Article

Perceptual and Physiological Responses to Carbohydrate and Menthol Mouth-Swilling Solutions

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Abstract: Carbohydrate and menthol mouth-swilling have been used to enhance exercise performance in the heat. However, these strategies differ in mechanism and subjective experience. Participants (n=12) sat for 60 min in hot conditions (35°C; 15±2%), following a 15 min control period, participants undertook three 15 min testing blocks. A randomised swill (Carbohydrate; Menthol; Water) was administered per testing block (one swill every three minutes within each block). Heart rate, tympanic temperature, thermal comfort, thermal sensation and thirst were recorded every three minutes. Data were analysed by ANOVA, with carbohydrate intake controlled for via ANCOVA. Small elevations in heart rate were observed after carbohydrate (ES: 0.22 ± 90% CI: -0.09 to 0.52) and water swilling (0.26; -0.04 to 0.54). Menthol showed small improvements in thermal comfort relative to carbohydrate (-0.33; -0.63 to 0.03) and water (-0.40; -0.70 to -0.10), and induced moderate reductions in thermal sensation (-0.71; -1.01 to -0.40 and -0.66; -0.97 to -0.35, respectively). Menthol reduced thirst by a small to moderate extent. These effects persisted when controlling for dietary carbohydrate intake. Carbohydrate and water may elevate HR, whereas menthol elicits small improvements in thermal comfort, moderately improves thermal sensation and may mitigate thirst; these effects persist when dietary carbohydrate intake is controlled for.

Keywords: Carbohydrate; Menthol; Thermal Comfort; Thermal Sensation; Thirst; Water

1. Introduction

Mouth swilling is an increasingly popular ergogenic strategy employed by athletes over short to moderate exercise durations [1-6], during nutrient restricted states [7-10] and may be appropriate during times of potential gastrointestinal distress [11,12]. Multiple nutritional stimuli are swilled, with each conferring a different ergogenic effect [5,6,13,14] and magnitude thereof, most likely due to affecting differing sensory pathways. More precisely, as the nutritional stimulus being swilled changes the cells targeted by and exposed to the swill also alter and the resultant ergogenic effect is the product of these interactions [15]. Nutritional stimuli that are swilled either directly or indirectly affect the brain and bypass the digestive system, so reducing energy intake, and the risk of gastrointestinal distress, which is frequently reported during prolonged endurance activity [11,12,16,17] when caffeine [18] and or carbohydrate are ingested [11,19,20].

Carbohydrate (CHO) is considered the gold-standard ergogenic mouth swilling strategy, with a wealth of literature documenting its efficacy in contrasting environments [21], nutritional states [22] and sports [23-25]. Mechanistically, CHO is shown to activate areas of the brain that are associated with behavioural, cognitive and emotional responses [3], with areas associated with motivation and motor control also stimulated [26]. Activation of these higher order and efferent regions of the brain, as supported by fMRI, provide strong explanation(s) for CHO mouth swilling's ergogenic effects to date, but CHO is also shown to affect receptors within the oral cavity [27], as are caffeine [28-30] and menthol [13,31,32].

Menthol is considered a trigeminal afferent, stimulating the trigeminal nerve [33,34] and associated TRPM8 receptors [35-37]. The trigeminal network innervates the ophthalmic, mandibular and maxillary regions, with menthol and other cold stimuli particularly affecting the maxillary region due to its proximity to the nasal and oral cavities [38], stimuli have almost direct access to nerve endings due to the lack of squamous epithelia covering mucosa [38]. Indeed, it is stimulation of this collection of nerves that is responsible for sphenopalatine ganglioneuralgia, or 'brain freeze' [39]. This potent response highlights the sensitivity and role within cold temperature detection of TRPM8 receptors, and is likely enhanced due to the thinness of the membrane within the oral cavity [13,40]. Sports scientists have recently begun to investigate menthol mouth swilling as a strategy to ameliorate feelings of thermal comfort (TC) and sensation (TS) and exercise performance in hot conditions [13,41-44], but menthol may also confer hedonic and thirst attenuating responses that are yet to be investigated by sports scientists, and may be of use to other high performing professions e.g. firefighters or the military. These effects may in part be confounded by exercise due to effects such as increased ventilation [45,46] and decreased salivary flow rate [47]; but may be enhanced in hot conditions due to menthol's stimulatory effect upon TRPM8 receptors and the long-documented preference for application of cold stimuli to the tongue and oral-cavity under thermally challenging circumstances [31,48,49].

Assessment of the effect of differing mouth swilling strategies on physiological and subjective measures, under resting conditions may further elucidate mechanistic differences between nutritional stimuli applied to the oral cavity, without confounding effects brought about by exercise. Therefore, the aim of this investigation was to quantify physiological and subjective responses to CHO and menthol mouth swilling, at rest under thermally challenging conditions.

2. Materials and Methods

Ethical approval for this investigation was granted by the Teesside University School of Social Sciences, Business and Law ethics board.

2.1 Participants

Twelve participants (11males and one post-menopausal female) took part in this investigation. Participants had a mean age of 31.45 years (± 90% CI: 26.88 to 36.02 years), and were 177.38 cm (172.99 cm to 181.76 cm) tall, weighing on average 75.87 kg (70.91 kg to 80.82 kg). Participants were made aware of the aim, procedure and risks of the study prior to providing informed written consent; participants were non-heat acclimated and were screened for medical issues that may have affected their ability to participate in the investigation prior to commencement.

2.2 Mouth swilling solutions

Solutions were prepared outside of the environmental chamber, under thermoneutral conditions (22 ± 0.5 ºc), and administered in 25ml aliquots. Five swills took place per swill condition; swills lasted ~10 seconds prior to expectoration, with swilling order randomised via a Latin square design, using a customised spreadsheet [50].

Menthol (MEN) was prepared to a 0.1% concentration, as per [45]. Briefly, a 5% menthol ethanol-based stock solution was diluted to the desired concentration using distilled water. The carbohydrate

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mouth-swill (CHO) was prepared from unflavoured Maltodextrin (MyProtein, Northwich, United Kingdom) was diluted to 10% concentration (100g.L^{-1}). Water acted as the placebo swill and a control period of no swilling was incorporated into each testing session (see Procedure). Quasi-single blinding was employed, whereby solutions were matched to be colourless, but were not matched for taste.

2.3 Procedure

This investigation employed a crossover design with testing order of treatments assigned via Latin square; two participants completed each possible trial order. All testing took place within an environmental chamber set at 35ºc and 10% humidity, with outcome measures assessed at three minute intervals.

Testing began with 15 minutes of passive sitting, during which time outcome data were recorded by the researcher, but no swilling took place. Following this control period (CON), participants swilled their assigned swill at three minute intervals; five swills were completed per condition. Once the final swill was completed and outcome measures recorded, participants exited the chamber. The experimental procedure is pictorially represented in Figure 1.

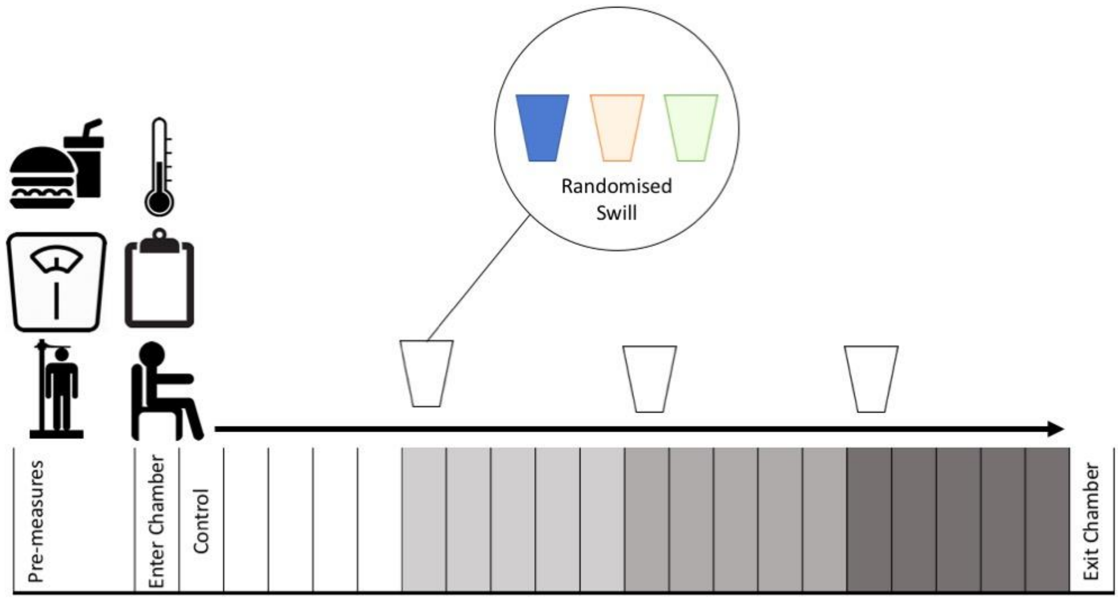

Figure 1 Experimental Procedure: Black vertical lines from entering the chamber represent three minute intervals; at each interval, physiological and subjective measures were assessed as indicated by the clipboard and thermometer. Blank vessels represent the commencement of a new randomised mouth swill; this is supported with a change in shade of time interval cells. Randomised swills included water (blue), carbohydrate (orange) and menthol (green).

2.4 Outcome Measures

2.4.1 Physiological measures

Tympanic temperature (T_{tym}) was assessed using a tympanic thermometer (±0.05°c; Squirrel SQ10 Data Logger, Grant Instruments, Cambridge, United Kingdom), with measures taken from the ear contralateral to participants' dominant hand. Temperature was assessed prior to the administration of mouth swills, so any potential increase in temperature caused by swilling or local irritation would be mitigated. Heart rate (HR) values were recorded 10 seconds prior to each three-minute interval via telemetry (Polar RS400; Polar, Helsinki, Finland).

2.4.2 Subjective measures

Subjective measures were assessed using validated rating scales, with accompanying descriptors. Thirst was assessed via a 10-point scale [51], ranging from 'Not at all thirsty' to 'Extremely thirsty'. Zhang *et al.*'s scales of TC and TS were used to assess these qualities [52]. Both scales range from -4 to +4, with polar descriptors of Very Uncomfortable: Very Comfortable, and Very Cold: Very Hot, respectively. As a point of difference, the TC scale contains values of -0 and +0 to numerically describe just uncomfortable and just comfortable, respectively [52].

2.4.3 Carbohydrate intake

Carbohydrate intake (g) was calculated for each participant from a written 24-h food recall, using specialist software (Nutritics, 2018). This was subsequently used as a covariate in statistical analyses.

2.5 Statistical Analyses

Normality was assessed for using Skewness and Kurtosis tests (acceptable Z scores not exceeding +1 or -1). Initially, a two-way multiple analysis of variance (MANOVA) was conducted to determine differences between time and beverage type on physiological and subjective outcome measures. Secondly, a two-way multiple analysis of covariance (MANCOVA) was conducted to determine differences between time and beverage type on outcome measures when controlling for carbohydrate intake. Significance was set at an *a priori* alpha level of $p<0.05$. Effect sizes are reported as standardised mean differences ± 90% confidence intervals (C.I.), with accompanying descriptors [53]. Ninety percent (90%) C.I. are used to differentiate between any observed significant results and the likely range in which true differences may occur [53,54], as opposed to another method of expressing a significant result.

3. Results

3.1 Carbohydrate Intake

Mean carbohydrate intake for participants was 69.92g (± 90% CI: 55.89g to 83.94g), with an absolute range of 203g. These values are considered low in relation to participants' bodyweight [55], hence being stated in absolute as opposed to relative values.

3.2 MANOVA

There was a statistically significant interaction effect between time and mouth-swill type on combined dependent variables, $F_{(20,750.507)} = 6.168$, $p<0.0001$; Wilks' $\Lambda = 0.604$. This interaction effect is attributed to the significant effect of mouth-swill type on combined dependent variables, $F_{(10,452)} = 2.419$, $p=0.008$; Wilks' $\Lambda = 0.901$, whereas time demonstrated a non-significant effect on combined dependent variables, $F_{(10,452)} = 1.090$, $p=0.368$. Pairwise comparisons were used to identify significant effects upon dependent variables between mouth-swill types.

3.2.1 Physiological outcomes

Small (ES: 0.26; -0.04 to 0.54), significant differences in HR were observed between CON and water ($p=0.018$). Small (0.22; -0.09 to 0.52) non-significant differences in HR were also recorded

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between CON and CHO. All other HR comparisons were non-significant and trivial. Tympanic temperature during the CON period was significantly different to all other conditions (values; $p<0.001$), displaying *Moderate* effects (MEN: 0.89; 0.56 to 1.19. CHO: 0.91; 0.59 to 1.22. Water: 0.88; 0.56 to 1.19) Tympanic temperature displayed *Trivial*, non-significant effects across all other comparisons i.e. between swills.

3.2.2 Subjective outcomes

Thermal comfort was significantly greater ($p<0.002$) in CON compared to water swilling (*Small*; -0.39; -0.69 to -0.09). Despite not reaching statistical significance ($p<0.062$) there were *small* (-0.32; -0.63 to -0.02) differences in TC between CON and CHO conditions too, whereas MEN was only *trivially* different to CON (-0.01; -0.29 to 0.31). Menthol improved TC by a *small* magnitude compared to CHO (-0.33; -0.63 to -0.03) and water (-0.40; -0.70 to -0.10). Carbohydrate and water swilling were *trivially* different (0.10; -0.20 to 0.40) with respect to TC. Thermal sensation was *moderately* and significantly reduced by MEN in comparison to CON, CHO and water (see Table 1). All other comparisons were *trivially* and non-significantly different. Thirst was significantly greater in CON compared to MEN ($p<0.001$) and water ($p<0.011$), but not CHO ($p=0.134$); magnitudes of swilling's ability to improve thirst varied from *small* to *moderate* (see Table 1). Menthol lowered thirst significantly in comparison to CON and CHO, but not water; these differences were *moderate* in nature. Further contrasts are outlined in Table 1.

3.3 MANCOVA

Upon controlling for carbohydrate intake, there was a significant effect of mouth-swill type upon combined dependent variables $F_{(10,298)} = 1.913$, $p<0.043$; Wilks' $\Lambda = 0.883$. Between subjects' comparisons revealed significant differences for TS ($p<0.004$) and thirst ($p<0.048$). Heart rate ($p<0.598$) and T_{tym} ($p<0.634$) responses were not significantly different between conditions when carbohydrate intake was controlled for, nor were differences in TC ($p<0.151$).

Despite non-significant differences in TC ($p<0.151$), when compared to both CHO (0.29; -0.09 to 0.65) and water (0.41; 0.04 to 0.78), MEN improved TC to a *small* extent. Pairwise comparisons demonstrated MEN significantly reduced TS in comparison to CHO (-0.36 units; $p<0.004$) and water (-0.37 units; $p<0.008$), exerting *moderate* (-0.63; -1.00 to -0.25) and *small* (-0.38; -0.75 to -0.01) effects respectively. Similar reductions in thirst were also observed, however in contrast to the unadjusted model MEN displayed a *moderate* (-0.67; -1.04 to -0.29) standardised mean difference in thirst compared to water of -0.69 units ($p<0.023$), with a *small* (-0.46; -0.83 to -0.08) difference in comparison to CHO (-0.49 units; $p<0.068$).

Table 1. Differences between mouth-swilling conditions for thermal sensation and thirst; significant effects are denoted by an asterisk (*). ES: Effect Size; C.I.: Confidence Interval

Variable	Swill	Comparison	*p* value	ES; 90% C.I.	Descriptor
Thermal Sensation	Control	Menthol	0.001*	0.66; 0.34 to 0.96	*Moderate*
		Carbohydrate	0.835	0.04; -0.26 to 0.34	*Trivial*
		Water	0.878	0.06; -0.24 to 0.36	*Trivial*
	Menthol	Control	0.001*	-0.66; -0.96 to -0.34	*Moderate*
		Carbohydrate	0.001*	-0.71; -1.01 to -0.40	*Moderate*
		Water	0.001*	-0.66; -0.97 to -0.35	*Moderate*
	Carbohydrate	Control	0.835	-0.04; -0.34 to 0.26	*Trivial*
		Menthol	0.001*	0.71; 0.40 to 1.01	*Moderate*
		Water	0.965	0.02; -0.28 to 0.33	*Trivial*
	Water	Control	0.878	-0.06; -0.36 to 0.24	*Trivial*
		Menthol	0.001*	0.66; 0.35 to 0.97	*Moderate*
		Carbohydrate	0.965	-0.02; -0.33 to 0.28	*Trivial*
Thirst	Control	Menthol	0.001*	0.75; 0.43 to 1.06	*Moderate*
		Carbohydrate	0.134	0.26; -0.05 to 0.56	*Small*
		Water	0.011*	0.33; 0.02 to 0.63	*Small*
	Menthol	Control	0.001*	-0.75; -1.06 to -0.43	*Moderate*
		Carbohydrate	0.022*	-0.55; -0.85 to -0.24	*Small*
		Water	0.263	-0.49; -0.79 to 0.18	*Small*
	Carbohydrate	Control	0.134	0.26; -0.56 to 0.05	*Small*
		Menthol	0.022*	0.55; 0.24 to 0.85	*Small*
		Water	0.259	-0.07; -0.37 to 0.23	*Trivial*
	Water	Control	0.011*	-0.33; -0.02 to 0.44	*Small*
		Menthol	0.263	0.49; -0.18 to 0.79	*Small*
		Carbohydrate	0.259	0.07; -0.23 to 0.37	*Trivial*

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4. Discussion

The aim of this study was to assess physiological and subjective responses to CHO and menthol mouth swilling, at rest under thermally challenging conditions, by employing a randomised tasting order in a quasi-blinded fashion.

Thermal Sensation was significantly improved, to a *moderate* degree by menthol in comparison to all other conditions. This finding has been reported repeatedly when menthol is applied to the oral cavity [41,43,44] and topically [56,57] by other researchers. However, we are the first group to document that this effect remains when nutrition (CHO intake) is accounted for statistically. This is important given the documented and potential use of menthol mouth swilling as an ergogenic aid during endurance exercise in thermally challenging conditions [13,43,58].

Further, this suggests that menthol mouth swilling has the potential to be incorporated alongside other nutritional practices that may not alter TS, such as CHO intake during or following exercise. Such findings may be of use to athletes undertaking heat acclimation training, whereby the heat stimulus may be applied actively i.e. during exercise [59-61] or passively via hot-water immersion [62,63] or a sauna [64] during recovery from exercise. Alternatively, in competition this finding allows athletes to pursue complementary nutrition and thermal ergonomic strategies, potentially mitigating commonly reported issues during prolonged exercise (in the heat) such as gastrointestinal distress [17,65] or taste fatigue [65]. This finding also has relevance to armed or emergency service personnel, who may have to report rapidly to situations in thermally challenging environments, potentially in varying states of nutritional preparedness.

Thirst, on the other hand, may be a key indicator of physiological readiness in these professions, and in prolonged endurance activity may also convey homeostatic information. Menthol mouth rinsing likely satiates thirst via a pre-absorptive pathway [66] through stimulation of oral cold receptors [66,67] concomitantly conferring an hedonic effect, effectively mimicking a cold beverage. The hedonic relationship between beverage temperature is well described in humans [68-70], and has been shown to occur in rodents even in the absence of thirst or water deficit [71]. Therefore, when implementing mouth-swilling protocols menthol's ability to attenuate thirst significantly, to a *small* to *moderate* extent is something practitioners and scientists must consider. It is not clear from this investigation whether a brief application of menthol in a mouth-swill can alter exercise or thermoregulatory behaviours to the extent that they become detrimental to the individual in question. It would be prudent to recommend that menthol mouth-swilling be employed in compensable heat stress, in exercise durations whereby muscle glycogen concentration is not also a limiting factor so reducing the need for further nutritional support e.g. events lasting ~60 min, or sports divided into periods of play. If athletes and practitioners still wish to employ menthol mouth-swilling in events outside of these constraints, then the co-implementation of other pre, or per-cooling strategies may be warranted [58,72,73] and these should be accompanied by athlete or user education strategies from the supporting practitioner(s).

Thermal Comfort was also improved to a *small* extent by menthol mouth swilling when compared to CHO (-0.33; -0.63 to -0.03) and water (-0.40; -0.70 to -0.10). Conversely, menthol was *trivially* different to CON, displaying a broader confidence interval than for TS. Thermal comfort may be susceptible to a time effect in this investigation despite the randomised swilling order, as evidenced by participants reporting that CON was more thermally comfortable to a *small* extent, in comparison to CHO (p<0.062) and water (p<0.002) swills. As time progressed, participants may have experienced greater awareness of tactile elements of their environment such as the wettedness of clothing, local skin wettedness or the texture of the chair on which they were sat, as longer exposure to a hot environment elicits and accumulates a greater volume of sweat, through which a participant must interact with their tactile environment. Incorporating local measures of TC and skin wettedness in subsequent investigations would allow for greater precision in this hypothesis.

Heart rate and T_{tym} behaved differently over the course of the investigation. This is somewhat counterintuitive as typically we would expect a concomitant increase in both metrics over time, but not necessarily in response to swills. The expected time course response would be attributed to progressive heat load [60,74]; yet in the present investigation each swill appeared to produce a different response, when compared to the CON period both water and CHO elicited *small* increases in HR, whereas menthol did not.

With respect to menthol our findings are in keeping with those of Shepherd and Peart, [75] who rebutted the results of Meamarbashi and Rajabi, who asserted that 10 days of supplementation with a peppermint oil solution, has a stimulatory effect [46] increasing maximal HR achieved during a maximal exercise test by 8%, and also increasing a complement of other exercise associated variables. The *small* increases in HR observed during water and CHO swilling appear counterintuitive from a sport and exercise scientist's perspective, as an increase in HR would confer a cost to the athlete, especially in a hot environment where factors such as increased sweat rate, resultant dehydration and increased skin blood flow already add to the thermal physiological strain experienced by the athlete [76]. Yet at rest when paired with a pleasant stimulus, these responses are perfectly normal [77,78]. Indeed, these responses have been noted to be goal directed [79,80], and increases in HR are associated with expectancy [78], a higher perceived reward value in healthy individuals [79,80]. Carbohydrate and water both confer hedonic responses by stimulating either receptors associated with fuel availability [6] or oral cold receptors [31], respectively and may convey a homeostatically derived sense of reward; thus an elevation in HR is probable. An alternative explanation for the elevation in HR in the present study is that of habituation [78]. Heart rate (HR) responses have been shown to be greater in response to an habituated 15.4% sucrose solution in comparison to water (control) or quinine solution (bitter); this response is consistent between exposures and independent of participant expectation [78]. Menthol mouth swilling on the other hand, may be too novel a stimulus for participants to be habituated to and subsequently elicit a HR response, but its ability to be of hedonic value in the current investigation is evidenced by improvements in TC and TS. Habituation to menthol mouth swilling requires further investigation; frequent users of oral hygiene products may present a logical starting population.

5. Conclusions

Menthol mouth swilling improves perceptions of TC and TS, and satiates thirst compared to mouth swilling other solutions. Carbohydrate intake can alter the perceptual characteristics of other swills, and thus the nutritional state of those undertaking mouth swilling strategies is a key consideration for supporting practitioners and users. Swilling carbohydrate and water may lead to *small* elevations in HR; this may be an anticipatory hedonic response, and or habituated. Menthol mouth swilling only *trivially* affects HR, but habituation to menthol mouth swilling warrants further exploration.

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