Title: Thermo-behavioural responses to orally applied L-menthol exhibit sex-specific differences during exercise in a hot environment

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Running head:

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Abstract

Aims: This study investigated the efficacy of L-menthol mouth-rinsing on thermal sensation and perceived effort in females and males, using a fixed-rating of perceived exertion (RPE) exercise protocol in a hot environment.

Methods: Twenty-two participants (eleven females, eleven males) completed two trials using a fixed-RPE protocol at an exercise intensity between 'hard' and 'very hard', equating to 16 on the RPE scale at ~35 °C. Participants adjusted power output to maintain RPE-16. In a randomised, double-blind, crossover design, L-menthol or a control mouthwash was administered at an orally neutral temperature (~32 °C) prior to exercise and at 10 min intervals thereafter. Measures of mechanical power output, core temperature, heart rate, perception of thermal sensation and thermal comfort, and whole-body sweat loss are reported.

Results: Thermal sensation was lowered by L-menthol in both sexes ($P < 0.05$), however during exercise this was only maintained for 40% of the trial duration in females. Thermal comfort did not differ between conditions ($P > 0.05$). No differences in exercise duration were observed compared to control, despite a ~4 % and ~6 % increase in male and females respectively. Power output increased by ~6.5 % males ($P = 0.039$) with no difference in females ~2.2% ($P = 0.475$), compared to control. Core temperature, heart rate and whole-body sweat loss was not different between condition or sex.

Conclusions: L-menthol lowered perceptual measures of thermal sensation in females, but did not attenuate a greater rate of rise in thermal sensitivity when exercising in a hot environment, compared to males. Males appeared to adopt a higher risk strategy by increasing power output following L-menthol administration in contrast to a more conservative pacing strategy in females. Therefore, there appear to be sex-specific differences in L-menthol's non-thermal cooling properties and subsequent effects on thermo-behavioural adjustments in work-load when exercising in a hot environment.

Introduction

There are a number of reported sex differences in thermoregulatory responses to exercise in hot environments (Fox et al., 1969; Gagnon and Kenny, 2012; Shapiro et al., 1980; Smith and Havenith, 2012). In eumenorrheic women, core temperature displays a biphasic rhythm across the menstrual cycle, with ~ 0.4 ^{o}C increase during the post-ovulatory luteal phase (Marshall, 1963) due to a change in thermoregulatory set point (Inoue et al., 2005; Pivarnik et al., 1992; Tenaglia et al., 1999). As a result, the threshold for thermoregulatory effector responses is increased (Inoue et al., 2005; Kolka and Stephenson, 1997; Stachenfeld et al., 2000), and an increase in cardiorespiratory strain has been reported (Janse de Jonge, 2003; Pivarnik et al., 1992). Indeed, heat tolerance is reduced by ~6-16 % during exercise tasks performed in the mid-luteal phase when compared to the early follicular phase (Avellini et al., 1980; De Jonge et al., 2012; Tenaglia et al., 1999). Hormonal contraceptive use is prevalent in females and female athletes (Martin et al., 2018; Rechichi et al., 2009) were suppression of endogenous hormone concentrations inhibits ovulation. However, phase-related changes in core temperature and effector responses are still apparent (~0.15 ^{o}C) (Grucza et al., 1993; Lei et al., 2019) and therefore should still be considered when examining strategies to enhance heat tolerance in females using hormonal contraceptives.

Behavioural thermoregulation is the first strategy to defend against a disruption in heat balance in hot environments, secondary to changes in body temperature (Flouris and Schlader, 2015). Subjective responses to physical activity, which include perceived exertion, thermal sensation and sensation of pain, are known to vary in females according to menstrual cycle phase (Gerrett et al., 2014; Hooper et al., 2011; Travlos and Marisi, 1996). In hot conditions, alteration of thermal sensitivity leads to behavioural reductions in exercise intensity following stimulation of peripheral thermoreceptors, which demonstrate regional sensitivity (Nakamura et al., 2008). However, there is clear variation in thermal sensitivity between sexes (Gerrett et al., 2014), with females able to detect warm (Gerrett et al., 2014; Golja et al., 2003; Lautenbacher and Strian, 1991) and cold stimuli (Golja et al., 2003) more strongly than males, independent of changes in body temperature. Higher sensitivity is reported around the head regions with respect to the extremities (Gerrett et al., 2014); however, the oral cavity is one of the most densely innervated parts of the body in terms of peripheral receptors (Haggard and de Boer, 2014). Limited research has been presented on sex differences in oral sensitivity, with one study reporting no differences in relation to sex or phases of the menstrual cycle (Abe et al., 2012). We have recently shown that ice slushy or L-menthol oral mouth-rinsing during advanced thermal stress can extend exercise performance (a conscious behaviour), despite no change in body temperature, in males (Jeffries et al., 2018). Female participants are significantly under-represented across the sports and exercise literature (Costello et al., 2014) and the

92 recent growth in research into L-menthol's ergogenic properties during exercise in hot and indoor
93 environments has not yet extended to females.

94 When administered orally, L-menthol non-thermally enhances cold sensations in the mouth (Eccles, 1994)
95 and inhibits the perception of warmth (Green, 1986), ultimately leading to a conscious reduction in thermal
96 sensation, which is particularly effective when exercising in a hot environment (Jeffries and Waldron,
97 2019). Fixed-RPE exercise protocols allow instantaneous thermo-behavioural adjustments in work-load to
98 be monitored, whereby individuals can integrate perceptual, peripheral and environmental cues to self-
99 determine work intensity. We have previously shown that L-menthol can increase work-load and extend
100 exercise time during a fixed-RPE protocol in hot conditions, in males (Flood et al., 2017). Considering the
101 reported greater thermal sensitivity in females, it is unknown if L-menthol may elicit comparable or
102 stronger effects than observed in males. In addition, L-menthol is typically delivered in a solution cooler
103 (19-23 °C) (Flood et al., 2017; Mündel and Jones, 2010; Riera et al., 2014; Stevens et al., 2015, 2016) than
104 oral temperature (~36 °C), hence potentiating the thermal cooling capacity of each mouth rinse (Green,
105 1985). Therefore, we delivered L-menthol at a temperature that would be thermally neutral in the mouth
106 to isolate L-menthol's efficacy in modulating perceived thermal sensation and resultant effects on
107 behaviour in both sexes when exercising in the heat.

108 Our aims were to investigate L-menthol mouth-rinsing in males and females using a fixed-RPE exercise
109 protocol in hot conditions. We hypothesised that females would exhibit a reduction in perceived thermal
110 sensitivity following non-thermal cooling provided by orally applied L-menthol that would be equally or
111 more effective in facilitating an increased work-load and extension in task performance as we have
112 previously described for males (Flood et al., 2017).

Materials and methods

Participants

Twenty-two non-acclimated participants, comprising eleven females (age = 22 ± 2 years; body mass = 65.3 ± 4.0 kg; stature = 167.6 ± 4.2 cm; maximal oxygen uptake, $\dot{V}O_{2max}$ = 43.5 ± 2.9 ml.min.kg^{-1}) and eleven males (age = 20 ± 1 years; body mass = 77.7 ± 8.9 kg; stature = 180.0 ± 6.0 cm; maximal oxygen uptake, $\dot{V}O_{2max}$ = 53.9 ± 6.9 ml.min.kg^{-1}) consented to take part in this study. A priori sample size was calculated using G*Power (version 3.1.9.6). Given the effect size (η_p^2 = 0.896; (Flood et al., 2017)) we reported previously for differences in power output using an RPE-16 protocol with L-menthol, a sample size of ten was deemed sufficient to identify differences between groups with a statistical power of 0.95. We recruited eleven participants to account for experimental mortality. Participants engaged in regular physical activity < 5-h per week. None of the participants had visited a hot country in the previous three months, all resided in the UK and experiments were conducted in one block during the winter months of January - March. Participants were instructed to avoid consumption of alcohol or caffeinated products for 24-h before each visit, as well as strenuous exercise 48-h before testing and to arrive fully hydrated. Ethical approval was provided by Newcastle University ethics committee, which was conducted in accordance with the 1964 Helsinki declaration.

Study design

A randomised, double-blind, crossover design examined L-Menthol mouth rinse in males and females during exercise in the heat using a fixed RPE protocol (Flood et al., 2017). Randomisation was conducted by generating random numbers for each condition for all participants using online software (Urbaniak and Plous, 2015) and blinding was performed by a person that was not on the research team and all solutions were administered with random letters. Participants were blinded to the original hypothesis of the study and informed that the effect of differing mouth-rinse flavours on exercise in the heat was being investigated. Participants visited the Laboratory on three separate occasions. During visit 1, participants conducted baseline testing to establish maximal oxygen uptake ($\dot{V}O_{2max}$) and power output at $\dot{V}O_{2max}$ (W_{max}), as well as being fully familiarised to the experimental protocol. During visits 2 and 3, the participants completed the fixed-RPE protocol either with L-menthol or control mouth rinse, which were all administered at approximate mouth temperature.

Experimental procedures

Menstrual phase determination

All females enrolled in this study were taking hormonal contraceptives (eight: combined oral contraceptive Rigenidon®; three: progesterone contraceptive implant Nexplanon®). Testing was designed to take place during the quasi early-to-mid follicular phase (approximately day 2–10). In females taking the combined oral contraceptive, where oestrogen and progesterone is downregulated, a quasi-follicular phase was calculated based on day 1 of the 28-day pill regime representing the beginning of the menstrual cycle. Females using progesterone implants, where a reduction in endogenous progesterone but not oestrogen is observed and hormonal fluctuations and menses may occur, we determined the correct phase using the forward counting method, which determines menstrual phases by counting the number of days from the previous onset of menses (Janse de Jonge, 2003). This method has acknowledged limitations due to variable follicular phases, particularly when using progesterone contraceptives and therefore we retrospectively calculated menstrual length by asking participants to report their next onset of menses, after testing was complete. All participants were tested $\pm$ 2 days of their calculated early-to-mid follicular phase. All experimental tests where timetabled to occur during an 8-day period (eg. day 2-10) based on the predicted quasi early-to-mid follicular phase with 72-h between tests.

Preliminary testing

Participants reported to the laboratory to conduct preliminary testing consisting of anthropometric measurements and an incremental ramp test. Participants then performed a self-paced warm-up for 5-min and were asked to select a preferred cadence that was standardised throughout the remaining experimental trials. The incremental ramp test began at 100 W, and work-load increased in one-min stages at a rate of 25 $W \cdot min^{-1}$ until volitional fatigue. All testing was conducted on an electronically-braked cycle ergometer (Velotron Racermate, USA). Expired gases were analysed using the Douglas bag method. Expired gases were collected by a mouthpiece connected to a 2-way Hans-Rudolph breathing valve (27000 series) (Hans Rudolph, inc. USA) and a 2-meter corrugated hose over a collection period ~45-s. At the end of the collection period, gas fractions (F_EO_2 and F_ECO_2) were analysed (Servomex, 5200 MiniMP, UK), volume of expired air (Harvard Apparatus, Kent, UK) and air temperature were measured for calculation of $\dot{V}O_{2max}$ by indirect calorimetry. All values were corrected to reflect standard temperature and pressures. $\dot{V}O_{2max}$ was determined as the highest average 30-s value obtained. RPE was recorded at the end of each 1-min stage by pointing to a 15-grade RPE scale held by an investigator. Following a 15-min rest period, two familiarisation exercises were conducted which were subsequently used with the intention of calibrating the participant's RPE-based selection of power output in the main trials. In the first exercise, participants conducted incremental ramp steps in accordance with the power output / RPE relationship derived from the incremental ramp test. The steps followed the order: RPE 11 for 4-min, RPE 13 for 3-min, and RPE 15 for 2-min. Participants were blinded to the RPE and asked to rate their own RPE to aid familiarisation with the RPE scale. The second exercise began at 110 W and involved participants controlling

resistance on the ergometer, whilst being blinded to actual power output, in order to achieve an RPE they perceived as equalling RPE-16 over a period of 5-min. The final power output was recorded as the power output at the level of cycling resistance that the participant indicated best represented an RPE-16. The latter test was used to demonstrate the reliability of the participant's ability to select a replicable exercise intensity at the desired RPE across the familiarisation and experimental trials prior to administration of the mouth rinse.

Experimental trials

Participants performed two randomised experimental trials in an environmental heat chamber in temperatures of 34.9 ± 0.5 ˚C and relative humidity 40.6 ± 2.2 %, separated by at least 72-h. For each participant, the experimental trials were conducted at the same time of day to eliminate the effect of circadian variation. Euhydration was established prior to exercise by identifying urine osmolality < 715 mOsm/Kg H_2O (Shirreffs and Maughan, 1998) (Pocket Osmochek, Vitech Scientific Ltd, West Sussex, UK) and average hydration was 388.8 ± 243.5 mOsmols/kg H_2O, across both conditions. Participants were instrumented with a heart rate chest strap then entered the heat chamber, resting for 10 minutes before baseline measures were recorded. Participants then conducted a standardised warm-up procedure, as outlined previously in the second familiarisation exercise, ramping to an RPE-16 over a 5 min period. Following 5-min of seated rest, participants then started the fixed-RPE protocol.

Fixed-RPE protocol

Participants were instructed to cycle at a power output that was perceived to represent an RPE of 16 on the 15-grade Borg scale (Borg, 1982) and to adjust their power output such that an RPE of 16 was maintained. An RPE of 16 represents a verbal cue of between 'hard' and 'very hard' on the Borg Scale. The highest average 30-s power output achieved during the first 3-min of the fixed RPE trial was recorded and participants exercised until their power output declined to 70 % of this initial value (Flood et al., 2017; Tucker et al., 2006). The trial was stopped when power output fell below this value for 30-s. Standardised feedback every ~2-min was given to remind participants to maintain an RPE of 16. Participants were encouraged to constantly reassess whether they were still exercising at RPE-16. They were blinded to distance covered, elapsed time, heart rate, power output.

Measurements

Physiological measures

Tympanic temperature was recorded every 6-min as an approximation of core temperature. Based on analysis conducted in our laboratory, tympanic temperature measured with the current device (Braun

Thermoscan IRT 6020, UK) underestimates rectal temperature by 0.5 ± 0.3 °C but correlates strongly (R^2 = 0.92) across a range of sub-maximal exercise intensities and environmental conditions. Participants recorded semi-nude (males: shorts; females: shorts and sports bra) body mass prior to entering the heat chamber and immediately following the completion of the experimental trial after wiping off sweat with a towel. No water was ingested during exercise in the heat. Heart rate was recorded continuously throughout the trials (Polar T31, UK) transmitting data onto a portable watch (Polar FT7, UK).

Perceptual measures

Participants were thoroughly briefed on the RPE scale during familiarisation sessions before commencing the fixed RPE trials as we have previously reported (please see for full description: Flood et al., 2017). Briefly, participants were instructed to pay close attention to how difficult the exercise felt, combining total exertion, fatigue, and physical stress in the heat, without considering one particular factor such as leg pain, shortness of breath or anticipation of how they might feel several minutes later. In addition, participants where familiarised with the thermal sensation scale and thermal comfort scale. Laminated scales were held in front of the participants during exercise and they were asked to indicate thermal comfort and sensation by pointing to the appropriate point on the scale. Thermal comfort (TC) was recorded on the Bedford 7-point analogue scale where -3 = “much too cool”, 0 = “comfortable”, and 3 = “much too warm” (Bedford, 1936). Thermal sensation (TS) was recorded on an adapted ASHRAE 9-point analogue sensation scale where -4 = “very cold”, 0 = “neutral”, and 4 = “very hot” (Zhang et al., 2004). Subjective ratings were recorded in 1.0 increments every 5 min during the experimental trials.

Mouth rinse formulation

Participants were given 25 ml solution to rinse 30-s prior to the main fixed RPE trial and at regular 10-min intervals (therefore delivered at -0:30, 9:30 and 19:30 min etc). They were instructed to swill around the mouth for 10-s before spitting into a bowl without swallowing. L-menthol solution was formulated from menthol crystals (≥ 99% food grade L-menthol, Sigma-Aldrich, UK) dissolved in de-ionised water heated to ~50 ^{o}C at a concentration of 0.64 mM (0.01 %) (Flood et al., 2017). The solution was then stored at 5 ^{o}C for up to 1 month. Prior to use, solutions were aliquoted for mouth-rinse and warmed to ambient laboratory temperature 31.8 ± 2.3^{o}C which was confirmed by a standard thermometer and recorded. A control mouth rinse was made using an apple flavoured non-calorific artificial sweetener, consisting of sucralose (FlavDrops, MyProtein, Norwich, UK) dissolved in 25 ml of deionised water and warmed to 32.1 ± 1.2 ^{o}C (Flood et al., 2017).

Statistical analysis

All statistical analyses were performed using SPSS (IBM SPSS statistics 22 Inc, USA). Sex differences between conditions were examined by collapsing time due to the statistical power required to conduct three-way analysis. Two-way analysis of variance (ANOVA) for repeated measures were then used to test for within-group effects across time in both conditions for each sex. If sphericity was violated a Greenhouse-Geisser correction was applied. When a significant interaction effect (condition x time) was reported, post-hoc pair-wise comparisons were made incorporating a Bonferroni adjustment. Magnitude of effect was calculated with partial eta-squared (η_p^2) according to the following criteria: 0.02, a small difference; 0.13, a moderate difference; 0.26 a large difference (Cohen, 1988). Differing trial durations meant that power data was normalized with respect to time. Trial duration, peak power and changes in body mass were analysed using a 2-tailed paired sample *t*-test and magnitude of effect calculated (Cohen's *d*) according to the following criteria: 0.2, a small difference; 0.5, a moderate difference; 0.8 a large difference (Cohen, 1988). Perceptual data, reported on an ordinal scale, was analysed using non-parametric alternatives. A Friedman test was conducted to assess repeated measures and a Wilcoxon signed-rank test to compare average data between sex. Magnitude of effect calculated by dividing the absolute standardised test z statistic by the square root of the number of pairs according to the following criteria: 0.1, a small difference; 0.3, a moderate difference; 0.5 a large difference (Cohen, 1988). Data are presented as mean ± SD, significance was set at $P < 0.05$.

Results

Exercise performance in males and females

Data for time and power output were normally distributed and showed no trial order effect (P > 0.05). During the pre-experimental warm-up were participants were instructed to self-select an RPE of 16 over 3-min from a starting intensity of 110 W, the final power output selected was not different between condition or sexes ($F_{(1,20)}$ = 0.019, P = 0.893; η_p^2 = 0.001), despite an observable ~30 W average difference between males (L-menthol: 170 ± 32 W; Control: 170 ± 25 W) and females (L-menthol: 139 ± 15 W; Control: 141 ± 22 W). Trial duration was not different between sex ($F_{(1,20)}$ = 1.119, P = 0.303; η_p^2 = 0.053) or condition ($F_{(1,20)}$ = 0.070, P = 0.794; η_p^2 = 0.003). However, in males (L-menthol: 34:54 ± 10:27 min; Control: 33:22 ± 10:36 min) there was a nominal ~4 % (92-s) increase in exercise time in the L-menthol condition and in females (L-menthol: 29:42 ± 7:43 min; Control: 27:51 ± 5:52 min) a ~6 % (111-s) increase in exercise time in the L-menthol condition.

*** Insert Figure 1 near here ***

Average power output across the trial was different between condition ($F_{(1,20)}$ = 5.917, P = 0.025; η_p^2 = 0.228), however the interaction effect indicated no differences between average power output in each condition and sex ($F_{(1,20)}$ = 1.137, P = 0.299; η_p^2 = 0.054). Power output decreased with time in males ($F_{(10,100)}$ = 122.114, P = 0.000; η_p^2 = 0.924) and females ($F_{(2.117,21.165)}$ = 11.294, P > 0.001; η_p^2 = 0.530). Across the trial, power output was higher in males in the L-menthol condition (L-menthol: 160 ± 26 W, Control: 150 ± 26 W (~6.5 %), ($F_{(1,10)}$ = 5.018, P = 0.039; η_p^2 = 0.334) with an interaction effect ($F_{(10,100)}$ = 2.016, P = 0.037, η_p^2 = 0.168) (Figure 1A). However, in females there was no difference between conditions (L-menthol: 127 ± 11 W, Control: 124 ± 14 W (~2.2%), ($F_{(1,10)}$ = 0.552, P = 0.475; η_p^2 = 0.052) and no interaction effect ($F_{(2.242,22.4525)}$ = 0.801, P = 0.474; η_p^2 = 0.074) (Figure 1A). During the first 10% of the exercise task, all participants achieved their peak power output which was different between males and females ($t_{(10)}$ = -4.083, P = 0.002, d = 1.44). In males, self-selected peak power was ~6 % higher in the L-menthol condition, with 8 out of 11 participants selecting a higher power output ($t_{(10)}$ = -2.247, P = 0.048, d = 0.38) (Figure 1B). However, no significant difference in peak power (~2%) was observed for females ($t_{(10)}$ = -0.627, P = 0.545, d = 0.15) (Figure 1B).

*** Insert Figure 2 near here ***

Subjective measures of thermal perception

Perceptual measures of thermal sensation increased with time in all conditions for males and females (P < 0.001). However, when collapsed for time there were differences between males and females (z = -2.357, P = 0.018, d = 0.71), with males reporting on average ~ 0.6 points lower on the scale for thermal sensation across both trials. In males, thermal sensation was lowered in the L-menthol condition across the entire trial, except at the 18-min time point (Start -0.81 (z = -2.714; P = 0.007; d = 0.82), 6-min -0.45 (z = -2.236; P = 0.025; d = 0.67), 12-min -0.50 (z = -2.049; P = 0.04; d = 0.61), 18-min -0.45 (z = -1.492; P = 0.136; d = 0.44, End -0.63 (z = -1.897; P = 0.05, d = 0.60)) (Figure 2A). In females, thermal sensation was lower only across the first 12-min of exercise in the L-menthol condition (Start -0.45 (z = -1.833; P = 0.050; d = 0.55), 6-min -0.41 (z = -2.121; P = 0.034; d = 0.64), 12-min -0.38 (z = -1.667; P = 0.048; d = 0.50), 18-min -0.05 (z = -0.333; P = 0.739; d = 0.10), End -0.18 (z = -0.973; P = 0.330; d = 0.29)) (Figure 2B). The rate at which thermal sensation increased across the first 18-min of the exercise trials was faster in females (L-menthol 0.12 units/min^{-1}, R^2 = 0.87; Control 0.13 units/min^{-1}, R^2 = 0.94) compared to males (L-menthol 0.07 units/min^{-1}, R^2 = 0.93; Control 0.08 units/min^{-1}, R^2 = 0.92), ($t_{(10)}$ = -2.294, P = 0.045, d = 0.97). Thermal comfort increased on the scale, denoting greater discomfort, across time in all conditions for males and females (P < 0.001). However, there were no differences at any time point for L-menthol and control conditions or between sex (P > 0.05) (Figure 2 C&D).

*** Insert Figure 3 near here ***

Physiological responses

Core temperature after the standardized warm-up was not different between conditions in males (L-menthol: 36.9 ± 0.4 ºC; Control: 36.9 ± 0.3 ºC) and females (L-menthol: 37.1 ± 0.4 ºC; Control: 37.2 ± 0.4 ºC) (P > 0.05). Core temperature increased with time in males ($F_{(4,40)}$ = 4.038, P < 0.001; η_p^2 = 0.905) and females ($F_{(1.53,15.33)}$ = 30.40, P < 0.001; η_p^2 = 0.752) but with no difference between condition for males ($F_{(1,10)}$ = 0.067, P = 0.801; η_p^2 = 0.007) and females ($F_{(1,10)}$ = 2.740, P = 0.129; η_p^2 = 0.215) (Figure 3 A&B). Heart rate increased with time in males ($F_{(1.25,12.46)}$ = 223.78, P < 0.001; η_p^2 = 0.957) and females $F_{(1.78,17.79)}$ = 371.11, P < 0.001; η_p^2 = 0.974) but with no difference between conditions for males ($F_{(1,10)}$ = 0.018, P = 0.897; η_p^2 = 0.002) and females ($F_{(1,10)}$ = 0.001, P = 0.992; η_p^2 = 0.030) (Figure 3 C&D). The change in body mass was not different between pre-to-post for the exercise task in males (Pre: 0.69 ± 0.3 kg; Post: 0.68 ± 0.2 kg) ($t_{(10)}$ = 0.126, P = 0.902, d = 0.04) and females (Pre: 0.36 ± 0.1 kg; Post: 0.39 ± 0.2 kg) ($t_{(10)}$ = -0.582, P = 0.574, d = 0.24). There were no differences between sex ($F_{(1,20)}$ = 0.179, P = 0.179; η_p^2 = 0.676).

Discussion

The aims of this study were to investigate the efficacy of L-menthol mouth-rinsing in males and females using a fixed-RPE exercise protocol in a hot environment. Oral application of L-menthol lowered perceptual measures of thermal sensation in males, but in females was only effective in the early stages of exercise in the heat. Females exhibited a faster rate of rise in reported thermal sensation in both conditions, when compared to males. Self-selected power output and exercise duration did not differ in females between the L-menthol and control condition (although exercise duration was increased ~6 %). In contrast, males showed a 6.5% increase in power output and a ~4 % increase in exercise duration in the L-menthol trial, replicating our previous findings (Flood et al., 2017). This refutes our primary hypothesis that L-menthol would be equally or more effective in females at reducing thermal sensation and facilitating a comparable increase in exercise work-load, to males. Consistent with the 'non-thermal' mechanistic basis of L-menthol's cooling effects (Jeffries and Waldron, 2019), there were no changes in core temperature, heart rate or sweat loss between conditions, despite the reported differences in thermal perception and performance.

Research examining sex-specific differences in thermal sensitivity tends to be largely confined to males. In the present study we tested male and female participants and controlled for potential differences in thermoregulation ascribed to the menstrual cycle (De Jonge et al., 2012; Marshall, 1963) by testing females during a calculated quasi-follicular phase. Baseline measures in both sexes confirmed that there were no differences in core temperature and no differences in the rise in core temperature during exercise (Figure 3 A&B). Thermal sensitivity encompasses the perceived intensity of temperature being sensed by the individual (Gagge et al., 1967). Psychological strategies that are effective at reducing thermal sensitivity have been successful in extending exercise tolerance in the heat (Cheung, 2010; Flouris and Schlader, 2015). We utilised a non-thermal cooling L-menthol mouth-rinse which was effective at reducing thermal sensation across the majority of the exercise test in males. However a significantly smaller reduction in thermal sensation was observed in females, indicating sex-specific differences in L-menthol's effectiveness during exercise in a hot environment. Indeed, L-menthol induced reductions in thermal sensitivity were only observed over the first 12 minutes of exercise in females. During exercise, the rise in perceived thermal sensation was faster in females than in males, reflecting a greater thermal sensitivity which did not differ between condition. That L-menthol was unable to modify this increase in thermal sensation in both sexes supports a possible reduced potency with subsequent administration that we have noted before (Flood et al., 2017). However, thermal sensitivity is also known to decrease during exercise (Gerrett et al., 2015; Ouzzahra et al., 2012), due to a reduction in transmission of sensory information along afferent fibres via exercise-induced analgesia (EIA) (Koltyn, 2000), therefore transmission of thermal sensory information may be reduced. Limited research exists investigating sex-specific differences in EIA with only one study

supporting no difference between males and females (tested in the follicular phase of the menstrual cycle) (Koltyn et al., 2014), and further work is needed.

Thermo-behavioural adjustments in work-load during the trials, enabled changes in perceived exertion to be observed using the fixed-RPE protocol. Males voluntarily adopted a higher power output (~6.5%) after rinsing with L-menthol which supported our previous observations (~4%) (Flood et al., 2017). However, in females, no difference in power output (>2%) following L-menthol-rinsing was observed. This was not anticipated in our initial hypothesis. That a lowering of thermal sensation was reported across both sexes in the early stages of the exercise trial suggests that the effectiveness of L-menthol in inducing non-thermal cooling cannot explain this discrepancy. Despite no significant changes, exercise time was extended in males (~4%) and females (~6 %). It is also unclear how L-menthol may have extended exercise performance in females compared to control conditions when any change in thermal sensation had dissipated beyond ~40 % of the exercise trial. Females are reported use thermal behaviour (such as modification of work-load) to a greater extent than males during exercise (Vargas et al., 2019) which may suggest that a more conservative pacing strategy was adopted. We have also previously proposed that L-menthol could act as a potential distractor to moderately uncomfortable stimuli, such as exercise in a hot environment, irrespective of its cooling properties (Jeffries et al., 2018), which is possible. It should be noted that exercise duration is an arbitrary measure of performance when using a fixed-RPE protocol and should be carefully interpreted. In females, it is possible that by not increasing power output, despite reporting a reduction in thermal sensation, exercise duration could be extended by consciously adopting a more conservative pacing strategy. If we approximate energy utilised (work done) by multiplying exercise duration (s) by power (J/s) across participants, in the L-menthol trial total work done was increased by 7% in males and 8% in females relative to the control trial. Therefore, the pacing strategy adopted by males in the L-menthol condition was inherently more aggressive by selecting a higher power output and yet this did not extend total work done beyond the more conservative strategy adopted by females. Typically, males exhibit different self-pacing strategies when compared to females. In repeated sprint study designs, males self-pace at higher exercise intensities, achieve higher total work and show greater power decrements than women, despite comparable cardiovascular strain (Billaut and Bishop, 2012; Panissa et al., 2016). The noted increase in fatigue is likely to be a consequence of their greater absolute initial sprint performance, rather than a sex-specific difference in fatigue (Billaut and Bishop, 2012). Behaviourally, in the case of decision making, males also appear to adopt a higher risk strategy based on physical fitness, or an alteration in motivation to perform exercise, compared to females (Deaner et al., 2015). When examining competition data, sex differences in marathon pacing in non-elites was larger for males in the 2007 Chicago marathon which was hot (27 °C) when compared to the 2009 Chicago marathon which was cool (3 °C), illustrating the greater propensity for a risky pacing strategy, despite unfavourable environmental conditions (Deaner et al., 2015).

Our findings refute our initial hypothesis that greater thermal sensitivities in females (Gerrett et al., 2014, 2015) and greater sensitivity to cold stimuli when compared to males (Gerrett et al., 2015) would potentiate L-menthol's effect. The oral cavity is one of the most densely innervated parts of the body in terms of peripheral receptors (Haggard and de Boer, 2014). Mouth-rinsing with L-menthol activates peripheral TRPM8 thermoreceptors on the oral mucosa transmitting information via the trigeminal system which mediates sensations such as burning, cooling and tingling (Laska et al., 1997). Despite reported sex-related differences in chemosensation, examination of irritants, including menthol, have failed to report sex-specific differences in trigeminal sensitivity (Ohla and Lundström, 2013). Psychophysical tests have identified that these differences may be due to differing cognitive appraisal between the sexes, therefore altering subjective perception (Lundström et al., 2005; Ohla and Lundström, 2013). However, in this study females reported a smaller reduction in thermal sensation following L-menthol mouth-rinsing, than males, which then dissipated. In thermally challenging environments, females tend to be more sensitive to warm stimuli than males and perceive a thermal stimulus to be hotter (Gerrett et al., 2014, 2015). Therefore the hot environment may have been perceived as a greater thermal threat thereby reducing or de-prioritising L-menthol's perceived cooling properties. At present it is clear that further research is required to understand these sex differences in behavioural thermoregulation.

Although not a primary aim of this study we administered L-menthol in a thermally neutral solution ~32 ^{o}C. This was important to experimentally establish L-menthol's efficacy when oral cooling facilitated by the delivery solution was removed. We and others have administered L-menthol in oral rinses between 19-23 °C, in males (Flood et al., 2017; Mündel and Jones, 2010; Riera et al., 2014; Stevens et al., 2015, 2016) and one study at ~40 °C (Gibson et al., 2019). Perceived sensation of cold in the oral cavity reaches zero at ~32 °C (the cold threshold) with perception beginning to shift to warmth as liquid temperature increases above ~35 °C (the warmth threshold), despite oral temperature being (~36 °C) (Green, 1986). Indeed L-menthol solutions below oral temperature have been suggested to feel cooler than water of the same temperature (Green, 1985). These have been demonstrated to potentiate exercise performance in hot humid conditions following neutral (23 ^{o}C), cold (3 ^{o}C) and ice-slushy (-1 ^{o}C) beverage ingestion during a 20-km time-trial (Riera et al., 2014). Therefore it was important to achieve oral temperature neutrality and therefore solutions were administered at ~32 ^{o}C to isolate L-menthol's true non-thermal cooling properties. We can confirm, as previously discussed, in males L-menthol was equally effective in enhancing exercise performance in the heat when delivered at ~32 ^{o}C when compared to our previous study ~19 ^{o}C using identical protocols and ambient conditions albeit a different participant group (Flood et al., 2017). Unfortunately we are unable to make the same comparisons in females.

Limitations

The females enrolled in this study all used hormonal contraception. Investigations into oral contraceptive users have reported that a phase-related elevation in core temperature (~0.15 ^{o}C) and concomitant increase in threshold effector responses is maintained during active and passive heating (Lei et al., 2019). In this study, to eliminate potential effects of the menstrual cycle and to primarily establish whether L-menthol can modulate exercise performance in the heat we tested females in the quasi-follicular phase of the menstrual cycle in contraceptive users. Eight out of eleven females in this study used the oral-combined contraceptive and three used a progesterone implant contraceptive. There are clear limitations with this combined approach as different contraceptive methods lead to fluctuations in hormone levels (Elliott-Sale et al., 2013); however, we attempted to test only in the predicted and quasi-follicular phase. Blood hormonal confirmation should be sought in future. However, this highlights some interesting future questions regarding L-menthol's effectiveness during different cycle phases of the menstrual cycle, particularly in females not using contraceptives where oscillations in body temperature could modulate L-menthol's efficacy, particularly in hot conditions. We also acknowledge the limitations with using tympanic temperature to inform changes in core temperature. Core temperature was not a primary outcome measure in this study and we have successfully shown no change in core temperature in a previous study that utilised the same experimental design (Flood et al., 2017), however future studies in combination with hormonal analysis should seek to measure core temperature more accurately.

Conclusion

In summary, L-menthol lowered perceptual measures of thermal sensation during the early stages of exercise in a hot environment in females, but did not attenuate a faster rate of rise in perceived thermal sensation in both conditions when compared to males. Following administration of L-menthol males adopted a higher risk strategy during exercise by increasing power output, however exercise duration was not significantly extended beyond control. Instead females appeared to adopt a more conservative pacing strategy and did not increase power output over control. In conclusion, L-menthol's non-thermal cooling properties and the subsequent modifications of exercise intensity described in males may not be the same in females. Therefore, there appear to be sex-specific differences in L-menthol's non-thermal cooling properties and subsequent effects on thermo-behavioural adjustments in work-load when exercising in a hot environment.

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Figure legends

Figure 1. A. Power output against trial duration expressed as a percentage of final time for males and females. Error bars have been removed for clarity. Asterisk denotes significant difference in average power between conditions in males (*P* = 0.039). B. Peak power output selected during the fixed-RPE trial for males and females. Solid lines indicate an increase and dashed lines a decrease between conditions. Asterisk denotes significant difference in peak power between conditions in males (*P* = 0.048). Conditions are indicated by colour, L-menthol (white) and control (black) and sex indicated on figures. All individual data is shown (n = 22).

Figure 2. A. Thermal sensation as reported during the fixed-RPE trial for males and B. females. C. Thermal comfort as reported during the fixed-RPE trial for males and D. females. Conditions are indicated by colour, L-menthol (white) and control (black) and sex indicated on figures. All data are shown as mean ± SD, (n = 22). Asterisk denotes significant difference between conditions at respective time points ($P < 0.05$).

Figure 3. A. Core temperature during the fixed-RPE trial for males and B. females. C. Heart rate during the fixed-RPE trial for males and D. females. Conditions are indicated by colour, L-menthol (white) and control (black) and sex indicated on figures. All data are shown as mean ± SD, (n = 22).

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643 Figure 1
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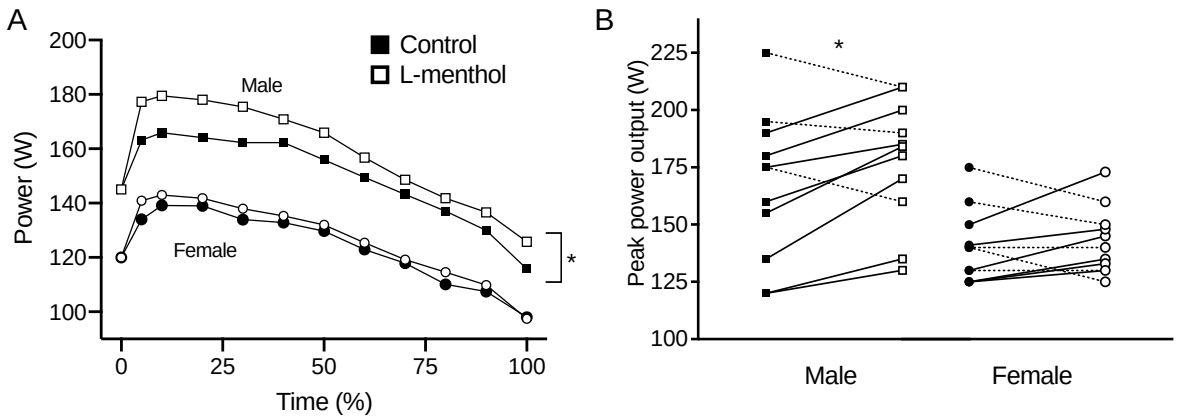

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Figure 2

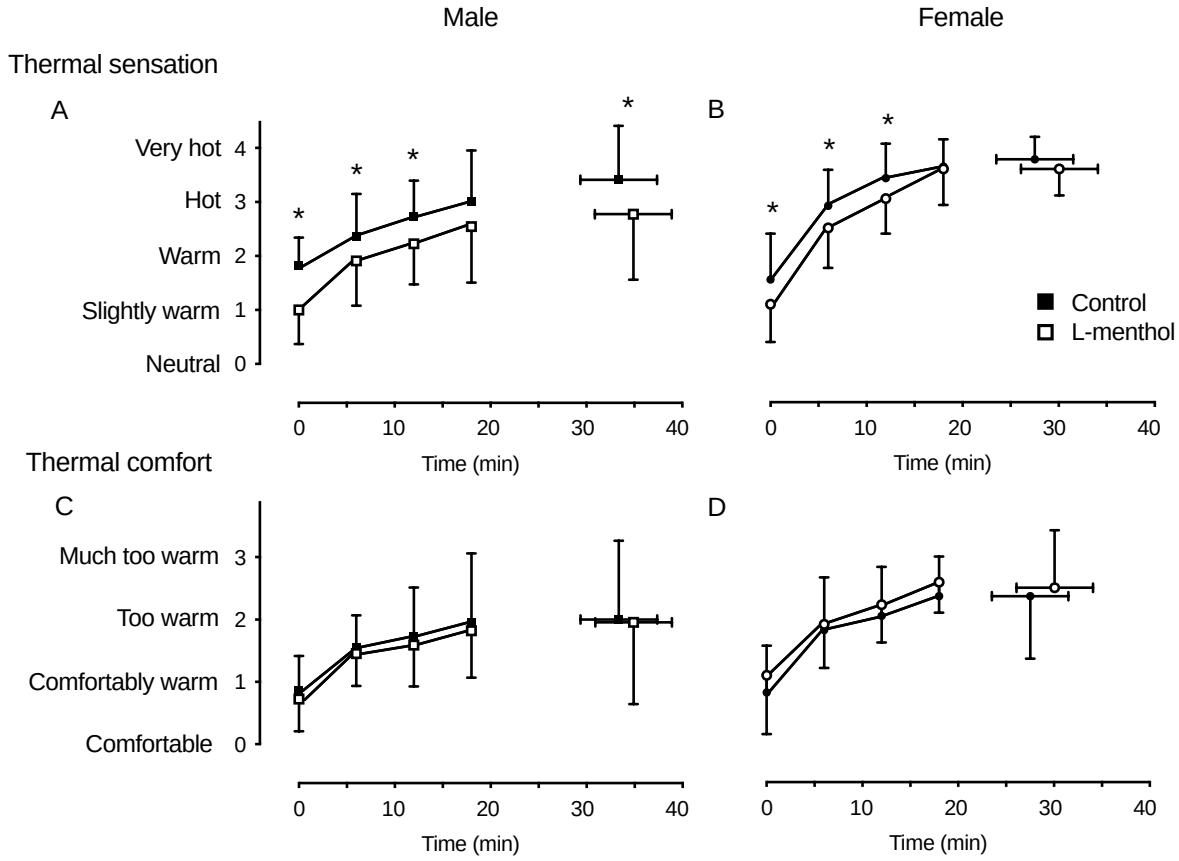
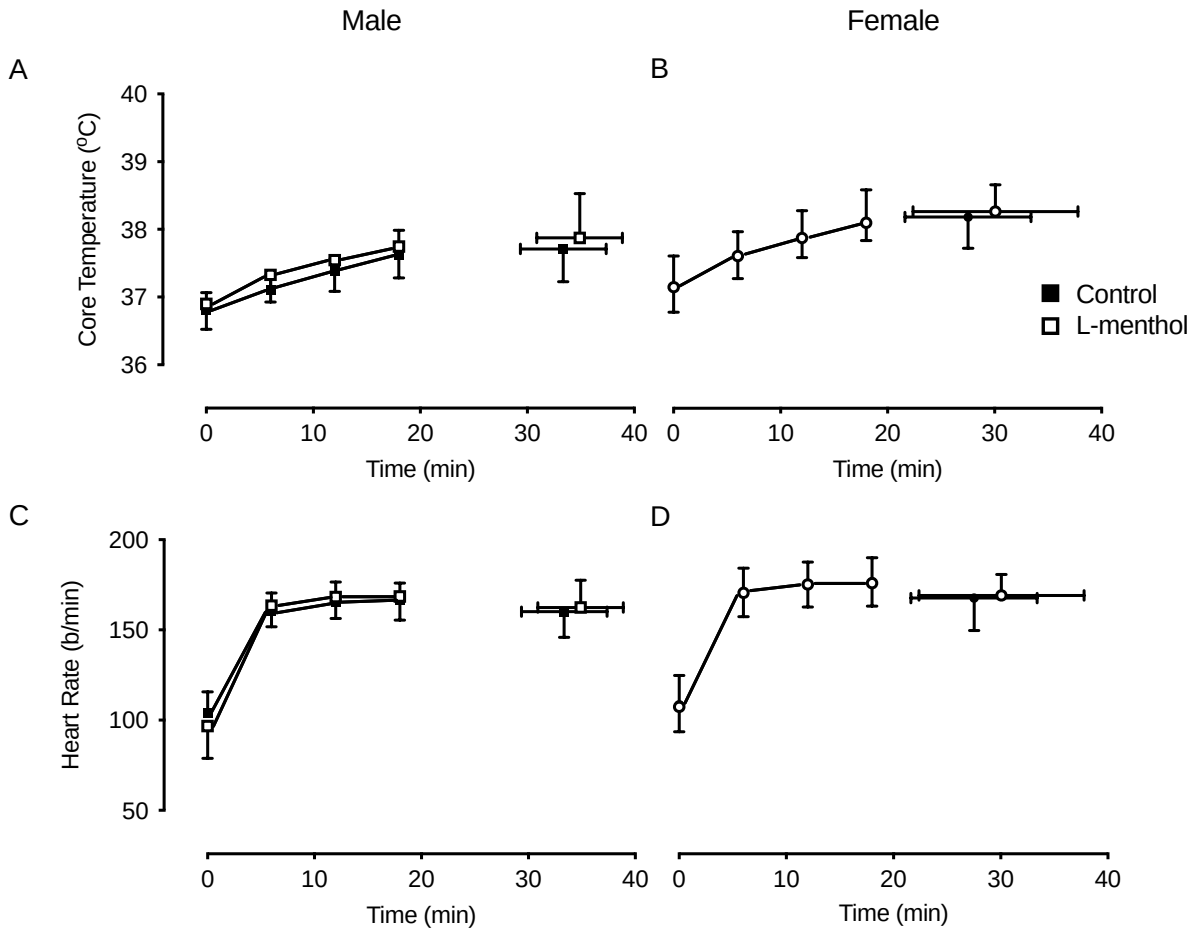

Male
Female
Thermal sensation
A
B
Very hot 4
Hot 3
Warm 2
Slightly warm 1
Neutral 0
*
Control
L-menthol
0 10 20 30 40
Time (min)
Thermal comfort
C
D
Much too warm 3
Too warm 2
Comfortably warm 1
Comfortable 0

Figure 3


Male
Female
A
B
C
D
Core Temperature (ºC)
Heart Rate (b/min)
Time (min)
Control
L-menthol