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Dilution Method of Menthol Solutions Affects Subsequent Perceptual Thermal Responses during Passive Heat Exposure in Non-Heat Acclimated Participants

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Abstract: Due to its volatility, the qualitative experience of menthol may be modulated by its preparation and combination with other compounds. One such method of preparation is dilution, with two dilution methods existing within the sport and exercise science literature, where menthol is used to impart feelings of oral cooling and improve thermal comfort and sensation during heat exposure. This study compared these two dilution methods; one using a solvent the other using temperature, via a randomized counterbalanced repeated measures design (n = 12; Height: 174.0 ± 8.5 cm Mass: 73.4 ± 13.3 kg Age: 28.7 ± 8.4 y; two exposures to each solution) to assess the effect of solution and heat exposure, upon thermal comfort, thermal sensation and associated physiological parameters in non-heat acclimated participants. Thermal comfort was significantly affected by solution (p = 0.041; η2 = 0.017) and time (p < 0.001; η2 = 0.228), whereas thermal sensation was significantly affected by time only (p = 0.012; η2 = 0.133), as was tympanic temperature (p < 0.001; η2 = 0.277). Small to moderate clear differences between solutions at matched time points were also observed. These trends and effects suggest that, depending upon the dilution method employed, the resultant perceptual effects are likely impacted; this also likely depends upon the timing of menthol administration within a heat exposure session.

Keywords: menthol; dilution; passive heat; thermal comfort; thermal sensation

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1. Introduction

Menthol is a naturally occurring flavor and fragrance molecule, which imparts sensations of cooling and freshness and is responsible for the characteristic taste(s) of the mint family. Different strains of mint possess differing quantities of menthol, and the related compound menthone [1]; likewise, differing isomers of menthol can have a 45-fold difference in their subjective qualities [2,3]. These qualitative experiences can occur even in individuals with impaired taste or smell, due to menthol's ability to act upon the trigeminal nerve and stimulate Transient Receptor Potential Melastatin-8 (TRP-M8) receptors [4,5].

Menthol is commonly used in oral hygiene products, confectionary and increasingly as a conduit for drug delivery within the digestive tract [6,7]. Menthol may also be used as a topical analgesic due to its ability to affect TRP channels, which are associated with both temperature perception and pain signaling/transduction [6,8]. This ability to alter perceived temperature is an important consideration when in hot environments and or in individuals with impaired abilities to thermoregulate [9], although these effects are likely responsible for menthol's appeal and seemingly ubiquitous use, too [10,11].

Within the last decade, menthol has emerged as a taste of interest with respect to sporting performance. Research has been completed in the areas of time to exhaustion [12–14], time trial performance [15–17] and repeated sprint activity [18,19]. A recent consensus statement [20] highlighted several areas where the research in menthol and performance is lacking, but also highlighted important safety considerations, concluding that if menthol is to be applied orally (e.g., as a mouth rinse or ingested), that solutions are prepared according to scientific literature and in a well-ventilated area [20]. Another related review pointed to the likely existence of individual sensory thresholds for menthol users and that this may be further altered by timing of administration [21], which has shown some variation in sport and exercise literature, but is yet to be examined methodically, that is, as an independent variable in orally applied menthol [22].

A factor not yet considered within the sport and exercise literature is that of the nature of dilution and menthol's subsequent volatility. This has been addressed to some extent within the food science and pharmaceutical literature, where menthol may be combined with other compounds that aim to alter the time course of menthol's characteristics across various preparations [23–25]. Two menthol dilution methodologies have been employed, within sport and exercise science. The more widely adopted methodology was initially devised by Mündel and Jones [26] and has subsequently been expanded upon, which consists of dissolving menthol crystals in deionized water to an experimental concentration of 0.01%, by heating the water to >40 °C [12,13], which mirrors menthol's melting point [27]. The less commonly used approach is to produce a stock solution, by dissolving menthol in ethanol and further diluting this to the desired experimental concentration, as per Best et al. [28]. This method takes advantage of menthol's chemical instability as an alcohol by dissolving it in another alcohol. Given the stark contrast in these dilution methodologies, and the potential scope for personalization of menthol-containing strategies for athletes or other menthol users, a deeper understanding of the perceptual characteristics each of these menthol solutions can alter, due to their dilution methodologies, is required.

Therefore, this paper aims to assess the physiological and perceptual responses to swilling two concentration matched menthol solutions, which are made by differing dilution procedures, during passive heat exposure.

2. Materials and Methods

2.1. Participants

Twelve (6 male, 6 female) non-heat acclimated participants (Table 1) volunteered to participate in the present study. None of the participants reported any illness or injuries that would impair or invalidate their participation, and none had travelled to a hot climate (≥28 °C; [29]) within the last 2 months prior to participation, so any confounding effects of partial heat acclimation were minimized. All participants had successfully completed a pre-screening health form and provided written informed consent prior to participation. Ethical approval was provided by the Human Ethics and Research Group of the Waikato Institute of Technology, Hamilton, New Zealand.

Table 1. Participant information (Mean ± Standard Deviation).

Metric	Female (*n* = 6)	Male (*n* = 6) [1]	Combined (*n* = 12)
Height (cm)	166.8 ± 1.7	181.2 ± 6.1	174.0 ± 8.5
Mass (kg)	63.6 ± 5.3	83.2 ± 11.5	73.4 ± 13.3
Age (y)	24.0 ± 6.9	33.3 ± 7.1	28.7 ± 8.4

[1] Please note that no between sex comparisons were made due to the relatively small, combined sample size.

2.2. Experimental Procedure

Participants visited the laboratory on two separate occasions for a duration of 60 min per visit. They were requested to wear lightweight exercise clothes, with an insulative value of approximately 0.45–0.61 CLO (e.g., T-shirt, shorts; [30,31]). All tests were carried out in a sealed environmental heat chamber and were conducted in hot environmental conditions (35 ± 0.2 °C, 40 ± 0.5% relative humidity), with no additional radiant heat load or convective cooling. During testing, participants wore a Polar heart rate monitor (RS400 Polar, Helsinki, Finland) around their torso. The monitors were connected to an iPad (10.2″, Apple, Cupertino, CA, USA) display using appropriate software (Polar Flow, Polar, Helsinki, Finland), with the heart rate recorded within the 10 s prior to each measurement interval. Participants also wore a tympanic thermistor, which was connected to a data logger (±0.5 °C; Squirrel SQ10 Data Logger, Grant Instruments, Cambridge, UK) to monitor their tympanic temperature throughout the testing period(s). Tympanic temperature was favored over rectal temperature, due to the less invasive nature of the measure and the passive nature of the heat exposure. Heart rate and tympanic temperature data were only visible to an experimenter who was situated outside of the chamber but could observe and communicate with participants through a window, to provide instruction and for health and safety reasons, as required.

During testing, participants were required to sit in the chamber for 10 min prior to any measures being recorded. During their first visit, participants would swill either solution A [28] or solution B [13] following 10 min of passive heat exposure (time point 1) and then swill the alternate solution 15 min later (time point 5). In their second visit, participants would swill the solutions in the opposing order to their first visit. Randomization for each of the participants’ solution order was conducted using a counter balanced approach. We have previously used a similar design to assess differing solutions applied during passive heat exposure [31], and the time-period between swills was devised following pilot data gathered during heat acclimation studies, which are yet to be published. Participants and facilitators were blinded to the type of solution and were informed of the effects of the different mouth rinse solutions. The participants swilled each menthol mouth rinse solution, for a duration of ~5 s, before expectorating the solution into a cup. Thermal comfort, thermal sensation, heart rate, tympanic temperature was recorded ~30 s prior, ~30 s post, ~5 min post, ~10 min post and ~15 min post swilling of the solutions (Time points 1–5 (first swill) and 5–9 (second swill)). In the latter half of the exposure, the 15 min post swill time point also acted as the 30 s prior time point (time point 5).

2.3. Perceptual Measurements

Thermal comfort (TC) was recorded using a 10-point scale, where −4 = “very uncomfortable”, and 4 = “very comfortable”. Thermal sensation (TS) has been recorded using a 9-point scale, where −4 = “very cold”, 0 = “neutral”, and 4 = “very hot” [32]. The TC scale contains values of −0 and +0 to numerically describe “just uncomfortable” and “just comfortable”, respectively [32]. These descriptions are encoded as −0.5 and +0.5, to ensure distinction between the thermal states and agreement with the direction of participants’ perceptions [31]. To help distinguish perceptions of TC and TS, participants were asked to imagine lying on a sun-lounger in a hot holiday destination. In this analogy, TC describes the degree of comfort experienced due to factors such as fluid availability, clothing material and skin wettedness and so forth, and their potential to influence this perception. TS describes the degree of perceived thermal load from environmental or metabolic heat sources, e.g., radiant heat, windspeed or exercise. Participants acknowledged the difference between the two sensations and were confident in their abilities to differentiate between the two perceptual measures [31]. Participants were also asked to report their preferred solution, at the completion of each visit. These data were collated and used to determine preference and assesses for a potential order effect.

2.4. Solution Formulation

Solution A was formulated by dissolving menthol crystals in ethanol, to produce a 5% menthol solution (50 g menthol per liter of ethanol). The menthol and ethanol mixture was then diluted using distilled water to a concentration of 0.1% as per Best et al. [28]. Solution B was formulated by dissolving menthol crystals in deionized water, which was heated to 40 °C with a concentration of 0.1% [13,14]. The solution was then cooled and stored in a refrigerator until use. Before use, both solutions were warmed to ~19.5 °C, that is, laboratory preparatory room temperature. The solutions were decanted into plastic glasses to a measure of 25 mL, where 0.5 mL of flavorless green food dye was also added and stirred through each solution until an even color was apparent. This was to ensure blinding of the solutions and enhance sensory expectancy, so as not to provide a potentially confounding visual stimulus compared to the solutions' taste [21,28].

2.5. Statistical Analysis

Descriptive data are presented in text and figures as means ± 90% confidence intervals (CI), unless otherwise stated. Statistical analysis was performed using JASP (JASP Team (2020). JASP (Version 0.13.1) [Computer Software]) and the *alpha* level set at $p \leq 0.05$. A two-way repeated measures ANOVA was used to test for within group effects across condition and time. Where sphericity could not be assumed, a Greenhouse–Geisser correction was applied. Simple main effects were also calculated with solution as the simple effect factor and time as a moderator factor. Eta squared is reported alongside analyses of variance and interpreted using the following thresholds: *Trivial* <0.1 *Small* 0.1–0.25 *Moderate* 0.25–0.37 *Large* >0.37. Post-hoc comparisons were performed, with a Bonferroni correction applied. Follow up paired-sample T tests were used to assess the alternative hypothesis that solutions were not equal at matched time points for TC and TS. Standardized mean differences (Cohen's *d*) were also calculated with accompanying 90% CI and interpreted as follows: *Trivial* <0.2 *Small* 0.2–0.6 *Moderate* 0.6–1.2 *Large* >1.2. These effects were considered clear, if 90% CI did not cross the threshold for small effects in both directions, where this did occur these effects were considered unclear [33,34]. No between sex comparisons were made due to the relatively small, combined sample size.

3. Results

3.1. Perceptual Measures

Thermal comfort (Figure 1) was significantly affected by solution ($F (1,11) = 5.385$; $p = 0.041$; $\eta2 = 0.017$) and time ($F (2.78,30.58) = 13.836$; $p < 0.001$; $\eta2 = 0.228$), but there was no significant solution x time interaction ($F (2.56,28.12) = 0.882$; $p = 0.476$; $\eta2 = 0.037$). Qualitatively, solution A reported lower thermal comfort scores than solution B at eight of the nine time points, differing significantly, via simple main effects, at time points 3 ($p = 0.027$) and 6 ($p = 0.040$). Time points 1, 3, 4 and 6 also demonstrated effect sizes that were small to moderate, with accompanying 90% confidence intervals that allowed for their interpretation as clear effects (Table 2). All other time points were non-significant and unclear.

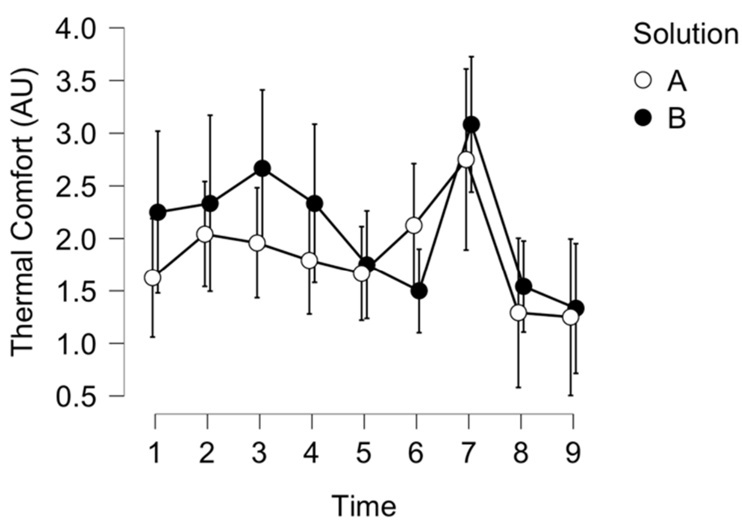

Figure 1. Participant ratings of thermal comfort following swilling solution A and B.

Table 2. Between solution differences at matched time points, including statistical significance, estimates of magnitude and uncertainty [2].

Parameter	Comparison (A–B) Timepoint	Raw Difference	*p* Value	Cohen's *d*	90 % C.I.	
					Lower	Upper
Thermal Comfort (AU)	**1**	**−0.625**	**0.119**	**−0.488**	**−0.982**	**0.026**
	2	−0.292	0.492	−0.205	−0.681	0.279
	3	**−0.708**	**0.027**	**−0.735**	**−1.259**	**−0.182**
	4	**−0.542**	**0.121**	**−0.485**	**−0.978**	**0.029**
	5	−0.083	0.782	−0.082	−0.556	0.396
	6	**0.625**	**0.040**	**0.670**	**0.128**	**1.186**
	7	−0.333	0.666	−0.128	−0.602	0.352
	8	−0.250	0.551	−0.178	−0.653	0.305
	9	−0.083	0.876	−0.04	−0.520	0.430
Thermal Sensation (AU)	1	0.167	0.438	0.232	−0.255	0.709
	2	0.083	0.674	0.125	−0.355	0.599
	3	−0.167	0.504	−0.200	−0.675	0.285
	4	**−0.500**	**0.082**	**−0.553**	**−1.053**	**−0.029**
	5	0.000	1.000	0.000	−0.475	0.475
	6	−0.167	0.504	−0.200	−0.675	0.285
	7	0.000	1.000	0.000	−0.475	0.475
	8	**0.417**	**0.241**	**0.358**	**−0.141**	**0.841**
	9	0.375	0.473	0.215	−0.271	0.691
Heart Rate (bpm)	1	−2.000	0.487	−0.207	−0.683	0.277
	2	−0.583	0.790	−0.079	−0.553	0.398
	3	−0.500	0.865	−0.050	−0.524	0.426
	4	**4.000**	**0.193**	**0.400**	**−0.103**	**0.886**
	5	0.833	0.754	0.093	−0.385	0.567
	6	**3.167**	**0.179**	**0.415**	**−0.090**	**0.902**
	7	**−3.833**	**0.141**	**−0.458**	**−0.949**	**0.053**
	8	−1.083	0.616	−0.149	−0.623	0.332
	9	1.000	0.558	0.174	−0.308	0.649
Tympanic temperature (°C)	**1**	**−0.200**	**0.235**	**−0.362**	**−0.846**	**0.137**
	2	−0.162	0.367	−0.271	−0.749	0.219
	3	**−0.183**	**0.121**	**−0.485**	**−0.979**	**0.029**
	4	**−0.088**	**0.012**	**−0.873**	**−1.419**	**−0.293**
	5	**−0.054**	**0.178**	**−0.415**	**−0.902**	**0.090**
	6	**0.125**	**0.003**	**1.118**	**0.486**	**1.712**
	7	**0.054**	**0.262**	**0.341**	**−0.156**	**0.823**
	8	0.046	0.596	0.157	−0.324	0.632
	9	**0.088**	**0.154**	**0.442**	**−0.066**	**0.932**

[2] Where confidence intervals do not overlap small effects in both directions, they are considered clear and marked in **bold**. We encourage readers to interpret these effects alongside their accompanying *p* values.

Thermal sensation (Figure 2) was significantly influenced by time (F (3.72,40.92) = 3.767; $p = 0.012$; η2 = 0.133), but not solution (F (1,11) = 0.035; $p = 0.854$; η2 = 2.545×10^{-4}), with no significant solution x time interaction (F (2.70,29.71) = 1.028; $p = 0.421$; η2 = 0.034). Qualitatively, solution A induced and retained improved thermal sensation for longer than solution B, earlier in the heat exposure, but this effect dissipated over time. This is supported by a clear *small* effect at time point 4 ($p = 0.082$; Table 1), before returning to non-significant differences and overlapping confidence intervals. Time points 4 and 8 also demonstrated effect sizes that were small to moderate, with accompanying 90%

confidence intervals that allowed for their interpretation as clear effects (Table 2). All other time points were non-significant and unclear.

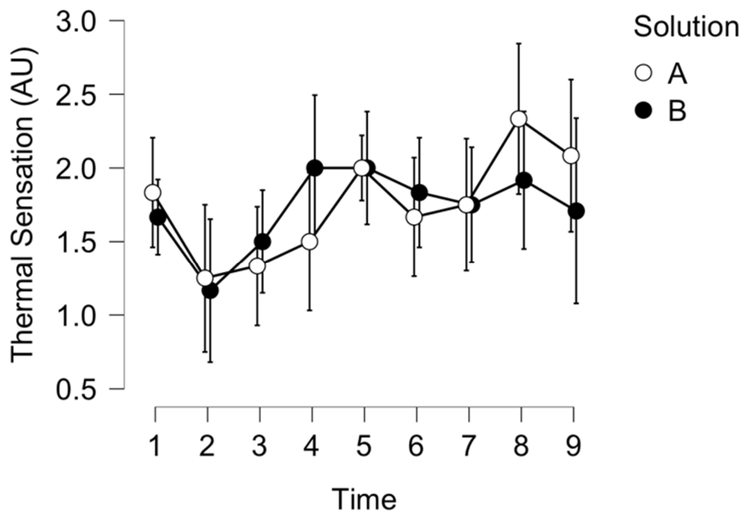

Figure 2. Participant ratings of thermal sensation following swilling solution A and B.

3.2. Physiological Measures

There were no significant effects for solution (F (1,11) = 0.024; $p = 0.879$; η2 = 8.802 × 10^{-5}) or time (F (8,88) = 1.254; $p = 0.278$; η2 = 0.051), nor a significant solution x time interaction for heart rate (F (3.02,33.21) = 0.984; $p = 0.413$; η2 = 0.038) (Figure 3). There was a significant effect of time (F (4.15,45.70) = 24.394; $p < 0.001$; η2 = 0.277), but not solution (F (1,11) = 0.760; $p = 0.402$; η2 = 0.009) nor solution x time interaction (F (1.91,21.02) = 1.978; $p = 0.165$; η2 = 0.070) for tympanic temperature (Figure 4).

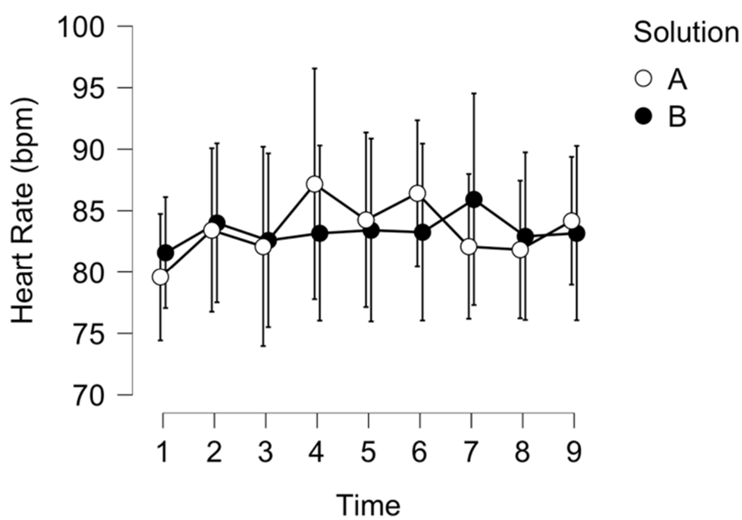

Figure 3. Participant heart rate responses following swilling solution A and B.

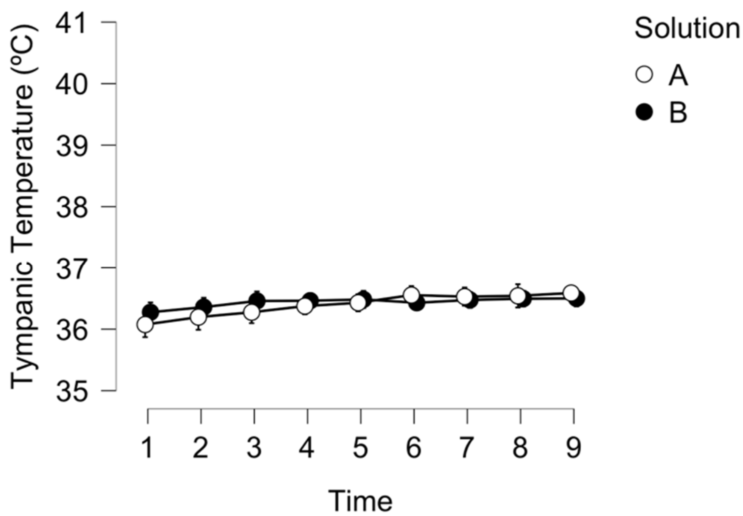

Figure 4. Participant tympanic temperature following swilling solution A and B.

3.3. Solution Preference

Seven out of 12 participants reported preferring Solution A (58.3%), with the remaining five participants reporting being unsure (41.7%). Out of a possible 24 trials (i.e., 2 × 12), 17 out of 24 participants (70.8%) reported preferring the first solution they swilled during either trial.

4. Discussion

This paper aimed to assess the perceptual and physiological effects of two menthol solutions created by way of differing dilution methods, during passive heat exposure. The two solutions showed distinct profiles for perceptual measures as time progressed, especially following swilling. Despite these idiosyncrasies, the majority of participants reported preferring solution A, with the remainder reporting being unsure.

Thermal comfort curves differed between solutions (Figure 2), with each solution also differing dependent upon timing of administration, and being sensitive to test duration (i.e., significant effect of time). Solution A induced an initially greater improvement in thermal comfort (delta between time points 1 and 2), but ratings of thermal comfort still remained lower than solution B, until time point 6, which coincided with the second administration of a swill, before again being rated lower. Solution B was rated higher for thermal comfort by a clear *small–moderate* extent at 5 (ES: 0.74; 90% CI: 0.18 to 1.26; p = 0.027) and 10 min (ES: 0.49; 90% CI: 0.03 to 0.98; p = 0.121) post-swilling, early in the exposure, corresponding to time points 3 and 4. This suggests that if participants are less thermally comfortable, solution A may induce an initially greater response, but the effects of solution B may be more likely to enhance or retain an already thermally comfortable state. Qualitatively, participants reported solution B as acting more quickly and being more pungent/potent than solution A, which was described as smoother; we propose that this is most likely due to the use of another solvent, beyond water, in the dilution process.

In contrast to thermal comfort responses, thermal sensation increased over time and, whilst these effects were attenuated by menthol administration, regardless of solution, to some extent. Participants felt hotter on average by the end of the exposure, but confidence interval breadth suggests a high degree of inter–individual variability (Figure 2). Earlier in the exposure, solution A induced a greater reduction in thermal sensation, which was then retained for longer (e.g., time point 4; ES: −0.55; 90% CI: −1.05 to −0.03; p = 0.082), compared to solution B. This was not the case later in the exposure as, whilst solution A still improves thermal sensation between points 5 and 6 (Figure 2), between time points 7 and 8 there is a marked increase, and solutions differ by 0.4 units (ES: 0.36; 90% CI: -.14 to

0.84; $p = 0.241$). We have previously considered a difference of ± 0.5 units as the smallest worthwhile change in thermal perception [22], as it represents a half-way point between verbal anchors, suggesting that the differences seen later in the bout, may be of practical importance in some individuals – possibly based upon individual sensory thresholds. Another phenomenon related to thermal sensation, was the reduction in thermal sensation observed between time points 8 and 9 in both solutions. This may be due to the participants' knowledge that the exposure was ending, and an impending relief effect lowering participants' perceptions. This could have been countered by incorporating a further 5 min of non-experimental sitting, post-data collection, but we have not experienced this phenomenon in other trials, nor is it well-documented in the heat-exposure literature.

Physiological parameters remained relatively stable throughout the heat exposure, independent of solution administration (Figures 3 and 4). Specifically, heart rate remained within 2 bpm between solutions, when matched for time point, with a mean difference of <5 bpm between time points across the entire exposure(s). Similarly, tympanic temperature differed by <0.4 °C across the entire exposure, and whilst post-hoc testing showed significant effects between time points even with the conservative Bonferroni correction (p values ranged from $p < 0.001$ to = 1.00), all participants' temperatures remained <37 °C, so were at low risk for heat related illness, with any differences likely not of practical importance. This supports previous work from our group, which has demonstrated that carbohydrate or water swilling may induce changes in heart rate during passive heat exposure, but menthol mouth swilling does not [31]. We acknowledge that both heart rate and tympanic temperature were significantly influenced by time, but the practical significance of these statistical conclusions are questionable, given their physiologically small magnitude, and the low-risk nature of the heat exposure within this study.

The preference findings are also interesting, and likely of most importance for practitioners, as they suggest that whilst solution A is preferred, there are a similar number of people (5/12) who may not be able to distinguish between the dilution characteristics of menthol solutions when matched for concentration. Despite individuals with heightened sensory abilities being well documented for a variety of tastes (e.g., super-tasters; [35,36]), individuals with lesser sensory abilities pose an important consideration for those practitioners looking to implement menthol in sporting or similarly thermally challenging occupational settings, as their responses to menthol may possibly be either blunted or less predictable.

Variation is to be expected within and between populations, due to variations in TRPM8 phenotype across latitudinal genotypes [37]. The present sample, whilst not genetically homogenous, were exposed to the same environmental constraints outside of the study and thus despite the potential for genetic variation, we feel that (the lack of) seasonal heat acclimation in the present sample would have exerted a stronger effect upon menthol sensitivity during passive heat exposure, than chance alone, thus the five individuals who were not able to distinguish between solutions may be considered low menthol responders—despite showing similar physiological responses to the rest of the group. This too is to be expected, as even those who have diminished, or are void of a, sense of taste or smell describe being able to experience menthol and menthol containing products [4,22].

Limitations and Future Research Directions

A potential limitation of the present work was that we chose to use passive, as opposed to active, heat exposure. Passive exposure serves as an experimental precursor to active exposure, possibly of differing exercise intensities and or simulated wind-speeds, to further manipulate heat storage within exercising individuals. Future research should aim to assess the independent and combined effects of exercise and environmental factors (beyond temperature) upon pertinent variables when menthol mouth-rinsing. These factors may further interact with the authors' chosen dilution method in an as yet unknown fashion, as they may enhance or decrease the (subjective) experiences of our participants reported above.

Another possible limitation is the use of a within and between session crossover design, where participants trial each solution in each session, in a counterbalanced order. This design is time and resource efficient, as it reduces session visits and ensures differing heat exposures (short and long) can be assessed; perceptual variables show a return to baseline or below (Figures 2 and 3); however, the impact upon physiological variables cannot be mitigated, but could likely be used as covariates in appropriately powered trials. This is likely more pertinent during active heat exposure, that is, when participating in exercise or increasing thermal load via increased clothing as the rate of change in physiological variables is likely to be greater, due to increased hyperthermia, than in the present study.

Future research should consider identifying those that respond most positively, most negatively and have diminished capacity for sensing menthol when applied to the oral cavity at rest (and during exercise) and assessing the reliability of these responses. Genotyping of this range of menthol sensitive individuals may also be of value, given the already documented latitudinal variation in TRPM8, mentioned above [37]. These findings may have clinical implications for migraine, allodynia and cold-sensitivity [38–40]. We acknowledge that these responses may be highly individual, and researchers who are interested in investigating individual responses to menthol administration are recommended to design their studies with an inherent degree of inter–individual variation and measurement error in mind; for further information please consult Atkinson and Batterham [41].

5. Conclusions

Based on the above, if rapid improvements in thermal comfort are required, solution A (0.1% menthol; diluted with ethanol and water) is recommended; whereas if lesser but more sustained effects are sought, choose solution B (0.1% menthol; heated to 40 °C following dilution in water). The inverse is to be considered for improvements in thermal sensation; however, thermal sensation effects are more likely to dissipate over time. These effects may be further modulated by the timing of menthol administration, for example, early or late in the heat exposure bout. Regardless of these general recommendations, if menthol is to be used during heat exposure, the personalization of a solution is encouraged, especially in those who may seem less sensitive to menthol.

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Institutional Review Board Statement: All participants gave their informed consent for inclusion before they participated in the study. The study was conducted in accordance with the Declaration of Helsinki, and the protocol was approved by the Ethics Committee of Waikato Institute of Technology Human Ethics in Research Group (CSSHPRN01072019)

Informed Consent Statement: Informed consent was obtained from all subjects involved in the study

Data Availability Statement: The data presented in this study are available on request from the corresponding author. The data are not publicly available due to institutional policies regarding data obtained during student-led research.

Conflicts of Interest: The authors declare no conflicts of interest.

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