to stimulate adiponectin generation, was also abolished in TRPC5 conditional knockout mice. These observations suggest that TRPC1 and TRPC5 establish a constitutively active hetero-multimeric channel in adipocytes that negatively regulates adiponectin.

4. CONCLUSION AND PERSPECTIVES

The involvement of TRP channels in energy metabolism, adipogenesis, adipose tissue inflammation, obesity, and related complications of both brown and beige adipocytes has been extensively explored in recent decades. Herein, we reviewed the emerging functions of TRP channels in brown adipocytes (Table 1 and Fig. 1). Modulation of [Ca²⁺] provides TRP channels intriguing targets in adipocytes to regulate energy metabolism for preventing and combating human obesity. However, many questions still remain unaddressed. First, how are these TRP channels activated endogenously? Second, activation of some TRP channels may cause opposite phenomena in thermogenesis (e.g., while TRPV4 activation impaired thermogenesis, TRPV2 activation facilitated thermogenesis). Third, which signaling pathways operate downstream of [Ca²⁺], increases via TRP channels activation? Fourth, how do membrane potential changes (sodium influx) affect the functions of brown adipocytes? Further animal and human studies are needed to address these questions and clarify the clinical significance of TRP channels. Such studies could pave the way for new clinical approaches to treat human obesity and related metabolic diseases.

Conflict of Interest The authors declare no conflict of interest.

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