ACTIVATION OF TRPM8 BY L-MENTHOL SKIN AND DIET TREATMENTS: EFFECT ON HUMAN METABOLISM AND THERMOREGULATION

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Introduction

Brown adipose tissue (BAT) in mammals functions to generate heat and maintain body temperature in cold environments via a process known as non-shivering thermogenesis. BAT activity has been positively correlated with resting metabolic rate, which suggests that it plays an important role in metabolism.

Transient receptor potential cation channel subfamily M member 8 (TRPM8) is the receptor for cold sensation and is located on the cell membrane of both brown adipocytes[1] and sensory neurons on the skin.[2] TRPM8 can be activated by both cold and exogenous chemical agonists, such as L-menthol. Indeed, both skin[3] and diet[1] L-menthol treatments evoke cold sensation and increase BAT activity. Moreover, frequent TRPM8 activation leads to a decrease in body weight gain which suggests that TRPM8 is involved in behavioral heat-gain responses and metabolism.

The activation of TRPM8 by L-menthol skin and diet treatments in animal models appear promising, but their efficacy in humans remains to be established. The aim of the present study was to examine, for the first time, the effect of dietary versus skin TRPM8 stimulation on metabolism and thermoregulation in adult male participants.

Materials and Methods

Nine healthy male volunteers were randomly assigned into either the L-menthol skin (ST; n=4) or the dietary (DT; n=5) treatment groups. Participants in both groups were treated with 10 mg/kg L-menthol [ST: gel; DT: capsule] and placebo (ST: water; DT: lactose capsule) in a random order on two different days. The L-menthol or water gel was applied on the SKIN group participants' neck, left arm and leg.

On the day prior to the first assessment, participants were asked to abstain from alcohol, coffee and passive smoke and to record their diet using a log and their physical activity using a pedometer. These data were used as a guide for them to follow on the day prior to the second assessment, and participants were asked to adhere to it as much as possible. Moreover, they were asked to fast for 12 hours prior to each assessment and wear the same clothes on both assessment days.

On the days of assessment fasted participants were asked to arrive to the laboratory at 8.30 and remained seated in a 24-25°C and 40-50% relative humidity environment. They were asked to drink water ad libitum as they were not allowed to consume food during data collection. Thereafter, body mass and percent body fat were measured and the various sensors were applied on the participants' body to record core temperature (Tc), heat storage (S), metabolic rate (M), and mean skin temperature (Tsk) at baseline, immediately following each treatment, and every hour thereafter for 7 hours. Tc was measured using a thin and flexible core temperature thermistor (Mon-A-Therm Core, Mallinkrodt Medical, St Louis, USA); body S and Tsk were measured non-invasively using partitional calorimetric techniques using probes placed on the forehead, abdomen, forearm, hand, quadriceps, shin and foot surfaces (Biomnic Ltd., Trikala, Greece); oxygen uptake and the

respiratory quotient were assessed using a portable gas analyser (Oxycon Mobile, CareFusion, San Diego, USA) and the exhaled gases from participants who were asked to breathe through a low resistance one-way valve attached to a face mask. Each assessment lasted 15 min.

Results

The placebo condition data were subtracted from the L-menthol condition data to eliminate the effect of diurnal variation. Kruskal–Wallis one-way ANOVA was used to assess the effect of each treatment on all variables showing a change across time for both ST and DT (Figure 1; $p<0.05$). Post hoc Mann-Whitney U tests showed that ST reduced Tsk within 2 hours and increased S, M, and Tc within 4 hours ($p<0.05$). A similar, albeit weaker, effect was observed following DT ($p<0.05$). Between-treatments comparisons showed that ST produced a strong vasoconstriction [evident by a greater reduction in Tsk ($p<0.05$)] that resulted in a greater increase in S, M, and Tc ($p<0.05$).

Conclusion

The present study examined the effect of DT versus ST TRPM8 stimulation on metabolism and thermoregulation in humans. We found that TRPM8 activation via L-menthol ST and DT result in cutaneous vasoconstriction and increased metabolic heat production. Moreover, the effects produced by ST appear to be stronger, as compared to those of DT.

Reference

1. Ma, S., et al., *Activation of the cold-sensing TRPM8 channel triggers UCP1-dependent thermogenesis and prevents obesity.* Journal of Molecular Cell Biology, 2012. **4**(2): p. 88-96.
2. Tajino, K., et al., *Cooling-Sensitive TRPM8 Is Thermostat of Skin Temperature against Cooling.* PLOSONE, 2011. **6** (3): p. e17504.
3. Tajino, K., et al., *Application of menthol to the skin of whole trunk in mice induces autonomic and behavioral heat-gain responses.* Am J Physiol Regul Integr Comp Physiol, 2007. **293**(5): p. R2128-35.

SUMMARY

Transient receptor potential melastatin 8 (TRPM8) is the receptor for cold sensation and is located on the cell membrane of brown adipocytes and sensory neurons on the skin, and can be activated by both cold and L-menthol. Indeed, both skin and dietary L-menthol treatments increase brown adipose tissue (BAT) activity and metabolism in mice, leading to reduced body weight. However, the effects of these treatments in humans remain incompletely understood. The aim of this study was to examine the effect of dietary versus skin TRPM8 stimulation on metabolism and thermoregulation in humans. Nine healthy male volunteers were randomly distributed into either L-menthol skin or dietary treatment groups. Participants in both groups were treated with 10 mg/kg L-menthol and placebo. Fasted participants remained seated in a 24-25°C and 40-50% relative humidity environment. Core temperature, heat storage, metabolic rate, and mean skin temperature were measured at baseline, immediately following each treatment, and every hour thereafter for 7 hours. Each assessment lasted 15 min. TRPM8 stimulation via L-menthol skin and dietary treatments result in cutaneous vasoconstriction and increased metabolic heat production. Moreover, the effects produced by skin treatment appear to be stronger, as compared to those of dietary treatment.

Figure legend

Figure 1. Median±SD change (L-menthol condition minus placebo condition) in mean skin temperature, core temperature, body heat storage, and metabolic rate in the skin and dietary treatment groups. Symbols are placed at the respective end of the SD bars: * = difference from baseline for the same treatment; ‡ = difference from the previous time-point for the same treatment; † = difference between treatments for the same time-point.