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Chronic L-menthol-induced browning of white adipose tissue hypothesis: A putative therapeutic regime for combating obesity and improving metabolic health

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ABSTRACT

Introduction: Obesity constitutes a serious global health concern reaching pandemic prevalence rates. The existence of functional brown adipose tissue (BAT) in adult humans has provoked intense research interest in the role of this metabolically active tissue in whole-body energy balance and body weight regulation. A number of environmental, physiological, pathological, and pharmacological stimuli have been proposed to induce BAT-mediated thermogenesis and functional thermogenic BAT-like activity in white adipose tissue (WAT), opening new avenues for therapeutic strategies based on enhancing the number of beige adipocytes in WAT.

Hypothesis: Recent evidence support a role of L-menthol cooling, mediated by TRPM8 receptor, on UCP1-dependent thermogenesis and BAT-like activity in classical WAT depots along with the recruitment of BAT at specific anatomical sites. L-Menthol-induced BAT thermogenesis has been suggested to occur by a β-adrenergic-independent mechanism, avoiding potential side-effects due to extensive β-adrenergic stimulation mediated by available beta receptor agonists. L-Menthol has been also linked to the activation of the cold-gated ion channel TRPA1. However, its role in L-menthol-induced UCP1-dependent thermogenic activity in BAT and WAT remains undetermined. White adipose tissue plasticity has important clinical implications for obesity prevention and/or treatment because higher levels of UCP1-dependent thermogenesis can lead to enhanced energy expenditure at a considerable extent. We hypothesize that chronic dietary L-menthol treatment could induce TRPM8- and TRPA1-dependent WAT adaptations, resembling BAT-like activity, and overall improve whole-body metabolic health in obese and overweight individuals.

Conclusions: The putative impact of chronic L-menthol dietary treatment on the stimulation of BAT-like activity in classical WAT depots in humans remains unknown. A detailed experimental design has been proposed to investigate the hypothesized L-menthol-induced browning of WAT. If our hypothesis was to be confirmed, TRPM8/TRPA1-induced metabolic adaptations of WAT to BAT-like activity could provide a promising novel therapeutic approach for increasing energy expenditure, regulating body weight, and preventing obesity and its related co-morbidities in humans.

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Introduction

Obesity and its related disorders represent major global health concerns. The World Health Organization estimates that the global prevalence of obesity has doubled since 1980 [1]. In 2014 the prevalence of overweight adults reached more than 1.9 billion, with 600 million of these being obese. Increased obesity and overweight prevalence rates are also recorded in children aged below 5 years reaching 42 million in 2013 [1]. Treating obesity, however, has proven to be a complex endeavor that has been mostly unsuccessful [2,3], supporting its epidemic prevalence rates in children and adults [1,4,5]. Therefore, novel therapeutic strategies are warranted for preventing and/or combating this health threatening condition.

The relatively recent discovery of functional brown adipose tissue (BAT) in adult humans has provoked a plethora of theories and experiments on the metabolic capabilities of BAT and its potential for the prevention and/or treatment of obesity and obesity-associated diseases, including type 2 diabetes, cardiovascular disease, and several cancers [6,7]. In human adults, BAT is found mainly in the cervical, supraclavicular, and paravertebral regions, while in infants it is located also in the interscapular and perirenal regions [8]. BAT is responsible for non-shivering thermogenesis (NST), an adaptive mechanism to maintain body temperature against cold environments by generating heat without shivering [7,9], mediated by increased uncoupling protein 1 (UCP1) activity. UCP1 is a mitochondrial transmembrane protein located in the inner mitochondrial membrane of brown adipocytes that enhances proton conductivity and provides an alternative pathway by which protons can re-enter the mitochondrial matrix. In most eukaryotic cells during cellular respiration, the protons that are normally pumped across the mitochondrial inner membrane due to the NADH and FADH2 oxidation generate an electrochemical gradient that is dissipated as they enter the mitochondrial matrix through ATP synthase resulting in ATP production. However, the presence of UCP1 in brown adipocytes provides a different biochemical pathway in these cells allowing protons to return to the mitochondrial matrix through UCP1 instead of ATP synthase. During this process, the energy of the electrochemical gradient is dissipated as heat [7,9].

Similarly to brown adipocytes, UCP1 is also expressed in a distinct type of adipocytes which are termed as beige adipocytes [extensively reviewed in [8,10–12]. Beige adipocytes, however, express a different gene pattern (e.g. transcription factors) than classical brown adipocytes [13–15]. Although beige adipocytes are characterized by BAT functional thermogenic properties (i.e., UCP1 expression and norepinephrine-induced thermogenesis) and are located in classical white adipose tissue (WAT), they are developmentally distinct from both brown and white fat precursors. Rodent studies proposed that only a subpopulation of beige adipocytes are located within WAT depots [13,16–18] exhibiting increased UCP1 activity in response to a number of stimuli, including cold exposure [19,20], β-adrenergic stimulation [16–18,21,22], peroxisome proliferator-activated receptor (PPARγ)-agonist stimulation [13,23–28], forced [14,29–32], or voluntary exercise [30].

Substantial evidence supports the putative role for BAT-mediated NST in increasing energy expenditure and regulating energy metabolism [33–36]. Indeed, the energy expended for heat production during prolonged and consistent BAT activity may be sufficient enough to regulate body weight and prevent obesity and its related comorbidities [7,37]. Despite these beneficial effects, current evidence suggests that BAT levels steadily decline with age and that BAT is less active in obese individuals [38,39]. Furthermore, studies in rodent (both *in vitro* and *in vivo*) and human (only *in vitro*) models have proposed a role for L-menthol in UCP1-NST stimulation and BAT-like activity, mediated by TRPM8 (transient receptor potential melastatin 8) [40–42].

L-Menthol is a chemical cooling agent naturally produced from mint oils or prepared synthetically [43] and also an agonist of the cold sensor TRPM8 [reviewed in [44]]. TRPM8 is predominantly located in a subpopulation of sensory neurons from dorsal root ganglia and trigeminal ganglia [45–47]. Interestingly, TRPM8 expression has also been recently discovered in brown and white adipocytes associated with an increase in UCP1 activation upon L-menthol administration [41,42]. Specifically, a dose-dependent effect of menthol on UCP1 protein expression has been demonstrated in brown adipocytes isolated from wild type mice, but not in those from TRPM8-deficient mice [41]. Studies on human white adipocytes cultured with L-menthol showed an increase in TRPM8 activation and UCP1-dependent thermogenesis, as evidenced by an increase in intracellular Ca^{2+}, UCP1 mRNA and protein levels, mitochondrial membrane potential, mitochondria number, heat production, basal and insulin stimulated glucose uptake [42]. *In vivo* studies on mice showed that 7-month L-menthol dietary treatment promoted TRPM8-mediated UCP1 activation in BAT through Ca^{2+}-dependent protein kinase A (PKA) phosphorylation, increased energy expenditure, improved glucose metabolism and protected against high-fat diet-induced obesity [41]. In addition, L-menthol skin application in mice activated TRPM8 and induced behavioral heat-gain responses, including increased NST, oxygen consumption and core body temperature [40]. The effects of L-menthol-induced stimulation of TRPM8 on UCP1-depedent thermogenesis mimic those thermoregulatory effects of cold exposure on BAT activity [48–51]. These findings are corroborating a putative role of L-menthol for the activation of β-adrenergic-independent signaling pathways mediated by TRPM8, regulating thermogenesis and metabolic homeostasis.

In vitro and *in vivo* studies in rodents have demonstrated that L-menthol activates the cold-gated ion channel TRPA1 (transient receptor potential ankyrin subtype 1 protein; reviewed in [52]) [53–55], although its role in mediating L-menthol-induced cold sensitivity has been disputed by others using also rodent animal [56] and cellular models [57]. Recently, a human study showed that concomitant activation of TRPA1 and TRPM8 following the simultaneous topical application of *trans*-cinnamaldehyde (TRPA1 activator) and L-menthol (TRPM8 activator) induced cold allodynia and hyperalgesia. These thermal responses were similar to those observed following the topical application of L-menthol alone, indicating a less pronounced role of TRPA1 in cold hypersensitivity and transduction [58].

It is evident that the putative role for TRPA1 in cold transduction remains an intense subject of debate. Recently, Chen et al. reported a species-dependent difference in TRPA1-mediated cold or L-menthol sensitivity, based on a single residue difference in S5 transmembrane domain of the TRPA1 receptor [59]. Rodent TRPA1 channels are activated by cold and not L-menthol, while human TRPA1 channels exhibit sensitivity to menthol but not cold [53,54,60]. Thus, it is intriguing to hypothesize that human TRPA1 could also contribute to L-menthol-induced UCP1-NST activation in WAT. Up to date, the putative relationship between TRPA1, L-menthol, and metabolic changes in BAT and WAT still remain to be firmly established.

Currently, the factors and the molecular mechanisms responsible for the recruitment of beige adipocytes in human classical WAT depots, a process known as 'browning', and the extent to which human beige cell development may occur remains to be fully understood. However, it is evident that the potential of enhancing the number and the UCP1 activation of beige adipocytes in WAT provides a promising method for increasing energy expenditure, regulating body weight, and preventing obesity and

obesity-associated diseases. Emerging evidence on the beneficial cooling effects of L-menthol on UCP1-mediated thermogenesis and enhanced number of beige adipocytes in BAT and WAT has prompted us to hypothesize that chronic dietary L-menthol treatment could induce WAT adaptations, resembling BAT-like activity, and overall improve whole-body metabolic health in obese and overweight individuals.

The hypothesis

Experimental studies in rodent models and human adipocytes indicate a potential role of L-menthol-induced TRPM8 activation in regulating core body temperature, UCP1-dependent NST, resting metabolic rate, energy expenditure, and glucose metabolism [40–42,61]. These findings implicate a putative contribution of L-menthol in BAT thermogenic capacity, a promising therapeutic regime to combat diet-induced obesity, not yet sufficiently studied in humans. Furthermore, the increased number of beige adipocytes in WAT and the UCP1 activation in response to physiological and pharmacologically-induced stimuli has attracted increased scientific attention as a novel therapeutic regime for combating obesity. It is, thus, intriguing to hypothesize that TRPM8 and TRPA1 activation, mediated by chronic dietary L-menthol treatment, could induce WAT adaptations that resemble BAT-like activity, and overall improve whole-body metabolic health in obese and overweight individuals (Fig. 1).

Evaluation of the hypothesis

Transient receptor potential (TRP) ion channels have been proposed to be the principal sensors of thermal stimuli in the peripheral nervous system [62,63]. TRPM8 is a temperature- and L-menthol-sensitive receptor, member of the TRPM subfamily [44,52,64], which appears to play an important role in thermoregulation [61]. A number of studies have suggested a physiological role of L-menthol-induced TRPM8 activation in UCP1-dependent thermogenesis in BAT and WAT, as listed in Table 1. Interestingly, long-term TRPM8 activation following dietary L-menthol was proposed to enhance BAT UCP1-dependent thermogenesis in wild type but not in TRM8-deficient mice, as evident by the enhanced UCP1 expression, energy expenditure, resting metabolic rate, and core body temperature only in wild type mice [41]. Further, chronic L-menthol dietary treatment prevented against obesity and improved glucose abnormal metabolism in mice on high-fat diet, with an increase in TRPM8-mediated UCP1 expression in BAT but not in WAT. On the other hand, L-menthol's cooling effects on UCP1-dependent thermogenesis were not observed in TRPM8-deficient mice, indicative of a physiological role of TRPM8 in the regulation of BAT activity and energy balance [41].

Evidence from human studies suggests a putative role of L-menthol-induced TRPM8 activation in WAT browning. Specifically, *in vitro* L-menthol stimulation of human white adipocytes induced TRPM8-dependent increased UCP1 expression, heat production, mitochondria number, mitochondrial membrane potential, and glucose uptake [42]. These findings indicate that TRPM8 stimulation could induce WAT adaptations to a BAT-like activity in response to L-menthol's cooling effects, a promising therapeutic target to regulate body weight and metabolic homeostasis. Our group showed that a single oral L-menthol administration generated minor effects on enhancing thermogenesis and metabolic rate in humans compared to a single skin L-menthol administration [37]. However, L-menthol absorption in the body was very high following the oral administration, thus it is logical to hypothesize that human metabolism and WAT thermogenic activity may increase following a long-term L-menthol oral administration. In this light, further studies are required to confirm whether daily L-menthol oral administration is promising candidate treatment for metabolic disease.

Another intriguing, yet controversial, issue is the role of TRPA1 in L-menthol-induced noxious cold transduction [55,56,58]. Gentry et al. reported that TRPM8-deficient mice showed L-menthol-induced cold hypersensitivity in response to intraplantar L-menthol injection, while TRPA1-null mice did not show this effect, supporting a role for TRPA1 in mediating L-menthol's effect on noxious cold sensation [55]. Conversely, Knowlton et al. demonstrated reduced neural responses to noxious cold stimuli as induced by intraplantar L-menthol injection in mice lacking TRPM8 channels, while TRPA1-deficient mice showed L-menthol-mediated neural activity comparable to wild-type mice [56]. These findings indicate that TRPA1 ion channel does not play a major role in noxious cold transduction. In humans, TRPA1-independent behavioral responses to L-menthol-induced cold hypersensitivity (cold allodynia and hyperalgesia), following a single topical L-menthol skin application, have been recently reported [58]. This discrepancy with the aforementioned results could be attributed to different experimental conditions, including species under study (human subjects or transgenic mice), L-menthol concentration and route of administration, as well as behavior, neural and thermal sensitivity assessment tests. Further investigation is thus necessary to elucidate the role for TRPA1 in noxious cold sensing following L-menthol administration.

To our knowledge, no human studies have been conducted so far to assess the impact of chronic L-menthol dietary treatment on UCP1-dependent thermogenesis in WAT and metabolism in obese and overweight individuals. To provide robust evidence for our L-menthol-induced WAT browning hypothesis, we are proposing a chronic L-menthol dietary intervention in physically inactive overweight and obese volunteers. The daily dose of orally administered L-menthol must remain within the tolerable upper intake levels to humans [65]. Participants will be also asked to assess thermal comfort and sensation following L-menthol oral administration, based on previously established scales [66]. To examine whether L-menthol-induced TRPM8 and TRPA1 activation can promote UCP1-dependent thermogenesis in subcutaneous WAT, histological and immunohistochemical analyses of WAT biopsies will be performed to detect the presence of beige adipocytes, as well as the protein expression of UCP1, TRPM8 and TRPA1. Further, to determine whether L-menthol-dependent chronic activation of TRPM8 and TRPA1 regulate WAT metabolic adaptation to BAT-like thermogenic activities, the expression, both at mRNA and protein levels, of key genes involved in the browning process and human metabolism will be assessed. The former genes include PPARγ, placenta-specific 8 (Plac8), retinaldehyde dehydrogenase 1 (Rald), and natriuretic peptide receptor A (NPRA), while the latter ones to be studied include leptin, adiponectin, fatty acid-binding protein 3 (FABP3), peroxisome proliferator-activated receptor-gamma coactivator 1 alpha (PGC-1α), peroxisome proliferator-activated receptor alpha (PPARα), phosphoglycerate kinase 1 (PGK1), solute carrier family 2, facilitated glucose transporter member 5 (SLC2A5), glucose transporter type 4 (GLUT4), and cytochrome C oxidase subunit IV isoform 1 (COX4i1).

The potential metabolic effects of L-menthol can be determined by partitional calorimetry, resting metabolic rate, glucose metabolism. Also, to identify the receptor(s) involved in the L-menthol-induced adaptive thermogenesis and increased metabolism, the expression of TRPM8 and TRPA1 may be assessed in white adipocytes both at mRNA and protein levels.

L-Menthol constitutes a novel activator of UCP1 in both BAT and WAT through the stimulation of TRPM8, independently of β-adrenergic signaling pathway, avoiding systemic side-effects

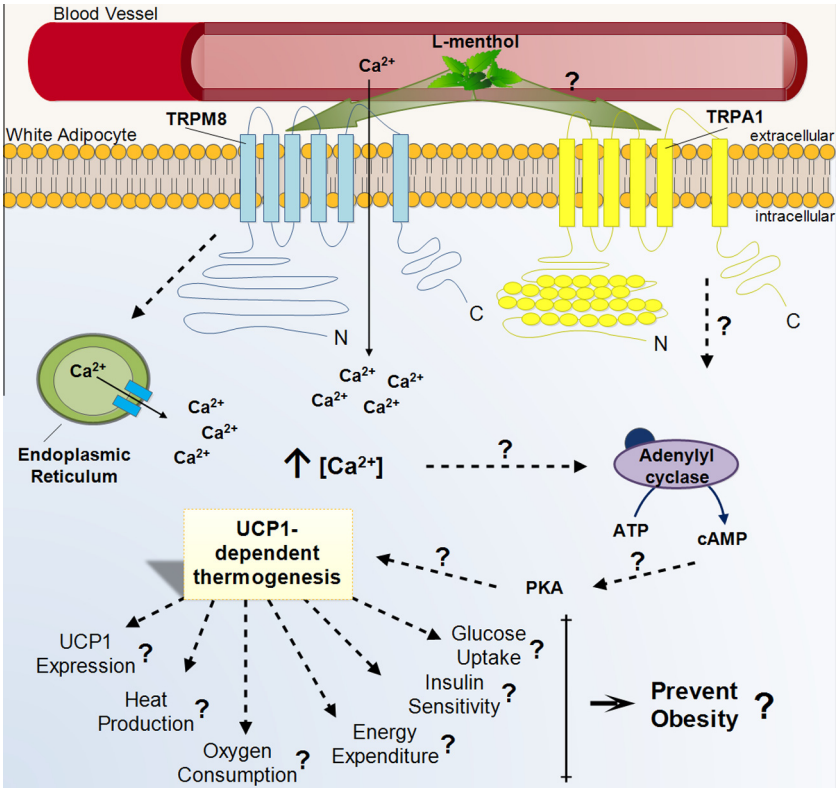

Fig. 1. Chronic L-menthol-induced WAT browning hypothesis model in humans. L-Menthol-induced browning of WAT hypothesis model is presented whereby chronic L-menthol dietary treatment can activate cold-sensitive TRPM8 receptors located on the cell membrane of human white adipocytes, thus inducing an intracellular [Ca^{2+}] increased and browning of WAT. Recent findings have proposed that L-menthol-mediated TRPM8 stimulation occurs in a Ca^{2+}-dependent protein kinase A (PKA) phosphorylation mechanism in brown adipocytes. A similar mechanism of L-menthol action in white adipocytes has yet to be determined. Enhanced adaptive UCP1-dependent thermogenesis in WAT can be associated with a number of thermogenic and metabolic processes, including increased UCP1 expression, heat production, oxygen consumption, energy expenditure, glucose uptake, and insulin sensitivity. Moreover, L-menthol has been also linked to the activation of the cold-gated ion channel TRPA1. Thus, similar results may occur whether TRPA1 is expressed on the cell membrane of human white adipocytes and exhibits sensitivity to L-menthol. The involvement of TRPA1 in L-menthol-induced human WAT browning remains undetermined. If our hypothesis was to be confirmed, L-menthol-induced metabolic adaptations from WAT to BAT-like activity might provide a safe and efficient therapeutic agent for the development of novel strategies for obesity prevention and/or treatment.

Table 1
Studies indicating the role of L-menthol-mediated TRPM8 activation in UCP1-dependent thermogenesis in BAT and WAT browning.

Study	Species/cellular models	L-Menthol administration	UCP1 thermogenesis in BAT	UCP1 thermogenesis in WAT	Molecular pathway
Tajino et al. [40]	*In vivo* TRPM8 (+/+) mice	Whole trunk skin application (10% L-menthol)	Yes	ND	ND
Ma et al. [41]	*In vitro* mature brown adipocytes isolated from C57BL/6J mice	Stimulation of cultured adipocytes (30, 100, 300 mM L-menthol)	Yes	No	Ca^{2+}-dependent PKA phosphorylation with no β-adrenergic-stimulation
	In vivo C57BL/6J mice	Chronic diet (chow diet + 0.5% L-menthol)	Yes	No	
Rossato et al.[42]	*In vitro* human differentiated white adipocytes	Stimulation of cultured adipocytes (100 μM L-menthol)	Yes	ND	ND

Note: UCP1 = uncoupling protein 1; BAT = brown adipose tissue; WAT = white adipose tissue; TRPM8 = transient receptor potential cation channel, subfamily M, member 8; ND = not determined, PKA = protein kinase A.

related to β-agonists. Further, it would be intriguing to investigate also the contribution of TRPA1 cold sensor to L-menthol's overall effect on BAT and WAT metabolic adaptations. Identifying putative factors and biological processes involved in L-menthol-induced browning effects on WAT could be critical to: (a) gain further knowledge on the role of this chemical cooling agent in metabolic homeostasis and its impact on human metabolic health, and (b) design novel and safe therapeutic strategies against obesity and its related co-morbidities.

Consequences of the hypothesis

An alarmingly high incidence of obesity has been recorded worldwide representing a serious public health concern with devastating impacts not only on life expectancy but also on health care economy [67,68]. Recent reports predict that the global market for drugs treating obesity will reach 5.3 million dollars by 2019 [3]. Despite the growing cost spent on anti-obesity agents, health professionals continue to express concern that the drugs fail to

provide lasting benefits for health and wellbeing, often accompanied with serious side effects [2,3]. The recent discovery of functional BAT in human adults [33,34,51,69] has attracted much research attention as a therapeutic agent for treating obesity.

In humans, cold exposure has long been used to stimulate BAT thermogenic capacity [70–73], which may occur through activation of sympathetic nervous system (SNS) [70,74]. Extensive animal studies showed that the β-adrenergic-dependent activation of SNS through agents such as PPARγ activators and thiazolidinediones induces BAT recruitment and/or enhances number of beige adipocytes in rodent WAT [23,25,75,76]. However, human trials of pharmaceutical agents stimulating BAT activity via β-adrenergic signaling pathways have led to unsuccessful results due to lack of efficacy or side effects accompanied the tested agents [48,77,78]. Experimental studies in rodents and humans provide evidence that the activation of β3-adrenergic receptors does not represent the only pathway to stimulate BAT-mediated NST [74,79]. Indeed, it seems that alternative pathways such as TRPM8-mediated UCP1 activation associated to Ca^{2+}-dependent PKA phosphorylation [41], or other tissues, including the skeletal muscle, can also stimulate NST, independently of SNS activation [74]. Thus, it is evident that novel factors activating UCP1-dependent thermogenesis in WAT depots as well as recruiting classical brown adipocytes may be identified.

Recent findings highlight the role of L-menthol-induced TRPM8 activation in UCP1-dependent thermogenesis, energy expenditure, glucose homeostasis, and protection against diet-induced obesity, promoting metabolic health [41]. The putative L-menthol-induced metabolic adaptation of WAT to a functional thermogenic BAT-like activity, independently of SNS stimulation, appears to be a promising therapeutic regime to regulate energy homeostasis and body weight, opening new avenues in treating individuals with obesity and its related co-morbidities. Therefore, investigating the impact of chronic L-menthol dietary treatment on energy balance and systemic metabolic homeostasis will shed light on the role of novel factors and mechanisms for the pathophysiology of obesity and the development of novel and safe therapeutic strategies.

Concluding remarks

The development of novel and safe therapeutic strategies for combating the global pandemic of obesity has become an urgent issue to be addressed. To our knowledge, the impact of TRPM8 and TRPA1 activation in response to chronic L-menthol dietary treatment on WAT thermogenic phenotype and metabolism in obese and overweight individuals remains unknown. It is hoped that, if our hypothesis was to be confirmed, the regulation of energy balance and metabolic homeostasis by L-menthol-dependent TRPM8 and/or TRPA1 activity could be therapeutically exploited to prevent and/or treat obesity and its related co-morbidities, promoting overall metabolic health.

Conflict of interest statement

The authors report no financial and personal relationships with other people or organisations that could inappropriately influence (bias) their work. The sponsors of the work had no role or contribution in the formulation of the hypothesis, the writing of the manuscript, or in the decision to submit the manuscript for publication.

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