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Influence of repeated daily menthol exposure on human temperature regulation and perception

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HIGHLIGHTS

- A single skin surface exposure to menthol enhances cool sensations and heat storage.
- Heat storage by menthol is mediated by a vasoconstrictor response.
- Repeated menthol exposure causes an habituation of cool sensation but not heat storage.
- 0.2% Menthol activation of thermoreceptors equals a 0.5 °C fall in skin temperature.

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ABSTRACT

A single exposure to menthol can, depending on concentration, enhance both cool sensations and encourage body heat storage. This study tested whether there is an habituation in either response after repeated-daily exposures. Twenty-two participants were assigned to one of three spray groups: Control (CON; $n = 6$), 0.05% L-menthol ($M_{0.05\%}$; $n = 8$), and 0.2% L-menthol ($M_{0.2\%}$; $n = 8$). On Monday (20 °C, 50% rh) participants were sprayed with 100 mL of solution and undertook 40 min of cycling at 45% of their peak power (Ex_1), from Tuesday to Thursday (30 °C, 50% rh) they were sprayed twice daily whilst resting (R_1 to R_6), Friday was a repeat of Monday (Ex_2). Thermal sensation (TS), thermal comfort, perceived exertion, irritation, rectal and skin temperature (T_{sk}), skin blood flow (SkBF) and sweat rate were monitored. A two-way ANOVA (alpha = 0.05) compared responses from the beginning (Ex_1, R_1) and end (Ex_2, R_5) of the testing week. $M_{0.2\%}$ induced significantly ($P < 0.05$) cooler TS at the beginning of the week (Ex_1, R_1) compared to the end (Ex_2, R_5), indicating habituation of TS; this was not observed in $M_{0.05\%}$. No other perceptual or physiological responses habituated. 0.2% Menthol caused a heat storage response, mediated by vasoconstriction, at the beginning and end of the week, suggesting the habituation of TS occurred in a pathway specific to sensation. In summary, the cooling influence of 0.2% menthol habituates after repeated-daily exposures, but with no habituation in heat storage.

1. Introduction

Menthol ($C_{10}H_{20}O$; molecular weight, 156) is a cyclic terpene alcohol produced from mint oils or prepared synthetically [11]. It is found in many active forms, but the L isomer is most commonly used in commercial products because it produces the strongest cooling effects and is nontoxic to humans [12]. Both menthol and temperatures below 28 °C activate the transient receptor potential melastatin-eight (TRPM8) family of ion channels, which are embedded in the terminals of primary afferent nerve endings [23,27]. These thermo-sensitive neurons are thought to project to the somatosensory cortex, where temperature is perceived [10] and towards the hypothalamus, where body temperature is regulated [25]. In this way, menthol is thought to influence both human perception and temperature regulation.

A growing number of studies support the notion that menthol influences human temperature regulation; an elevation in deep body temperature can be observed after it is applied to the skin surface of resting and exercising humans [14,19,20], but it is not yet clear whether this heat storage response is driven by a reduction in skin blood flow at rest and/or a withdrawal of sudomotor function during exercise. The magnitude of body heat storage is probably influenced by the size of the surface area stimulated by menthol, and the dose of menthol used, although this requires clarification. Regardless, when a stimulus is strong enough to induce such a change in homeostasis, adaptation theory suggests that the physiological outcome (i.e. heat storage) resulting from the forcing function (i.e. menthol exposure) progressively reduces with repeated exposures (i.e. it habituates) [34]. This often follows from a shift in the deep body temperature threshold for vasoconstriction,

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vasodilation and sweating [34]. Therefore, repeated exposure to menthol may attenuate the heat storage response, perhaps through a withdrawal of vasoconstrictor tone, and an increase in skin blood flow; but this has not been tested.

Although there is a large body of research describing menthol's perceptual influence, most studies are psycho-physical in nature and assess perceptual responses to small applications of menthol on the forearm of resting participants. Far fewer studies have applied menthol to larger body surface areas, especially during exercise, so its influence on more global measures of perception, like thermal comfort or perceived exertion, is not well understood. The findings from these few studies are in general agreement with the psycho-physical literature in that menthol elicits cool sensations [1,2,14,30] and irritation [14,20] when applied to large body surface areas, but it is not clear whether menthol improves thermal comfort during rest or exercise [14], lowers perceived exertion during exercise [14,20], or improves exercise performance [1,2].

The influence of repeated menthol exposure on perception has received little attention, and those studies which have been conducted have separated menthol exposures (oral cavity) by minutes, not hours or days [8,9]. Given the paucity of research in this area, studies assessing cold adaptation in humans might give clues about the repeated influence of menthol on thermal sensation. A single exposure to menthol is comparable to a single cold exposure in that each gives rise to cool sensations. The distinction being that menthol achieves this by direct stimulation of the TRPM8 cold receptor [23,27] without changing skin temperature [14], whilst a cold exposure achieves this sensation by first lowering skin temperature, which increases the firing rates of cold receptors and brings about cool sensations. With this distinction in mind, repeated exposures to either cold air [6,21,22] or cold water [15,32] have been shown to cause an habituation of cool sensations and/or thermal discomfort. These findings suggest that repeated exposure to menthol may result in an habituation of thermal sensation, but this has not been tested.

The aim of this experiment was to examine whether the perceptual (i.e. cool sensations) and/or thermoregulatory (heat storage) responses that accompany menthol exposure undergo any habituation after repeated exposures. It was hypothesised that there would be no habituation in either response following repeated exposure to menthol.

2. Methods

This experiment received ethical approval from the BioSciences Research Ethics Committee at the University of Portsmouth.

2.1. Participants

Twenty-two participants volunteered for this study. They were assigned to their testing condition according to the order in which they were enrolled, such that participants one through four were assigned to CON, $M_{0.05\%}$, $M_{0.2\%}$ and CON, respectively. This pattern continued until groups were filled. Participant characteristics are shown in Table 1.

Table 1
Mean (SD) participant age, height and weight.

Spray group	Age	Weight (kg)	Height (m)
Water ($n = 6$)	21.6 (1.3)	78.8 (5.5)	1.80 (0.05)
0.05% Menthol ($n = 8$)	19.6 (0.9)	70.5 (6.5)	1.78 (0.08)
0.2% Menthol ($n = 8$)	19.7 (1.5)	76.7 (15.3)	1.82 (0.09)

There were no significant differences in participant mass or height between conditions ($P > 0.05$).

2.2. General design

Participants were divided into one of three groups; Control (CON, $n = 6$), 0.05% menthol ($M_{0.05\%}$, $n = 8$), and 0.2% menthol ($M_{0.2\%}$, $n = 8$). Prior to testing all participants completed a peak power-output test (PO_{peak}). Testing always began on a Monday with a pre-intervention exercise test (Ex_1) and ended on a Friday, with a post-intervention exercise test (Ex_2). On Tuesday, Wednesday and Thursday participants underwent two resting exposures each day ($R_{1–6}$), once in the morning and once in the afternoon, each separated by 3 h. The testing schedule is displayed in Table 2.

2.3. Exercise sessions (Ex_1 and Ex_2)

Prior to the testing week, participants performed an incremental test until exhaustion on a Monark cycle ergometer. Peak O_2 uptake ($\dot{V}O_{2peak}$) was defined as the highest O_2 uptake attained during the test, analysed retrospectively from the gas collected in Douglas bags, provided that the participant also attained either their age-predicted maximal heart rate during the test, or they reached a respiratory exchange ratio of greater than 1.1 [17].

Exercise testing was undertaken on a Monday (Ex_1) and Friday (Ex_2). Each participant entered the environmental chamber (20 °C; 60% relative humidity [rh]) wearing a long sleeve breathable shirt, shorts, training shoes and socks and remained seated at rest on a cycle ergometer for 10 min. Participants then underwent either 0.05% or 0.2% menthol spraying or water spraying, and remained seated for five additional minutes. At the 15th minute they began to cycle at 45% of their previously determined peak power ($PO_{45\%}$), until T_{re} rose by 0.5 °C. At this point the test was terminated. Expired gas was collected both at rest (6th minute) and again just prior to the termination of exercise. The timeline for Ex_1 and Ex_2 is displayed in Fig. 1.

To minimize potential deterioration in performance due to dehydration, participants were instructed to drink 500 mL of water before going to bed the previous evening before testing, and 500 mL 2 h before arrival at the laboratory. They were provided with tap water throughout the test. Participants arrived at the laboratory, were weighed naked (before and after testing) and equipped with a heart rate monitor (Team System Polar, UK). They then self-inserted a calibrated rectal thermistor (Grant Instruments (Cambridge) Ltd., Royston, UK) 15 cm beyond their anal sphincter. Eight calibrated skin thermistors (Grant Instruments, Cambridge, UK) were secured by single pieces of adhesive tape (Tegaderm™ Film, 3M, UK) at eight different sites (left chest, right scapula, left biceps, left dorsal hand, right vastus medialis, left hamstring, right tibialis anterior, right dorsal foot). Mean skin temperature ($\overline{T}_{msk}$) was calculated using an eight site weighted formula developed by Olesen [26], and mean body temperature ($\overline{T}_b$) was calculated using Burton's formula [7]. Participants were further instrumented with one ventilated sweat capsule (with a surface area of 0.787 cm^2, and flow rate of 60 $mL \cdot min^{-1}$) on the lower back (Q-Sweat Quantitative Sweat Measurement System, Model 1.0, WR Medical Electronics Co., MN, USA). Ventilated sweat capsule data were recorded four times a second and averaged by the minute. Upon entering the chamber, participants were instrumented with laser Doppler fibre optic probes to measure skin blood flow (SkBF) at the left index finger (Moor Instruments Ltd., Axminster, England, UK). Laser Doppler data were recorded once per second, but as flux data can be highly variable within and between

Table 2
Participant testing schedule.

	Mon	Tue	Wed	Thu	Fri
am	Ex_1	R_1	R_3	R_5	Ex_2
pm		R_2	R_4	R_6	