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Review

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Menthol to Induce Non-shivering Thermogenesis via TRPM8/PKA Signaling for Treatment of Obesity

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Increasing basal energy expenditure via uncoupling protein 1 (UCP1)-dependent non-shivering thermogenesis is an attractive therapeutic strategy for treatment of obesity. Transient receptor potential melastatin 8 (TRPM8) channel activation by cold and cold mimetics induces UCP1 transcription and prevents obesity in animals, but the clinical relevance of this relationship remains incompletely understood. A review of TRPM8 channel agonism for treatment of obesity focusing on menthol was undertaken. Adipocyte TRPM8 activation results in Ca^{2+} influx and protein kinase A (PKA) activation, which induces mitochondrial elongation, mitochondrial localization to lipid droplets, lipolysis, β-oxidation, and UCP1 expression. Ca^{2+}-induced mitochondrial reactive oxygen species activate UCP1. In animals, TRPM8 agonism increases basal metabolic rate, non-shivering thermogenesis, oxygen consumption, exercise endurance, and fatty acid oxidation and decreases abdominal fat percentage. Menthol prevents high-fat diet-induced obesity, glucose intolerance, insulin resistance, and liver triacylglycerol accumulation. Hypothalamic TRPM8 activation releases glucagon, which activates PKA and promotes catabolism. TRPM8 polymorphisms are associated with obesity. In humans, oral menthol and other TRPM8 agonists have little effect. However, topical menthol appears to increase core body temperature and metabolic rate. A randomized clinical control trial of topical menthol in obese patients is warranted.

Key words: Obesity, Weight loss, Mitochondrial uncoupling proteins, Brown adipose tissue, Thermogenesis

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INTRODUCTION

Obesity and overweight are two of the most urgently pressing medical challenges facing contemporary healthcare. While the foundational approaches of caloric restriction and exercise focus on reducing energy intake and increasing activity energy expenditure, respectively, modifying resting energy expenditure remains a challenge. One way to do so would be to increase adipose tissue non-shivering thermogenesis. Non-shivering thermogenesis results primarily from expression of uncoupling protein 1 (UCP1), which allows hydrogen ions to be transported down an electrochemical gradient into the mitochondrial matrix without producing adenosine triphosphate, thereby dissipating the energy of the proton motive force as heat.[1] This uncouples the proton motive force from mitochondrial respiration.

The primary site of thermogenesis is brown adipose tissue, which is rich in iron-containing mitochondria that give rise to its distinct color. Functional brown adipose tissue appears to persist even into adulthood in humans,[2,3] but its metabolic activity tends to decrease in association with age and obesity.[2,4,5] In addition, there are distinct subpopulations of "brite" and "beige" adipocytes that are transcriptionally distinct from both classical brown and white adipocytes, and that can be stimulated to attain a brown adipose tissue-like phenotype featuring uncoupled mitochondria.[2,6,7] It has been reported that human brown adipose tissue is more similar to murine beige adipose tissue than murine brown or white adi-

pose tissue at the molecular level.[6]

Therapeutically, attempts have been made to stimulate human adipose tissue uncoupling and non-shivering thermogenesis to increase basal energy expenditure. Cold exposure stimulates brown adipose tissue activation in humans.[8-12] Ice pack application has been shown to beige subcutaneous white adipose tissue and upregulate UCP1 in lean and obese individuals.[11] Pharmacologically, much of the efforts in this area have focused on β3-adrenergic receptors. β3-adrenoceptors are activated by norepinephrine, production of which by the sympathetic nervous system is increased by cold and other stressors. Of the β3-adrenoceptor agonists tested, ZD7114 and ZD2079,[13,14] L-796568,[14,15] and TAK-677 have shown negative results in terms of decreasing adiposity, although TAK-677 (0.5 mg twice daily) did significantly increase energy expenditure by approximately 13 kcal/day.[14,16] Mirabegron, by contrast, has shown positive results in human studies on the parameters of adipose tissue beiging[11] and metabolic activity,[17] non-esterified fatty acid release,[18] and basal energy expenditure.[17,19] This provides proof-of-concept that stimulating adipose tissue through thermogenesis might be a viable complementary strategy to promote weight loss as part of multi-component treatment protocols to combat obesity. However, β3-adrenoceptors are not the only receptors activated by cold to induce thermogenesis: transient receptor potential melastatin 8 (TRPM8) channels fulfill both of these criteria as well.[2,20-22]

The TRPM8 channel is the primary cold receptor of the murine peripheral nervous system.[20] It is activated not only by cold, but also by icilin,[23] testosterone,[24] borneol (a traditional Chinese herb and terpene),[25] and cooling agents (i.e., cold mimetics) such as menthol and eucalyptol.[2,20,26-28] It is expressed in brown, beige, and white adipocytes,[29-31] as well as in prostate cells, prostate cancer cells,[24] dorsal root ganglion sensory neurons,[2,24] trigeminal ganglia sensory neurons,[2] N41 hypothalamic cells,[32] and hippocampal neurons.[24]

Emerging evidence has shown that TRPM8 activation leads to protein kinase A (PKA) activation, UCP1 upregulation, increased thermogenesis, and protection from obesity.[2,20-22,26,30,33] Topical menthol has been shown in rodent models to increase body temperature and non-shivering thermogenesis.[26,34,35] However, the evidence remains fragmentary, and the plausibility of translating TRPM8 agonists into clinical applications to treat obesity and overweight status remains unclear. To analyze whether TRPM8 agonism with menthol or other agents may promote clinically relevant weight loss in preclinical animal models and patients who are overweight or obese, we performed a review of TRPM8 agonism focusing on the cold mimetic menthol for treatment of obesity and overweight individuals.

We conducted a literature search in PubMed using the following keywords: "TRPM8 weight loss," "TRPM8 thermogenesis," "TRPM8 obesity," "TRPM8 overweight," "TRPM8 adipocyte," "menthol weight loss," "menthol thermogenesis," "menthol obesity," "menthol overweight," and "menthol adipocyte." Results on the association between menthol cigarettes and obesity and other cardiometabolic risk factors[36,37] were discarded. Studies about menthol in conditions that are not associated with overweight status or obesity (e.g., colitis[38]) were also discarded. Preclinical, epidemiological, and clinical studies were reviewed.

TRPM8 CHANNELS IN ENERGY HOMEOSTASIS

Table 1 summarizes the effects induced by the TRPM8 agonists menthol and icilin. *In vitro*, treating mature adipocytes with 1 μM bioavailable menthol for one hour increased RNA expression of genes associated with adipose tissue beiging, namely UCP1, peroxisome proliferator-activated receptor γ coactivator 1α (PGC1α), tumor necrosis factor receptor superfamily member 9, and Homeobox C10.[22] Menthol was more effective at increasing UCP1 expression and uncoupled respiration in white adipocytes than in brown adipocytes.[39] In white adipocytes, menthol and icilin significantly increased UCP1 mRNA and protein levels, thermogenesis, glucose uptake, mitochondrial membrane potential, and mitochondrial elongation and clustering around lipid droplets independent of genes involved in mitochondrial biogenesis through TRPM8 activation and consequent Ca^{2+} influx.[29] In cultured mouse white adipocytes, menthol significantly increased PGC1α and UCP1 mRNA levels, effects which were significantly blocked by the PKA inhibitor KT5720 and apparently even more effectively by the cell membrane permeable calcium chelator BAPTA-AM.[30] Indeed, activation of TRPM8 channels with menthol was found to induce cyto-

Table 1. The effects induced by menthol and icilin

Drug	Effect	Reference
Menthol	Induces adipose tissue beiging	22,30
	Upregulates uncoupling protein 1	21,22,29,30,40
	Upregulates peroxisome proliferator-activated receptor γ coactivator 1α	30,40
	Increases thermogenesis	21,29,35,41-43
	Increases basal energy expenditure	26,43
	Prevents liver triacylglycerol accumulation	44
	Prevents insulin resistance	44
	Improves glucose homeostasis	21,30
	Increases glucose uptake	29
	Increases mitochondrial membrane potential	29
	Induces mitochondrial elongation and clustering around lipid droplets	29
	Increases exercise endurance	40
	Decreases blood lactate and triglyceride levels	40
	Increases locomotor activity in normal but not high-fat diet fed mice	21
	Promotes glucagon release	44
	Increases oxygen consumption	35,45
	Decreases the respiratory coefficient	45
	Increases shivering	35
	Increases vasoconstriction	35,43
	Increases heat-seeking behavior	35
	Prevents weight gain	21,30,44
	Decreases abdominal fat percentage	46
Icilin	UCP1 upregulation and thermogenesis	29
	Increases glucose uptake	29
	Increases mitochondrial membrane potential	29
	Induces mitochondrial elongation and clustering around lipid droplets	29
	Promotes glucagon release	44
	Increases wet-dog shakes	47
	Induces synergistic reversal of diet-induced obesity, dyslipidemia, and glucose intolerance when combined with dimethylphenylpiperazinium	33

plasmic Ca^{2+} influx, Ca^{2+}-dependent PKA phosphorylation, and PKA-dependent UCP1 protein upregulation in adipose tissue,[21] suggesting that TRPM8 channels activate the PKA/UCP1 pathway.[21,48]

TRPM8 channels may affect energy homeostasis in non-adipose tissues as well. In mice, TRPM8-activated sensory nerves promote brown adipose tissue thermogenesis.[49] Menthol upregulated UCP1 and PGC1α mRNA expression in C2C12 myotubes and mouse skeletal muscle via TRPM8 activation, and dietary menthol increased exercise endurance and decreased blood lactate and triglyceride levels.[40] Hypothalamic TPRM8 activation appears to promote glucagon release and increased energy expenditure. In mice, acute oral and topical menthol or icilin increased serum glucagon level via TRPM8 activation, and treating mature white 3T3L1 adipocytes with serum from menthol-treated mice increased energy expenditure in a manner that was blocked by a glucagon receptor antagonist.[44] In N41 hypothalamic cells, TRPM8 activation mediated the effects on Ca^{2+} currents of the thyroid hormone metabolite 3-iodothyronamine,[32] central intracerebroventricular administration of which increased glucagon and endogenous glucose production.[50] Glucagon has an acute hyperglycemic effect but also increases energy expenditure and decreases food intake in rodents when co-administered with glucagon-like peptide-1.[51,52]

In vivo, TRPM8 knockout mice had attenuated UCP1 protein expression in their brown adipose tissue.[53] TRPM8 inhibition decreased deep body temperature in mice and rats.[54] TRPM8 knockout mice displayed hypothermia, hyperphagia, decreased fat oxidation, and obesity.[55] Even in thermoneutral conditions, TRPM8 activation by topical menthol application increased oxygen consumption and decreased the respiratory coefficient, suggesting that TRPM8 activation may promote fat oxidation.[45] TRPM8 agonism with intragastric menthol or 1,8-cineole increased thermogenesis in mice.[41] Topical menthol increased core body temperature, shivering, oxygen consumption, tail skin vasoconstriction, and heat-seeking behavior in mice.[35] Intraperitoneal injection of icilin increased wet-dog shakes in mice,[47] and dietary menthol increased locomotor activity in wild-type mice but not those fed a high-fat diet even though it increased thermogenesis in both.[21] These findings suggest that TRPM8 activation can increase energy expenditure both by increasing locomotor activity and independent of activity level through non-shivering thermogenesis.

Topical menthol significantly increased basal metabolic rate despite unchanged food intake in a murine model of diet-induced obesity.[26] Dietary menthol treatment attenuated high-fat diet-induced obesity and improved glucose homeostasis and white adipose tissue beiging in a rodent model.[30] Via the TRPM8/Ca^{2+}/PKA/UCP1 pathway, dietary menthol prevented diet-induced obesity and glucose intolerance in mice.[21] In broiler chickens, supplemental peppermint leaves or menthol increased body weight and dietary intake, decreased the percentage of breast and leg muscle lost due to cooking, and decreased abdominal fat percentage.[46]

Interestingly, menthol or peppermint was also associated with decreased mortality in these birds.[46] Chronic oral (50 and 100 mg/kg/day for 12 weeks) or topical menthol in high-fat diet fed mice prevented weight gain and adipose tissue hypertrophy, as well as liver triacylglycerol accumulation and insulin resistance.[44] Combining subcutaneously injected icilin and dimethylphenylpiperazinium to target the appetite-suppressing nicotinic acetylcholine receptor α3β4 resulted in synergistic reversal of diet-induced obesity, dyslipidemia, and glucose intolerance.[33]

Recently, Sakellariou et al.[2] proposed that chronic administration of oral menthol to obese individuals may induce sustained weight loss by increasing adipose tissue thermogenesis. The *TRPM8* gene has been found to be differentially expressed in two individuals with familial obesity and one non-obese individual from the same Thai family. Furthermore, its minor allele frequency was low, indicating a possible causal variant.[56] In a Turkish population, the rs12472151 polymorphism of the *TRPM8* gene was associated with metabolic syndrome.[57]

In 16–18 healthy adult individuals, oral administration of 0.2 mL of a TRPM8 agonist cooling flavor in 200 mL tomato juice did not alter energy expenditure or substrate utilization.[58] However, in seven swimmers and seven physical education students, topical menthol decreased rectal temperature loss caused by immersion in cold water, suggesting that topical menthol may have increased thermogenesis.[42] In 20 healthy adult individuals, topical menthol significantly increased metabolic rate (18%), cutaneous vasoconstriction, body heat storage, and rectal temperature compared to control and oral menthol.[43] Oral menthol underperformed relative to topical menthol due in part to increased glucuronidation and elimination of oral menthol compared to topical menthol.[43]

CONCLUSION

Overall, the few associated studies performed in human participants suggest that topical menthol might effectively promote increased basal energy expenditure and weight loss through non-shivering thermogenesis, whereas oral TRPM8 agonists (at least at the low doses used in these studies) may not.[42,43,58] One explanation for this difference is that oral menthol is more readily glucuronidated and excreted than topical menthol.[43] However, a complementary explanation is that topical menthol may reach the target adipose tissue more directly and thereby achieve greater concentrations there than oral menthol. Therefore, future studies in human participants should study the effects of topical menthol or icilin, as topical administration is the most promising drug delivery method.

Another conclusion that can be drawn from this review is that there is a TRPM8/Ca^{2+}/PKA/UCP1 pathway whose activation uncouples respiration and increases non-shivering thermogenesis, and it is active and physiologically relevant in adipose tissue, especially in white adipose tissue (Fig. 1).[21,22,29,39,48] The pleiotropic effects of PKA activation in this context, however, warrant further mechanistic discussion to glean additional therapeutic insights. Previously, PKA activation has been shown to promote lipolysis[59,60] and β-oxidation.[61] In one of the studies reviewed, menthol or icilin was found to not only upregulate UCP1, but also to induce mito-

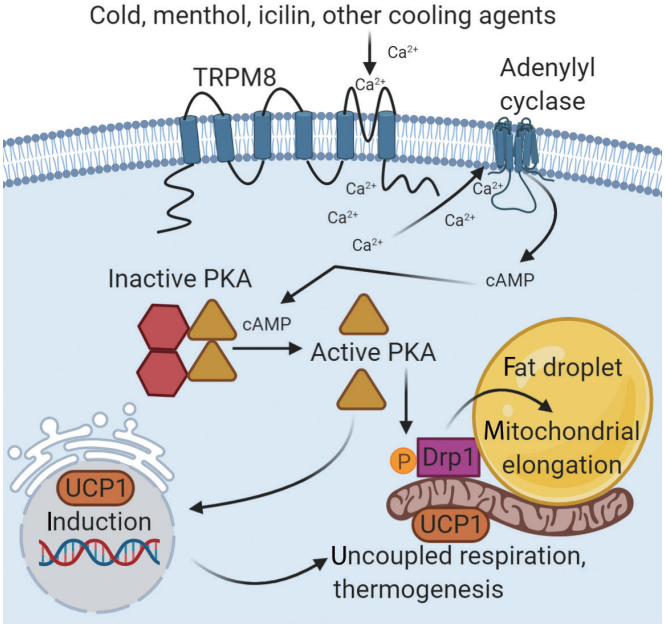

Figure 1. Mechanisms of transient receptor potential melastatin 8 (TRPM8)-mediated uncoupled respiration and mitochondrial elongation. Cold, menthol, icilin, and other cooling agents open brown, beige, and white adipocyte TRPM8 channels.[2,20,31,23-30] Extracellular Ca^{2+} ions influx through TRPM8 channels into the adipocyte cytoplasm.[29] Peri-plasma membrane Ca^{2+} activates adenylyl cyclase 1 and 8, which generate cyclic adenosine monophosphate (cAMP).[62] cAMP activates protein kinase A (PKA) to induce uncoupling protein 1 (UCP1) transcription.[2,20-22,26,30,33] Once translated, UCP1 proteins are imported into mitochondria and localized to the inner mitochondrial membrane, where they allow protons to diffuse down an electrochemical gradient into the mitochondrial matrix, dissipating the energy of the proton motive force as heat instead of generating adenosine triphosphate.[1] PKA also phosphorylates and thereby activates dynamin-related protein 1 (Drp1), which induces mitochondrial fusion and elongation around lipid droplets.[29,63]

chondrial elongation and localization around lipid droplets via TRPM8 activation and Ca^{2+} influx.[29] This probably occurred via PKA activation and PKA phosphorylation of dynamin-related protein 1 (Drp1).[63] Drp1 is a large GTPase that executes mitochondrial fission, whereby mitochondria split to form two daughter mitochondria. PKA activation inhibits Drp1, promoting mitochondrial fusion and elongation (Fig. 1).[63]

TRPM8 activation transduces its signal into the cell via Ca^{2+} influx and raises the mitochondrial membrane potential, making it more positive and promoting depolarization. This result is intriguing[29] since cytoplasmic Ca^{2+} becomes sequestered in the mitochondria, dissipating the mitochondrial membrane potential,[64] increasing mitochondrial reactive oxygen species (ROS) production,[65] and eventually promoting permeability transition opening and cell death.[64] Therefore, it appears that TRPM8 activation may lead to mild, physiological mitochondrial Ca^{2+} accumulation. This would explain the more positive mitochondrial membrane potential, and it would also predict that TRPM8 activation should lead to mitochondrial ROS production. Consistent with this relationship, interscapular brown adipose tissue activation in mice increased mitochondrial ROS production in this tissue.[66] Furthermore, ROS were found to activate UCP1 by sulfenylating its Cys253 residue.[66] Moreover, experiments with antioxidants showed that mitochondrial ROS are required to prevent hypothermia and to increase energy expenditure upon cold exposure.[66] Therefore, antioxidants might be contraindicated while administering topical menthol to ensure its efficacy, although further experiments are required to evaluate this possibility.

The connection between hypothalamic TRPM8 signaling and glucagon production[32,44] is also intriguing, since cold exposure acutely raises glucagon level,[67] and both TRPM8 channels and glucagon increase PKA activity.[21,68] This makes it appear as if glucagon were a hormone messenger of TRPM8 agonism, with both signals converging on PKA activation and PKA-driven catabolism.

Therefore, this literature review concludes that menthol should be studied further in patients to test whether it can deliver clinically-relevant increases in non-shivering thermogenesis, basal energy expenditure, and weight loss.[42,43,58] Menthol raises the basal metabolic rate by activating the TRPM8/Ca^{2+}/PKA, PKA/UCP1, and PKA/Drp1 pathways in white adipose tissue, resulting in beiging.[21,22,29,30,48] Cold, menthol, and glucagon promote catabolism via PKA activation.[21,32,44,67,68] A clinical trial of menthol for weight loss in obese patients is warranted.[42,43,58,66]

CONFLICTS OF INTEREST

The authors declare no conflict of interest.

AUTHOR CONTRIBUTIONS

Study concept and design, drafting of the manuscript, and critical revision of the manuscript: all authors.

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