

# Involvement of thermosensitive TRP channels in energy metabolism

Kunitoshi Uchida<sup>1,2,3</sup> · Katsuya Dezaki<sup>4</sup> · Takeshi Yoneshiro<sup>5</sup> · Tatsuo Watanabe<sup>6</sup> · Jun Yamazaki<sup>3</sup> · Masayuki Saito<sup>7</sup> · Toshihiko Yada<sup>4</sup> · Makoto Tominaga<sup>1,2</sup> · Yusaku Iwasaki<sup>4</sup>

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**Abstract** To date, 11 thermosensitive transient receptor potential (thermo-TRP) channels have been identified. Recent studies have characterized the mechanism of thermosensing by thermo-TRPs and the physiological role of thermo-TRPs in energy metabolism. In this review, we highlight the role of various thermo-TRPs in energy metabolism and hormone secretion. In the pancreas, TRPM2 and other TRPs regulate insulin secretion. TRPV2 expressed in brown adipocytes contributes to

differentiation and/or thermogenesis. Sensory nerves that express TRPV1 promote increased energy expenditure by activating sympathetic nerves and adrenaline secretion. Here, we first show that capsaicin-induced adrenaline secretion is completely impaired in TRPV1 knockout mice. The thermogenic effects of TRPV1 agonists are attributable to brown adipose tissue (BAT) activation in mice and humans. Moreover, TRPA1- and TRPM8-expressing sensory nerves also contribute to potentiation of BAT thermogenesis and energy expenditure in mice. Together, thermo-TRPs are promising targets for combating obesity and metabolic disorders.

Kunitoshi Uchida, Katsuya Dezaki, and Takeshi Yoneshiro contributed equally to this work.

✉ Kunitoshi Uchida  
uchida@college.fdcnet.ac.jp

✉ Yusaku Iwasaki  
ysk-iwasaki@jichi.ac.jp

- <sup>1</sup> Division of Cell Signaling, Okazaki Institute for Integrative Biosciences (National Institute for Physiological Sciences), National Institutes of Natural Sciences, 5-1 Higashiyama, Myodaiji, Okazaki, Aichi 444-8787, Japan
- <sup>2</sup> Department of Physiological Sciences, SOKENDAI (The University of Advanced Studies), 38 Nishigounaka, Myodaiji, Okazaki, Aichi 444-8585, Japan
- <sup>3</sup> Department of Physiological Science and Molecular Biology, Fukuoka Dental College, 2-15-1 Tamura, Sawara-ku, Fukuoka, Fukuoka 814-0193, Japan
- <sup>4</sup> Division of Integrative Physiology, Department of Physiology, Jichi Medical University School of Medicine, 3311-1 Yakushiji, Shimotsuke, Tochigi 320-0498, Japan
- <sup>5</sup> Diabetes Center, University of California, San Francisco, 35 Medical Center Way, San Francisco, CA 94143-0669, USA
- <sup>6</sup> Faculty of Future Industry, Happy Science University, 4427-1 Hitotsumatsu-hei, Chosei-mura, Chiba 299-4325, Japan
- <sup>7</sup> Hokkaido University, Kita18-Nishi9, Kita-ku, Sapporo, Hokkaido 060-0818, Japan

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## Introduction

Most transient receptor potential (TRP) channels are non-selective cation channels. The name TRP comes from the prototypical member in *Drosophila*, in which a mutation resulted in abnormal transient receptor potential to continuous light [1]. TRP channels are now divided into seven subfamilies: TRPC (canonical), TRPV (vanilloid), TRPM (melastatin), TRPML (mucolipin), TRPP (polycystin), TRPA (ankyrin) and TPRN (NomPC). In mammals, there are six TRP subfamilies and 28 channels. TRP channels are expressed in many tissues and have a wide variety of physiological functions, including detection of various physical and chemical stimuli in vision, taste, olfaction, hearing, touch, and thermosensation [2, 3]. 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 was considered

to be a breakthrough for research concerning temperature sensing [4]. Since then, several TRP channels having thermosensitive abilities have been identified in mammals, with 11 thermosensitive TRP (thermo-TRP) channels reported in mammals to date (Table 1). 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 we can discriminate. TRPV1 and TRPV2 are activated by elevated temperatures, whereas TRPM8 and TRPA1 are activated by cool and cold temperatures. TRPV3, TRPV4, TRPM2, TRPM4, and TRPM5 are activated by warm temperatures. In addition, TRPM3 was shown to be a sensor for noxious heat and TRPC5 was identified as a candidate cold sensor [5, 6]. Thermo-TRP channels usually function as ‘multi-modal receptors’ that respond to various chemical and physical stimuli. For example, TRPV1, activated by noxious heat (>42 °C), is also a receptor for several pungent agents such as capsaicin, an active ingredient in chili peppers, as well as by low pH. Activation of these channels could contribute to changes in intracellular Ca<sup>2+</sup> concentrations ([Ca<sup>2+</sup>]<sub>i</sub>) and control of membrane potentials in

many cell types, except TRPM4 and TRPM5, which are not permeable of divalent cations. Thermo-TRP channels expressed in sensory neurons and skin can act as ambient temperature sensors. On the other hand, thermo-TRP channels are also expressed in tissues that are not exposed to dynamic temperature changes, suggesting that these channels have other physiological roles that are unrelated to sensation of temperature changes.

The prevalence of excess weight and obesity is increasing at an alarming rate worldwide. According to the World Health Organization, in 2014, ~39% (1.9 billion) and 13% (600 million) of adults were overweight and obese, respectively [7]. These numbers are expected to increase in the future [8]. Obesity is a major risk factor for metabolic syndromes, including type 2 diabetes, as well as for cardiovascular and cerebral diseases. The fundamental cause of excess weight and obesity is an energy imbalance between energy intake and energy expenditure. Recent studies showed that several thermo-TRP channels are key molecules in the regulation of energy metabolism. In this review, we focus on the involvement of thermo-TRP channels, especially those expressed in the pancreas, brown

**Table 1** Properties of thermosensitive TRP channels

		Temperature threshold	Tissue distribution	Other stimuli
Heat	TRPV1	>42 °C	Sensory neuron, brain, skin	Capsaicin, proton, capsiate, gingerol, shogaol, allicin, shanshool, camphor, resiniferatoxin, vanillotoxin, 2-APB, propofol, anandamide, arachidonic acid metabolic products (by lipoxygenases), monoacylglycerol, NO, extracellular cation
	TRPV2	>52 °C	Sensory neuron, brain, spinal cord, lung, liver, spleen, colon, heart, immunocyte	Probenecid, 2-APB, cannabidiol, mechanical stimulation
Warm	TRPV3	>32 °C	Skin, sensory neuron, brain, spinal cord, stomach, colon	Camphor, carvacrol, menthol, eugenol, thymol, 2-APB
	TRPV4	>27–41 °C	Skin, sensory neuron, brain, kidney, lung, inner ear, bladder	4 $\alpha$ -PDD, bisandrographolide, citric acid, arachidonic acid metabolic products (by epoxygenases), anandamide, hypoosmolality, mechanical stimulation
	TRPM2	>36 °C	Brain, immunocyte, pancreas etc.	(cyclic) ADPribose, $\beta$ -NAD, H <sub>2</sub> O <sub>2</sub> , intracellular Ca <sup>2+</sup>
	TRPM3	Warm-heat	Brain, sensory neuron, pancreas, eye	Ca <sup>2+</sup> store depletion, pregnenolone sulfate, nifedipine, clotrimazole
	TRPM4	Warm	Heart, liver, immunocyte, pancreas etc.	Intracellular Ca <sup>2+</sup>
	TRPM5	Warm	Taste cell, pancreas	Intracellular Ca <sup>2+</sup>
Cold	TRPM8	<27 °C	Sensory neuron	Menthol, icilin, eucalyptol
	TRPC5	Cold	Brain, sensory neuron, liver, heart, kidney	G <sub>q/11</sub> -coupled receptors, diacylglycerol, Gd <sup>3+</sup>
	TRPA1	<17 °C	Sensory neuron, inner cell	Allyl isothiocyanate, carvacrol, cinnamaldehyde, allicin, diallyl trisulfide, miogadial, miogatrial, capsiate, acrolein, icilin, tetrahydrocannabinol, menthol (10–100 $\mu$ M), formalin, H <sub>2</sub> O <sub>2</sub> , alkalization, intracellular Ca <sup>2+</sup> , NSAIDs, propofol/isoflurane/desflurane/etomidate/octanol/hexanol etc.

2-APB 2-aminoethoxydiphenyl borate, NO nitric oxide, 4 $\alpha$ -PDD 4 $\alpha$ -phorbol-didecanoate, ADPribose adenosine diphosphate ribose,  $\beta$ -NAD  $\beta$ -nicotinamide adenine dinucleotide, H<sub>2</sub>O<sub>2</sub> hydrogen peroxide, NSAIDs non-steroidal anti-inflammatory drugs

adipocytes and sensory nerves, in energy metabolism and the secretion of the metabolically important hormones insulin and adrenaline.

## Thermo-TRP in the pancreas and regulation of insulin secretion

### TRPM2 channel in $\beta$ -cells

In pancreatic  $\beta$ -cells, glucose metabolism-induced closure of ATP-sensitive  $K^+$  ( $K_{ATP}$ ) channels and membrane depolarization trigger opening of voltage-dependent  $Ca^{2+}$  channels, which induces  $Ca^{2+}$  influx and subsequent insulin secretion. During glucose-stimulated insulin secretion in  $\beta$ -cells, induction of background inward current promoted by the opening of non-selective cation channels (NSCCs) might facilitate depolarization after glucose metabolism-induced closure of the  $K_{ATP}$  channels. Glucose metabolism evokes not only  $K_{ATP}$  channel inhibition but also increases NSCC currents. We reported that the NSCC transient receptor potential melastatin 2 (TRPM2) channel in  $\beta$ -cells plays an essential role in glucose-induced and incretin-potentiated insulin secretion [9]. TRPM2 is expressed in  $\beta$ -cells [10] and the increase in glucose-induced NSCC activity is due to opening of TRPM2 channels, since this glucose effect was attenuated in  $\beta$ -cells from TRPM2-deficient mice [11]. These effects of glucose on  $K_{ATP}$  channel inhibition and NSCC (TRPM2) activation may synergistically and effectively depolarize the  $\beta$ -cell membrane to trigger acute insulin secretion. This mechanism may contribute to the priming of insulin release from  $\beta$ -cells, since glucose-induced TRPM2 activation occurs before glucose-induced  $K_{ATP}$  channel inhibition [11].

Intestinal incretin hormones, such as glucagon-like peptide-1 (GLP-1) secreted from L-cells and glucose-dependent insulinotropic polypeptide (GIP) secreted from K-cells after meal, potentiate glucose-induced insulin release by cytosolic cAMP production via Gs-coupled receptors [12, 13]. GLP-1 activates NSCC currents and depolarizes the membrane potential through cAMP production in  $\beta$ -cells. In wild-type mice, the non-selective TRPM2 blocker 2-aminoethoxydiphenyl borate (2-APB) inhibits GLP-1-mediated increases in NSCC currents. Furthermore, GLP-1 has no effect on insulin secretion in TRPM2-deficient mice [11, 14]. These results demonstrate that TRPM2 activation is an important pathway for incretin-potentiated insulin secretion.

Considering the mechanism of TRPM2 channel stimulation mediated by cAMP signaling via Gs-coupled receptors, Gi/Go-mediated inhibition of cAMP production is expected to attenuate TRPM2 channel activity. Ghrelin, an acylated 28-amino acid peptide produced predominantly

in the stomach, was discovered as the endogenous ligand for the growth hormone secretagogue-receptor (GHS-R), which is widely expressed throughout the body. Ghrelin inhibits glucose-stimulated insulin secretion in vitro in perfused pancreas tissue and isolated islets [15, 16]. We found that the insulinostatic action of ghrelin is produced via pertussis toxin-sensitive Gi-proteins in  $\beta$ -cells that in turn attenuate cAMP and  $[Ca^{2+}]_i$  signaling in  $\beta$ -cells and insulin release from islets [17, 18]. Moreover, ghrelin markedly counteracts glucose (8.3 mM)-induced activation of TRPM2 currents in islet  $\beta$ -cells from wild-type mice but not TRPM2 knockout (TRPM2-KO) mice [19]. These results suggest that ghrelin suppresses glucose-induced insulin secretion at least partly by inhibiting TRPM2 channels. Furthermore, ghrelin potently attenuates GLP-1-induced cAMP generation and insulin release from islet  $\beta$ -cells, whereas ghrelin receptor antagonists potentiate GLP-1-induced cAMP generation and insulin release [20]. Consistent with ghrelin signaling, we recently found that adrenaline attenuates TRPM2 activation via Gi-mediated inhibition of cAMP signaling in  $\beta$ -cells [21].

The gastric hormones GLP-1 and ghrelin have reciprocal actions on cAMP levels and TRPM2 channel activity in islet  $\beta$ -cells. GLP-1 and ghrelin are released in a reciprocal pattern: following a meal, the GLP-1 plasma level rises while ghrelin levels fall [22]. These changes may collaborate to effectively elevate cAMP and activate TRPM2 channels in  $\beta$ -cells, leading to rapid and efficient insulin release for regulated postprandial glucose disposal. As such, the development of approaches that would specifically intervene in TRPM2 signaling in  $\beta$ -cells might provide a potential therapeutic tool to treat patients with type 2 diabetes.

### Other TRP channels in $\beta$ -cells

Several TRP family members are reportedly expressed in  $\beta$ -cells, although their physiological role is unclear and mechanistic insights into the regulation of these channels during insulin release are limited. TRPM3 expressed in  $\beta$ -cells functions as an ionotropic steroid receptor that links insulin release [23], although the inhibition of TRPM3 channels does not affect glucose-induced insulin secretion [24]. TRPM4 is a  $Ca^{2+}$ -activated NSCC that may play a key role in controlling the membrane potential and electrical activity of insulin-secreting INS1 cells [25]. TRPM5 is also activated by  $Ca^{2+}$  and plays a role in insulin release [26]. GLP-1 stimulates insulin secretion in part by promoting TRPM4 and TRPM5 activation [27]. Activation of TRPA1 channels expressed in  $\beta$ -cells by the agonist allyl isothiocyanate stimulates insulin release from insulinoma cells and primary  $\beta$ -cells [28]. TRPA1-mediated depolarization may act synergistically with  $K_{ATP}$  channel blockade

to facilitate insulin release in  $\beta$ -cells. Interestingly, the anti-diabetic drug glibenclamide inhibits  $K_{ATP}$  channels but activates human TRPA1 channels [29], suggesting that part of the insulin-secreting action of glibenclamide could be attributed to TRPA1 activation.

## Roles of thermo-TRP channels in brown adipose tissue

### Trpv2

TRPV2 was initially reported to be activated by noxious heat (temperature threshold  $>52$  °C) [30]. TRPV2 was also found to act as a mechano-sensor that is activated by membrane stretch and cell swelling [31]. 2-APB and lysophosphatidylcholine (LPC) act as TRPV2 agonists [32, 33], whereas ruthenium red and SKF96365 antagonize TRPV2 activity [32], although these ligands are not specific for TRPV2. TRPV2 is dominantly expressed in the central and peripheral nervous systems and is involved in axon outgrowth in developing neurons and intestinal movement [34–36]. In addition, the phenotype of TRPV2 knockout (TRPV2-KO) mice suggests that TRPV2 is involved in phagocytosis of macrophages [37].

TRPV2 was recently shown to be expressed in brown adipose tissue (BAT) and primary brown adipocytes. In particular, the increase in TRPV2 expression levels during brown adipocyte differentiation suggests that TRPV2 could have important roles in differentiated brown adipocytes. A study by Sun et al. showed that TRPV2-KO mice have alterations in the mRNA levels for genes that are related to mitochondrial oxidative metabolism [38]. For instance, mice lacking TRPV2 have decreased amounts of mRNA of UCP1, which is a mitochondrial transporter protein and plays important roles in energy balance and regulation, and peroxisome proliferator-activated receptor gamma coactivator 1-alpha (*PERM1*) expression that are accompanied by increases in expression of genes related to lipid accumulation, such as lipoprotein lipase (*LPL*), and cluster of differentiation 36 (*CD36*). Furthermore, in morphological terms, TRPV2-KO BAT cells were larger overall and had larger lipid droplets compared to wild-type BAT. These differences could be explained by the impaired thermogenic activity seen for TRPV2-KO BAT. TRPV2-KO mice are unable to maintain body temperature upon exposure to cold, but these mice had locomotor activity and sympathetic nerve activity that were similar to wild-type mice. In addition, increases in *UCP1* mRNA and protein in BAT were impaired in TRPV2-KO mice exposed to cold temperature (4 °C). TRPV2 expression levels in BAT were also increased following exposure of wild-type mice to cold. Thermogenesis in mice can be assessed by measuring

interscapular BAT (iBAT) temperature using an inserted temperature probe. The iBAT temperature could be increased by systemic administration of the  $\beta$ 3-adrenergic receptor agonist BRL37344 to anesthetized wild-type mice, but not TRPV2-KO mice. Consistent with this impairment, TRPV2-KO mice showed heavier white adipose tissue (WAT) and increased accumulation of lipid droplets in BAT. Moreover, TRPV2-KO mice fed a high-fat diet had a significant increases in body weight and metabolically active tissues.

These findings raise several questions: (1) how is TRPV2 activated downstream of sympathetic nerve activation? (2) what are the mechanisms involved in increased TRPV2 expression? and (3) what is the involvement of calcium signaling in BAT thermogenesis? Stimuli that activate TRPV2 include membrane stretch (mechanical tension), as well as the chemical agonists LPC, LPI, and the endocannabinoids [31, 33, 39, 40]. Insulin growth factor-1 (IGF-1) is reported to enhance translocation of TRPV2 from intracellular compartments to the plasma membrane following stretch-mediated activation of TRPV2 [41]. In brown adipocytes, TRPV2 could be activated downstream of adenylyl cyclase (AC), based on the finding that enhancement of *UCP1* mRNA expression mediated by the AC activator forskolin was almost abolished in primary brown adipocytes from TRPV2-KO mice [38]. Although the precise mechanism of TRPV2 activation in BAT is unclear, several studies indicated that TRPV2 agonists and stimulations could work synergistically both in vitro and in vivo. There is little evidence for the dependence of calcium influx on sympathetic nerve activation of brown adipocytes, although increases in intracellular calcium concentrations have been observed in these cells. Calcium is thought to be released from mitochondria or the ER followed by store-operated calcium entry. On the other hand, activation of TRPM8 by menthol in brown adipocytes reportedly enhances calcium-dependent PKA phosphorylation (also described below). This result suggests that a calcium influx pathway that promotes entry of extracellular calcium directly into the cell may exist, and TRPV2 could be a candidate channel that promotes this entry. Another important event in brown adipocyte function is PKA phosphorylation. Given that chelation of intracellular calcium by BAPTA-AM attenuates increases in *UCP1* expression in brown adipocytes treated with the  $\beta$ -adrenergic receptor agonist isoproterenol [38], it is possible that calcium influx could modulate *UCP1* expression increase through PKA phosphorylation. Furthermore, TRPV2 activation could enhance PKA phosphorylation levels [42], although further experiments are necessary to clarify the precise mechanisms of thermogenesis mediated by TRPV2 activation.

TRPV2 mRNA expression can also be seen in primary pre-adipocytes, but its expression is significantly increased in differentiated brown adipocytes compared to pre-adipocytes [43]. Pre-adipocytes isolated from mouse iBAT showed impaired differentiation to brown adipocytes in the presence of non-selective TRPV2 agonists (2-APB and LPC). However, application of the TRPV2 agonists 3 days after differentiation began had no effect, indicating that TRPV2 activation could be critical for the early stages of pre-adipocyte differentiation [43]. Mechanical stimulation, which could activate TRPV2, also inhibited brown adipocyte differentiation. In addition, brown adipocyte differentiation was enhanced in TRPV2-KO mice. Calcium influx is reported to suppress brown adipocyte differentiation through a calcineurin-dependent pathway [44]. Indeed, the calcineurin inhibitors cyclosporine A and FK506 partially recovered TRPV2 activation-induced inhibition of brown adipocyte differentiation [43]. These results demonstrated that TRPV2 is involved in the differentiation of brown adipocytes, and could regulate the number of brown adipocytes. Although the phenotype related to impaired differentiation is not seen in TRPV2-KO BAT, a compensatory effect could be present in these mice, particularly given the importance of BAT functions.

Taken together, these findings suggest that TRPV2 plays two different roles related to the developmental stages of brown adipocytes. First, in pre-adipocytes, TRPV2 could inhibit the differentiation of brown adipocytes, and second, in differentiated brown adipocytes TRPV2 could facilitate thermogenesis.

### Other TRP channels

TRPV1 is reported to be expressed in the 3T3-L1 and HB2 adipocyte cell lines, brown adipocytes, and BAT [45–47]. Application of the TRPV1 agonist (capsaicin) to 3T3-L1 adipocytes caused upregulation of the expression of genes related to thermogenesis and “browning” [46]. However, the physiological roles of TRPV1 in BAT have not been well clarified.

TRPM8 is also expressed in BAT and menthol-induced activation of TRPM8 expressed in brown adipocytes upregulates UCP1 expression, which requires the activation of PKA [48]. Chronic dietary application of menthol significantly increases the core body temperature and locomotor activity in wild-type mice, whereas these effects are absent in both TRPM8-KO and UCP1-KO mice [48]. In addition, TRPM8 was demonstrated to be expressed in a human white adipocyte cell line and its expression level is elevated during the differentiation of adipocyte. TRPM8 activation induced UCP1 expression, mitochondrial activation and heat production [49]. Although this study suggests that TRPM8 stimulation enhances non-shivering

thermogenesis, the roles of TRPM8 in BAT are still largely unclear [48].

TRPV4 is expressed in both BAT and WAT [50]. Reduction of TRPV4 expression enhances the expression of genes related to thermogenesis such as *PGC-1 $\alpha$*  and *UCP1* without changing adipogenesis in 3T3-F442A adipocytes [50]. TRPV4 activation causes a rapid phosphorylation of ERK1/2 and JNK1/2, which further suppresses the expression of thermogenic genes [51]. Consistent with this finding, TRPV4-KO mice exhibit increased muscle oxidative capacity and resistance to diet-induced obesity [50]. Another report indicated that TRPV4 is expressed in WAT and BAT [50]. Interestingly, induction of thermogenic gene expression upon TRPV4 inhibition by GSK205 leads to the development of metabolically active brown fat-like features in WAT [50]. Calcium influx through TRPV4 has an opposite effect to that seen for TRPV2 in BAT thermogenesis. Thus, how and when calcium influx occurs during thermogenesis in BAT could have important regulatory implications.

### Activation of thermo-TRP channels in sensory nerves increases energy expenditure via sympathetic nerve activation and via enhancement of adrenaline secretion

#### Findings on the effects and mechanisms of thermo-TRP from animal studies

It is empirically known that consumption of spicy foods or drinks can enhance thermogenesis by increasing energy expenditure. In traditional Chinese medicine, many spicy foods were shown to induce warming sensations in the body. In 1986, Henry and Emery provided evidence to link consumption of hot foods and enhanced energy expenditure, in that individuals who consumed spicy foods containing chili or mustard sauces had an approximately twofold increase in O<sub>2</sub> consumption after the meal [52]. Red hot peppers (*Capsicum* sp.) are a representative spicy food, and its pungent ingredient is capsaicin. Also in 1986, Kawada et al. reported that intraperitoneal injection of capsaicin increased O<sub>2</sub> consumption and that the addition of capsaicin to a high-fat diet prevented accumulation of visceral WAT and obesity in rats [53, 54]. The effect of capsaicin on increasing energy expenditure has since been supported by numerous studies involving both humans and animals [55, 56].

After cloning and identification of the capsaicin receptor TRPV1 in 1997 [4], the mechanism of action by which capsaicin and TRPV1 agonists increase energy expenditure and thermogenesis has been determined in greater detail. Many pungent ingredients derived from spices activate

TRPV1 [57], including capsaicin in hot peppers [4], piperine and its analog in black pepper [58, 59], and gingerol and shogaol in ginger [60, 61]. Interestingly, some compounds with no or very low pungency have been identified as TRPV1 agonists, such as the capsaicin analog capsiate present in “CH-19 sweet” peppers [62, 63], [10]-shogaol from ginger [61], and 1-monoacylglycerol that has various acyl moieties in wheat, mioga (*Zingiber mioga*) and onion [64]. TRPV1 agonists with no or low pungency can also have high lipophilicity, which could render these molecules unable to access the termini of trigeminal nerves in the oral cavity that is covered with epithelium [61, 63, 64].

Oral administration of the TRPV1 agonists capsaicin and capsiate increases energy expenditure ( $O_2$  consumption) and core body temperature, and these responses are significantly blunted by a TRPV1 antagonist [65] and abolished in TRPV1-KO mice [66], suggesting that TRPV1 is an essential receptor for increasing energy expenditure and heat production. TRPV1 is markedly expressed in peripheral sensory neurons derived from the dorsal root ganglion, the trigeminal ganglion and the nodose ganglion [4, 67, 68]. Denervation of TRPV1-expressing sensory nerves by systemic pretreatment with excess capsaicin can completely block enhancement of  $O_2$  consumption and increases in body temperature [69]. Therefore, TRPV1 agonists induce incremental energy expenditure and thermogenesis via TRPV1-expressing sensory nerves.

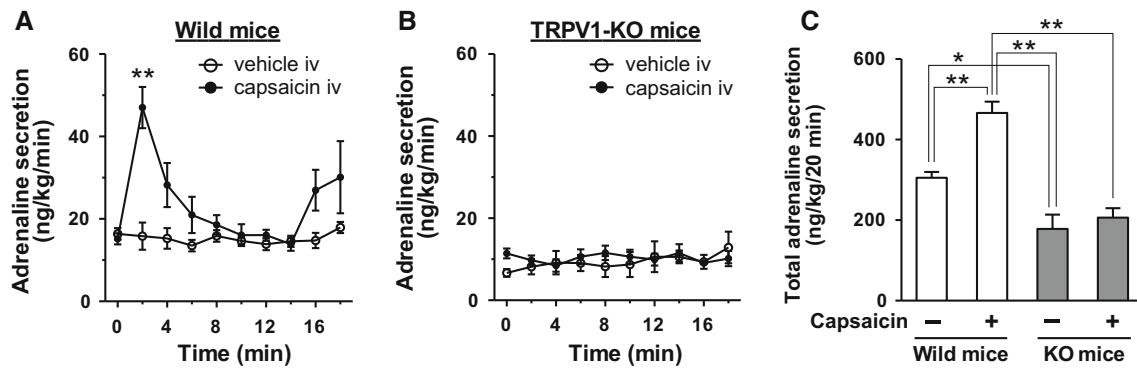
Adrenaline produced by the adrenal medulla is an important stimulating hormone that increases energy expenditure and thermogenesis, as well as possibly contributing to diet-induced energy expenditure. Meal intake induces increases in plasma adrenaline and  $O_2$  consumption, and meal-induced energy expenditures can be blunted by the  $\beta$ -blocker propranolol [70]. Administration of capsaicin enhances adrenal sympathetic nerve activity [71] and adrenaline secretion from the adrenal medulla [72]. Pretreatment with a  $\beta$ -blocker and adrenal demedullation largely attenuate capsaicin-induced increases in  $O_2$  consumption [54, 69]. Capsaicin-induced adrenaline secretion is inhibited by chemical denervation of sensory nerves [73], pretreatment with the TRPV1 antagonist capsazepine [74], and in TRPV1-KO mice (Fig. 1). In Fig. 1, basal adrenaline level in adrenal vein in TRPV1-KO mice were lower than that in wild-type mice. Previous reports indicate that TRPV1-KO mice show a decreased sympathetic activity although basal body temperature, heart rate and blood pressure are normal [75–77], therefore the lowering of adrenaline secretion in TRPV1-KO might be due to decreasing activity of sympathoadrenal nerves. Moreover, not only the pungent TRPV1 agonist capsaicin but also the low pungency TRPV1 agonists capsiate [78] and [10]-shogaol [61] could induce adrenaline secretion.

These results indicate that peripheral administration of TRPV1 agonists can evoke adrenaline secretion via sensory—central—sympathoadrenal reflexes to increase energy expenditure (Fig. 2). In addition, Osaka et al. reported that the rostral ventrolateral medulla, which is the site of the premotor area that contains sympathoadrenal preganglionic neurons, is a critical locus for capsaicin-mediated increases in energy expenditure [79].

Increases in  $O_2$  consumption by capsaicin are partially retained in adrenal-demedullated rats [69], suggesting that mechanisms other than those involving adrenaline could underlie the increase in energy expenditure. Capsaicin and capsiate activate sympathetic efferent nerves innervating iBAT, thereby inducing expression of UCP1 and heat production [66, 69, 80, 81]. Sympathetic denervation of iBAT partly attenuates capsaicin-induced energy expenditure [69]. The administration of capsaicin or capsiate into the GI tract increases the activity of sympathetic efferent nerves innervating iBAT and induces thermogenesis in iBAT. These effects can be impaired by the denervation of vagal afferents and extrinsic nerves connected to the jejunum [66, 81]. Subchronic intake of the non-pungent TRPV1 agonist capsiate or 1-monoolein elevates UCP1 expression in iBAT and prevents accumulation of visceral fat promoted by a high-fat diet [64, 80]. Furthermore, the anti-obesity effects of capsiate are completely abolished in UCP1-KO mice [82]. Together, these findings indicate that TRPV1-expressing sensory nerves, especially vagal afferent sensory nerves, participate in regulating activity of sympathetic nerves innervating the iBAT as well as energy expenditure (Fig. 2).

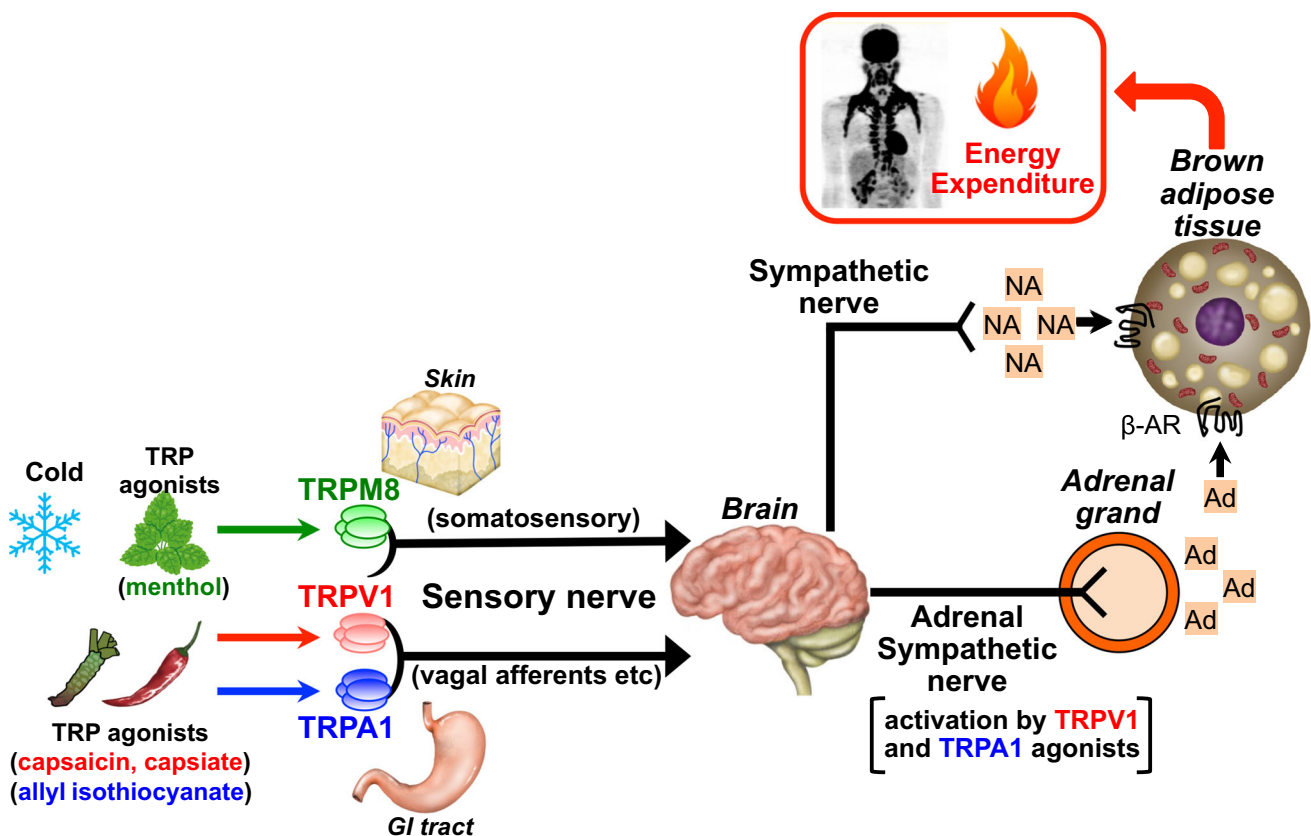
Many spicy foods contain both TRPV1 agonists and TRPA1 agonists [57]. For example, allyl isothiocyanate in mustard [83], cinnamaldehyde in cinnamon [83], and allicin and diallyl trisulfide in garlic [84, 85] are TRPA1 agonists. Additionally, we identified miogatriol from mioga (*Zingiber mioga*) as a potent and low pungency TRPA1 agonist [86]. TRPA1 is co-expressed with TRPV1 in dorsal root ganglion neurons and nodose ganglion neurons [87, 88], and similar to that of TRPV1 agonists, the TRPA1 agonists allyl isothiocyanate and cinnamaldehyde enhance adrenaline secretion via the sensory—central—sympathoadrenal reflex [89]. Allyl isothiocyanate and cinnamaldehyde induce UCP1 expression in iBAT [90–92] and heat production [93]. Therefore, sensory neurons expressing both TRPV1 and TRPA1 might be a crucial subclass that regulates energy expenditure and thermogenesis (Fig. 2).

Cold exposure is known to enhance thermogenesis to maintain body temperature. TRPM8 is a cold receptor that is activated by moderate cold (<25–28 °C) and “cooling-mimetic” compounds such as menthol from mint [94, 95]. TRPM8 is abundantly expressed in different



**Fig. 1** Capsaicin-induced adrenaline secretion is completely impaired in TRPV1 knockout mice. We investigated the effect of capsaicin on adrenaline secretion in male adult C57BL/6 wild-type mice and TRPV1 knockout mice (obtained from Dr. D. Julius, University of California, San Francisco) according to the previous report with a slight modification [89]. A mouse anesthetized with  $\alpha$ -chloralose and urethane (0.1 and 1 g/kg, respectively) was placed on heated pad, and the rectal temperature was maintained at 36.5–37.5 °C. Heparinized saline (500 IU/ml, 25  $\mu$ l) was injected through the femoral vein, then sampling of adrenal blood from the adrenal vein was started with a 2-min interval. Immediately after

collecting the first fraction, capsaicin (0.05 mg/kg) or vehicle (saline containing 2% ethanol and 10% Tween-80) was administered into the femoral vein. Plasma adrenaline was purified with active alumina and measured by HPLC-electrochemical detection. Intravenous administration of capsaicin significantly increased adrenaline secretion in wild-type mice (a) but not TRPV1 knockout mice (b). There was a significant difference between vehicle and capsaicin in a but not b (treatment effect,  $p < 0.01$  by two-way ANOVA). \*\* $p < 0.01$  by Bonferroni's test. c Total adrenaline secretion for 20 min in a and b. \* $p < 0.05$ , \*\* $p < 0.01$  by one-way ANOVA followed by Tukey's test. Each value is the mean  $\pm$  SEM ( $n = 5$ )



**Fig. 2** Mechanisms of increasing energy expenditure and thermogenesis by sensory nerves expressing thermo-TRP channels. Ad adrenaline, NA noradrenaline, GI tract gastrointestinal tract,  $\beta$ -AR  $\beta$ -adrenergic receptor. Modified with permission from Ref. [126]

subpopulations of sensory neurons (from dorsal root, trigeminal and nodose ganglion) that express TRPV1 and TRPA1 [87, 96–98]. Previous reports using TRPM8-

deficient mice show that TRPM8 mediates cold sensations to induce avoidance behavior towards innocuous cold [99–101]. Moreover, recent studies demonstrated that

TRPM8 is a crucial channel for cold-defense thermoregulation. The core body temperature in wild-type mice is maintained during cold exposure, but the core temperature of TRPM8-KO mice and wild-type mice treated with a TRPM8 antagonist is decreased [102, 103]. Moreover, activation of TRPM8 by cold stimulation or menthol administration increases core temperature in wild-type mice but not TRPM8-KO mice [93, 102]. Cold exposure activates and increases c-Fos expression in the lateral parabrachial nucleus, which is relay area for cutaneous cold signals from primary sensory neurons to the preoptic hypothalamus [104, 105]; c-Fos expression is blunted by treatment with TRPM8 antagonists [103]. Taken together with these data, activation of TRPM8, presumably on sensory nerves, increases heat production (Fig. 2). TRPM8 expression has also been detected outside of sensory nerves in tissues such as the bladder, prostate, brown adipocyte, liver, gastrointestinal mucosa and several types of tumors. As such, the above-mentioned results using TRPM8-KO mice and TRPM8 antagonists might contribute to an understanding of TRPM8 mechanisms beyond that in sensory nerves. Future studies using site-specific loss of function approaches could clarify the mechanism by which TRPM8 in sensory nerves regulates thermogenesis.

### TRP-activated brown fat thermogenesis in humans

In humans, with the exception of newborns, the prevalence of BAT has long been believed to be negligible. However, recent radionuclide imaging studies revealed the existence of considerable amounts of BAT in healthy adults [106, 107]. The metabolic activity of human BAT can be assessed by fluorodeoxyglucose (FDG)-positron emission tomography (PET) combined with X-ray computed tomography (CT), which is a powerful diagnostic tool for malignant tumors. Although the principal substrate for BAT thermogenesis is fatty acids, glucose utilization is greatly enhanced in parallel with the activation of UCP1, a key molecule of BAT thermogenesis [108]. Thus, glucose utilization assessed by FDG uptake could serve as an index of BAT thermogenic activity.

Cold exposure is the most powerful and physiological stimulus for BAT activation. Cold acts on TRP expressed in sensory nerves to enhance sympathetic nerve activity and trigger  $\beta$ -adrenergic receptor-mediated intracellular cascades in brown adipocytes, and finally to activate UCP1 and thermogenesis (Fig. 2) [104, 109]. In fact, the activity of BAT as assessed by FDG-PET/CT is greatly increased after acute cold exposure or administration of  $\beta$ -adrenergic receptor agonists, but is reduced under warm conditions or by pretreatment with a  $\beta$ -adrenergic blocker [106, 110, 111]. BAT activity is positively associated with cold-induced non-shivering thermogenesis (CIT), suggesting that BAT

contributes to whole-body energy expenditure in humans [112]. BAT activity decreases with age and these decreases are associated with excessive accumulation of body fat with age [113]. Inactivation and reduction of BAT are now accepted to be associated with obesity and insulin resistance [114, 115]. As such, methods that re-activate and recruit BAT could promote reductions in body fat. Indeed, repeated mild cold exposure at 16 °C for 2 h every day for 6 weeks results in increases in BAT activity and CIT in healthy lean subjects [116]. More importantly, such cold acclimation decreases body fat mass, in parallel with the changes in BAT activity and CIT. This result is in line with a recent report showing increased BAT mass and CIT after cold acclimation in obese subjects [117]. Thus, BAT is a significant anti-obesity target in humans [118].

Nevertheless, increased exposure to cold would be difficult and uncomfortable in daily life. As mentioned above, some thermo-TRP channels are activated by various chemical substances, including food ingredients. Given that chemical activation of TRPV1 in the gastrointestinal tract by capsaicin and capsiate activates UCP1 in BAT in mice [82], oral administration of these ingredients may be a more feasible method to recruit BAT in humans. We found that single oral ingestion of capsinoids, which include capsiate, dihydrocapsiate, and nordihydrocapsiate, increases whole-body energy expenditure in human individuals with metabolically active BAT, but not in those without active BAT [119]. Furthermore, daily ingestion of capsinoids augments BAT activity [120] and CIT [116] even in individuals with low BAT activities. Thermogenic and fat-reducing effects of capsinoids have also been shown in an obese population [55, 121, 122]. Taken together, the anti-obesity effects of capsinoids as TRPV1 agonists may be attributable to the thermogenic activity of recruited BAT.

The mechanism of capsinoid-induced activation of BAT has been characterized for the most part by studies in small rodents. As noted above, the primary action site of capsinoids would be TRPV1 on sensory nerves in the gastrointestinal tract. Consistent with the crucial role of TRPV1 in capsinoid-mediated effects in mice [66], the beneficial effects of capsinoids are greatly attenuated in individuals who carry a mutated (Val585Ile) TRPV1 [121]. Although TRPV1 is expressed in brown adipocytes, the direct action of capsinoids toward TRPV1 in human BAT may be unlikely because orally ingested capsinoids are rapidly hydrolyzed, and thus are usually undetectable in the general circulation in humans. Although TRPV1 activators affect BAT and body fat similarly to cold exposure, it should be noted that TRPV1 is not a cold sensor but instead is a sensor of noxious stimuli, including exposure to temperatures above 42 °C. Thus, human BAT is likely to be activated by nociceptive stimuli such as high temperature and capsinoids. In agreement with this idea, Sidossis et al.



[123] recently demonstrated in humans that chronic adrenergic stress induced by burn trauma results in browning of WAT.

In addition to capsinoids, several dietary substances activate TRPV1 and BAT thermogenesis. For example, dietary supplementation with eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) up-regulates UCP1 expression in both BAT and WAT, thereby preventing diet-induced obesity [124]. These effects are largely dependent on the presence of TRPV1 and activation of sympathetic nervous system (SNS) [76]. Despite the evidence in mice, effects of EPA and DHA on human BAT have not been determined. Additionally, as noted above, TRPV1-expressing sensory nerves also express cold-sensitive TRPA1, which is involved in BAT activation induced by cold exposure. TRPA1 is activated by various pungent compounds, such as allyl- and benzyl-isothiocyanates in wasabi (Japanese horse radish) and cinnamaldehyde in cinnamon. These compounds are known to increase thermogenesis and UCP1 expression in small rodents [125]. Furthermore, TRPM8 is also the most likely candidate receptor to sense lower temperatures and is known to be involved in BAT activation [125]. A representative TRPM8 agonist is menthol, a cooling and flavor compound in mint [93]. Using TRPM8- and UCP1-deficient mice, Ma et al. documented that dietary menthol activates UCP1-dependent thermogenesis in a TRPM8-dependent manner [48]. These findings concerning the chemical activation of the TRPs-SNS-BAT axis in small rodents provide further impetus for the identification of common food ingredients that can activate and recruit BAT in humans.

## Conclusions and perspectives

The obesity pandemic is a serious global health problem because it is a major risk factor for metabolic syndromes including insulin resistance, impaired insulin secretion, hyperglycemia, dyslipidemia, and hypertension. Normal regulation of glucose and lipid metabolism is indispensable for healthy biological activity. Moreover, enhancement of energy expenditure together with a reduction in food intake is effective in reducing obesity. Eleven thermo-TRP channels have been identified in the 20 years since the first thermo-TRP channel, TRPV1, was cloned. Furthermore, these thermo-TRPs not only sense temperature but also regulate events related to energy metabolism, such as insulin secretion by the pancreas, differentiation and/or thermogenesis in brown adipocytes, and energy expenditure mediated by sensory nerve—brain—sympathetic reflexes. We anticipate that further studies on the physiological and pathophysiological roles of thermo-TRPs may

open novel avenues for treating metabolic disorders, including obesity and diabetes.

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## Compliance with ethical standards

**Conflict of interest** All authors have no conflicts of interest related to this manuscript.

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