

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

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27 **Running head:**

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31

32 **Abstract**

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

36
37 *Methods:* Twenty-two participants (eleven females, eleven males) completed two trials using a fixed-RPE
38 protocol at an exercise intensity between 'hard' and 'very hard', equating to 16 on the RPE scale at ~35 °C.
39 Participants adjusted power output to maintain RPE-16. In a randomised, double-blind, crossover design,
40 L-menthol or a control mouthwash was administered at an orally neutral temperature (~32 °C) prior to
41 exercise and at 10 min intervals thereafter. Measures of mechanical power output, core temperature,
42 heart rate, perception of thermal sensation and thermal comfort, and whole-body sweat loss are reported.

43
44 *Results:* Thermal sensation was lowered by L-menthol in both sexes ($P < 0.05$), however during exercise
45 this was only maintained for 40% of the trial duration in females. Thermal comfort did not differ between
46 conditions ($P > 0.05$). No differences in exercise duration were observed compared to control, despite a ~4
47 % and ~6 % increase in male and females respectively. Power output increased by ~6.5 % males ($P = 0.039$)
48 with no difference in females ~2.2% ($P = 0.475$), compared to control. Core temperature, heart rate and
49 whole-body sweat loss was not different between condition or sex.

50
51 *Conclusions:* L-menthol lowered perceptual measures of thermal sensation in females, but did not
52 attenuate a greater rate of rise in thermal sensitivity when exercising in a hot environment, compared to
53 males. Males appeared to adopt a higher risk strategy by increasing power output following L-menthol
54 administration in contrast to a more conservative pacing strategy in females. Therefore, there appear to
55 be sex-specific differences in L-menthol's non-thermal cooling properties and subsequent effects on
56 thermo-behavioural adjustments in work-load when exercising in a hot environment.

57

58 **Introduction**

59

60 There are a number of reported sex differences in thermoregulatory responses to exercise in hot
61 environments (Fox et al., 1969; Gagnon and Kenny, 2012; Shapiro et al., 1980; Smith and Havenith, 2012).
62 In eumenorrhic women, core temperature displays a biphasic rhythm across the menstrual cycle, with ~
63 0.4 °C increase during the post-ovulatory luteal phase (Marshall, 1963) due to a change in
64 thermoregulatory set point (Inoue et al., 2005; Pivarnik et al., 1992; Tenaglia et al., 1999). As a result, the
65 threshold for thermoregulatory effector responses is increased (Inoue et al., 2005; Kolka and Stephenson,
66 1997; Stachenfeld et al., 2000), and an increase in cardiorespiratory strain has been reported (Janse de
67 Jonge, 2003; Pivarnik et al., 1992). Indeed, heat tolerance is reduced by ~6-16 % during exercise tasks
68 performed in the mid-luteal phase when compared to the early follicular phase (Avellini et al., 1980; De
69 Jonge et al., 2012; Tenaglia et al., 1999). Hormonal contraceptive use is prevalent in females and female
70 athletes (Martin et al., 2018; Rechichi et al., 2009) where suppression of endogenous hormone
71 concentrations inhibits ovulation. However, phase-related changes in core temperature and effector
72 responses are still apparent (~0.15 °C) (Grucza et al., 1993; Lei et al., 2019) and therefore should still be
73 considered when examining strategies to enhance heat tolerance in females using hormonal
74 contraceptives.

75 Behavioural thermoregulation is the first strategy to defend against a disruption in heat balance in hot
76 environments, secondary to changes in body temperature (Flouris and Schlader, 2015). Subjective
77 responses to physical activity, which include perceived exertion, thermal sensation and sensation of pain,
78 are known to vary in females according to menstrual cycle phase (Gerrett et al., 2014; Hooper et al., 2011;
79 Travlos and Marisi, 1996). In hot conditions, alteration of thermal sensitivity leads to behavioural
80 reductions in exercise intensity following stimulation of peripheral thermoreceptors, which demonstrate
81 regional sensitivity (Nakamura et al., 2008). However, there is clear variation in thermal sensitivity between
82 sexes (Gerrett et al., 2014), with females able to detect warm (Gerrett et al., 2014; Golja et al., 2003;
83 Lautenbacher and Strian, 1991) and cold stimuli (Golja et al., 2003) more strongly than males, independent
84 of changes in body temperature. Higher sensitivity is reported around the head regions with respect to the
85 extremities (Gerrett et al., 2014); however, the oral cavity is one of the most densely innervated parts of
86 the body in terms of peripheral receptors (Haggard and de Boer, 2014). Limited research has been
87 presented on sex differences in oral sensitivity, with one study reporting no differences in relation to sex
88 or phases of the menstrual cycle (Abe et al., 2012). We have recently shown that ice slushy or L-menthol
89 oral mouth-rinsing during advanced thermal stress can extend exercise performance (a conscious
90 behaviour), despite no change in body temperature, in males (Jeffries et al., 2018). Female participants are
91 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).

113 **Materials and methods**

114

115 **Participants**

116

117 Twenty-two non-acclimated participants, comprising eleven females (age = 22 ± 2 years; body mass = 65.3
118 ± 4.0 kg; stature = 167.6 ± 4.2 cm; maximal oxygen uptake, $\dot{V}O_{2\max} = 43.5 \pm 2.9$ ml.min.kg⁻¹) and eleven
119 males (age = 20 ± 1 years; body mass = 77.7 ± 8.9 kg; stature = 180.0 ± 6.0 cm; maximal oxygen uptake,
120 $\dot{V}O_{2\max} = 53.9 \pm 6.9$ ml.min.kg⁻¹) consented to take part in this study. A priori sample size was calculated
121 using G*Power (version 3.1.9.6). Given the effect size ($\eta_p^2 = 0.896$; (Flood et al., 2017)) we reported
122 previously for differences in power output using an RPE-16 protocol with L-menthol, a sample size of ten
123 was deemed sufficient to identify differences between groups with a statistical power of 0.95. We recruited
124 eleven participants to account for experimental mortality. Participants engaged in regular physical activity
125 < 5-h per week. None of the participants had visited a hot country in the previous three months, all resided
126 in the UK and experiments were conducted in one block during the winter months of January - March.
127 Participants were instructed to avoid consumption of alcohol or caffeinated products for 24-h before each
128 visit, as well as strenuous exercise 48-h before testing and to arrive fully hydrated. Ethical approval was
129 provided by Newcastle University ethics committee, which was conducted in accordance with the 1964
130 Helsinki declaration.

131

132 **Study design**

133

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

145

146 **Experimental procedures**

147

148 *Menstrual phase determination*

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

163

164 *Preliminary testing*

165 Participants reported to the laboratory to conduct preliminary testing consisting of anthropometric
166 measurements and an incremental ramp test. Participants then performed a self-paced warm-up for 5-min
167 and were asked to select a preferred cadence that was standardised throughout the remaining
168 experimental trials. The incremental ramp test began at 100 W, and work-load increased in one-min stages
169 at a rate of $25 \text{ W}\cdot\text{min}^{-1}$ until volitional fatigue. All testing was conducted on an electronically-braked cycle
170 ergometer (Velotron Racermate, USA). Expired gases were analysed using the Douglas bag method.
171 Expired gases were collected by a mouthpiece connected to a 2-way Hans-Rudolph breathing valve (27000
172 series) (Hans Rudolph, inc. USA) and a 2-meter corrugated hose over a collection period ~ 45 -s. At the end
173 of the collection period, gas fractions ($F_{\text{E}O_2}$ and $F_{\text{E}CO_2}$) were analysed (Servomex, 5200 MiniIMP, UK),
174 volume of expired air (Harvard Apparatus, Kent, UK) and air temperature were measured for calculation
175 of $\dot{V}O_{2\text{max}}$ by indirect calorimetry. All values were corrected to reflect standard temperature and pressures.
176 $\dot{V}O_{2\text{max}}$ was determined as the highest average 30-s value obtained. RPE was recorded at the end of each
177 1-min stage by pointing to a 15-grade RPE scale held by an investigator. Following a 15-min rest period,
178 two familiarisation exercises were conducted which were subsequently used with the intention of
179 calibrating the participant's RPE-based selection of power output in the main trials. In the first exercise,
180 participants conducted incremental ramp steps in accordance with the power output / RPE relationship
181 derived from the incremental ramp test. The steps followed the order: RPE 11 for 4-min, RPE 13 for 3-min,
182 and RPE 15 for 2-min. Participants were blinded to the RPE and asked to rate their own RPE to aid
183 familiarisation with the RPE scale. The second exercise began at 110 W and involved participants controlling

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

190

191 *Experimental trials*

192 Participants performed two randomised experimental trials in an environmental heat chamber in
193 temperatures of 34.9 ± 0.5 °C and relative humidity 40.6 ± 2.2 %, separated by at least 72-h. For each
194 participant, the experimental trials were conducted at the same time of day to eliminate the effect of
195 circadian variation. Euhydration was established prior to exercise by identifying urine osmolality < 715
196 mOsm/Kg H₂O (Shirreffs and Maughan, 1998) (Pocket Osmocheck, Vitech Scientific Ltd, West Sussex, UK)
197 and average hydration was 388.8 ± 243.5 mOsmols/kg H₂O, across both conditions. Participants were
198 instrumented with a heart rate chest strap then entered the heat chamber, resting for 10 minutes before
199 baseline measures were recorded. Participants then conducted a standardised warm-up procedure, as
200 outlined previously in the second familiarisation exercise, ramping to an RPE-16 over a 5 min period.
201 Following 5-min of seated rest, participants then started the fixed-RPE protocol.

202

203 *Fixed-RPE protocol*

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

213

214 **Measurements**

215

216 *Physiological measures*

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

219 Thermoscan IRT 6020, UK) underestimates rectal temperature by 0.5 ± 0.3 °C but correlates strongly ($R^2 =$
220 0.92) across a range of sub-maximal exercise intensities and environmental conditions. Participants
221 recorded semi-nude (males: shorts; females: shorts and sports bra) body mass prior to entering the heat
222 chamber and immediately following the completion of the experimental trial after wiping off sweat with a
223 towel. No water was ingested during exercise in the heat. Heart rate was recorded continuously throughout
224 the trials (Polar T31, UK) transmitting data onto a portable watch (Polar FT7, UK).

225

226 *Perceptual measures*

227 Participants were thoroughly briefed on the RPE scale during familiarisation sessions before commencing
228 the fixed RPE trials as we have previously reported (please see for full description: Flood et al., 2017).
229 Briefly, participants were instructed to pay close attention to how difficult the exercise felt, combining total
230 exertion, fatigue, and physical stress in the heat, without considering one particular factor such as leg pain,
231 shortness of breath or anticipation of how they might feel several minutes later. In addition, participants
232 were familiarised with the thermal sensation scale and thermal comfort scale. Laminated scales were held
233 in front of the participants during exercise and they were asked to indicate thermal comfort and sensation
234 by pointing to the appropriate point on the scale. Thermal comfort (TC) was recorded on the Bedford 7-
235 point analogue scale where -3 = “much too cool”, 0 = “comfortable”, and 3 = “much too warm” (Bedford,
236 1936). Thermal sensation (TS) was recorded on an adapted ASHRAE 9-point analogue sensation scale where
237 -4 = “very cold”, 0 = “neutral”, and 4 = “very hot” (Zhang et al., 2004). Subjective ratings were recorded in
238 1.0 increments every 5 min during the experimental trials.

239

240 *Mouth rinse formulation*

241 Participants were given 25 ml solution to rinse 30-s prior to the main fixed RPE trial and at regular 10-min
242 intervals (therefore delivered at -0:30, 9:30 and 19:30 min etc). They were instructed to swill around the
243 mouth for 10-s before spitting into a bowl without swallowing. L-menthol solution was formulated from
244 menthol crystals ($\geq 99\%$ food grade L-menthol, Sigma-Aldrich, UK) dissolved in de-ionised water heated to
245 ~ 50 °C at a concentration of 0.64 mM (0.01 %) (Flood et al., 2017). The solution was then stored at 5 °C for
246 up to 1 month. Prior to use, solutions were aliquoted for mouth-rinse and warmed to ambient laboratory
247 temperature 31.8 ± 2.3 °C which was confirmed by a standard thermometer and recorded. A control mouth
248 rinse was made using an apple flavoured non-calorific artificial sweetener, consisting of sucralose
249 (FlavDrops, MyProtein, Norwich, UK) dissolved in 25 ml of deionised water and warmed to 32.1 ± 1.2 °C
250 (Flood et al., 2017).

251

252 **Statistical analysis**

253

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

271

272 **Results**

273 *Exercise performance in males and females*

274

275 Data for time and power output were normally distributed and showed no trial order effect ($P > 0.05$).
276 During the pre-experimental warm-up were participants were instructed to self-select an RPE of 16 over
277 3-min from a starting intensity of 110 W, the final power output selected was not different between
278 condition or sexes ($F_{(1,20)} = 0.019, P = 0.893; \eta_p^2 = 0.001$), despite an observable ~30 W average difference
279 between males (L-menthol: 170 ± 32 W; Control: 170 ± 25 W) and females (L-menthol: 139 ± 15 W; Control:
280 141 ± 22 W). Trial duration was not different between sex ($F_{(1,20)} = 1.119, P = 0.303; \eta_p^2 = 0.053$) or condition
281 ($F_{(1,20)} = 0.070, P = 0.794; \eta_p^2 = 0.003$). However, in males (L-menthol: $34:54 \pm 10:27$ min; Control: $33:22 \pm$
282 $10:36$ min) there was a nominal ~4 % (92-s) increase in exercise time in the L-menthol condition and in
283 females (L-menthol: $29:42 \pm 7:43$ min; Control: $27:51 \pm 5:52$ min) a ~6 % (111-s) increase in exercise time
284 in the L-menthol condition.

285

286 *** Insert Figure 1 near here ***

287

288 Average power output across the trial was different between condition ($F_{(1,20)} = 5.917, P = 0.025; \eta_p^2 =$
289 0.228), however the interaction effect indicated no differences between average power output in each
290 condition and sex ($F_{(1,20)} = 1.137, P = 0.299; \eta_p^2 = 0.054$). Power output decreased with time in males ($F_{(10,100)}$
291 $= 122.114, P = 0.000; \eta_p^2 = 0.924$) and females ($F_{(2.117,21.165)} = 11.294, P > 0.001; \eta_p^2 = 0.530$). Across the trial,
292 power output was higher in males in the L-menthol condition (L-menthol: 160 ± 26 W, Control: 150 ± 26 W
293 (~6.5 %), ($F_{(1,10)} = 5.018, P = 0.039; \eta_p^2 = 0.334$) with an interaction effect ($F_{(10,100)} = 2.016, P = 0.037, \eta_p^2 =$
294 0.168) (Figure 1A). However, in females there was no difference between conditions (L-menthol: 127 ± 11
295 W, Control: 124 ± 14 W (~2.2%), ($F_{(1,10)} = 0.552, P = 0.475; \eta_p^2 = 0.052$) and no interaction effect ($F_{(2.242,22.4525)}$
296 $= 0.801, P = 0.474; \eta_p^2 = 0.074$) (Figure 1A). During the first 10% of the exercise task, all participants
297 achieved their peak power output which was different between males and females ($t_{(10)} = -4.083, P = 0.002,$
298 $d = 1.44$). In males, self-selected peak power was ~6 % higher in the L-menthol condition, with 8 out of 11
299 participants selecting a higher power output ($t_{(10)} = -2.247, P = 0.048, d = 0.38$) (Figure 1B). However, no
300 significant difference in peak power (~2%) was observed for females ($t_{(10)} = -0.627, P = 0.545, d = 0.15$)
301 (Figure 1B).

302

303 *** Insert Figure 2 near here ***

304

305 *Subjective measures of thermal perception*

306

307

308 Perceptual measures of thermal sensation increased with time in all conditions for males and females ($P <$
309 0.001). However, when collapsed for time there were differences between males and females ($z = -2.357,$
310 $P = 0.018, d = 0.71$), with males reporting on average ~ 0.6 points lower on the scale for thermal sensation
311 across both trials. In males, thermal sensation was lowered in the L-menthol condition across the entire
312 trial, except at the 18-min time point (Start -0.81 ($z = -2.714; P = 0.007; d = 0.82$), 6-min -0.45 ($z = -2.236; P$
313 $= 0.025; d = 0.67$), 12-min -0.50 ($z = -2.049; P = 0.04; d = 0.61$), 18-min -0.45 ($z = -1.492; P = 0.136; d = 0.44,$
314 End -0.63 ($z = -1.897; P = 0.05, d = 0.60$)) (Figure 2A). In females, thermal sensation was lower only across
315 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 $-$
316 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 ($z = -0.333;$
317 $P = 0.739; d = 0.10$), End -0.18 ($z = -0.973; P = 0.330; d = 0.29$)) (Figure 2B). The rate at which thermal
318 sensation increased across the first 18-min of the exercise trials was faster in females (L-menthol 0.12
319 $\text{units}/\text{min}^{-1}, R^2 = 0.87$; Control $0.13 \text{ units}/\text{min}^{-1}, R^2 = 0.94$) compared to males (L-menthol $0.07 \text{ units}/\text{min}^{-1},$
320 $R^2 = 0.93$; Control $0.08 \text{ units}/\text{min}^{-1}, R^2 = 0.92$), ($t_{(10)} = -2.294, P = 0.045, d = 0.97$). Thermal comfort increased
321 on the scale, denoting greater discomfort, across time in all conditions for males and females ($P < 0.001$).
322 However, there were no differences at any time point for L-menthol and control conditions or between sex
323 ($P > 0.05$) (Figure 2 C&D).

324

325

*** Insert Figure 3 near here ***

326

327 *Physiological responses*

328

329 Core temperature after the standardized warm-up was not different between conditions in males (L-
330 menthol: 36.9 ± 0.4 °C; Control: 36.9 ± 0.3 °C) and females (L-menthol: 37.1 ± 0.4 °C; Control: 37.2 ± 0.4 °C)
331 ($P > 0.05$). Core temperature increased with time in males ($F_{(4,40)} = 4.038, P < 0.001; \eta_p^2 = 0.905$) and
332 females ($F_{(1.53,15.33)} = 30.40, P < 0.001; \eta_p^2 = 0.752$) but with no difference between condition for males ($F_{(1,10)} = 0.067, P = 0.801; \eta_p^2 = 0.007$) and females ($F_{(1,10)} = 2.740, P = 0.129; \eta_p^2 = 0.215$) (Figure 3 A&B).
333 Heart rate increased with time in males ($F_{(1.25,12.46)} = 223.78, P < 0.001; \eta_p^2 = 0.957$) and females $F_{(1.78,17.79)}$
334 $= 371.11, P < 0.001; \eta_p^2 = 0.974$) but with no difference between conditions for males ($F_{(1,10)} = 0.018, P =$
335 $0.897; \eta_p^2 = 0.002$) and females ($F_{(1,10)} = 0.001, P = 0.992; \eta_p^2 = 0.030$) (Figure 3 C&D). The change in body
336 mass was not different between pre-to-post for the exercise task in males (Pre: 0.69 ± 0.3 kg; Post: $0.68 \pm$
337 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) ($t_{(10)} = -0.582,$
338 $P = 0.574, d = 0.24$). There were no differences between sex ($F_{(1,20)} = 0.179, P = 0.179; \eta_p^2 = 0.676$).

340

341

342

343 **Discussion**

344

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

358 Research examining sex-specific differences in thermal sensitivity tends to be largely confined to males. In
359 the present study we tested male and female participants and controlled for potential differences in
360 thermoregulation ascribed to the menstrual cycle (De Jonge et al., 2012; Marshall, 1963) by testing females
361 during a calculated quasi-follicular phase. Baseline measures in both sexes confirmed that there were no
362 differences in core temperature and no differences in the rise in core temperature during exercise (Figure
363 3 A&B). Thermal sensitivity encompasses the perceived intensity of temperature being sensed by the
364 individual (Gagge et al., 1967). Psychological strategies that are effective at reducing thermal sensitivity
365 have been successful in extending exercise tolerance in the heat (Cheung, 2010; Flouris and Schlader,
366 2015). We utilised a non-thermal cooling L-menthol mouth-rinse which was effective at reducing thermal
367 sensation across the majority of the exercise test in males. However a significantly smaller reduction in
368 thermal sensation was observed in females, indicating sex-specific differences in L-menthol's effectiveness
369 during exercise in a hot environment. Indeed, L-menthol induced reductions in thermal sensitivity were
370 only observed over the first 12 minutes of exercise in females. During exercise, the rise in perceived thermal
371 sensation was faster in females than in males, reflecting a greater thermal sensitivity which did not differ
372 between condition. That L-menthol was unable to modify this increase in thermal sensation in both sexes
373 supports a possible reduced potency with subsequent administration that we have noted before (Flood et
374 al., 2017). However, thermal sensitivity is also known to decrease during exercise (Gerrett et al., 2015;
375 Ouzzahra et al., 2012), due to a reduction in transmission of sensory information along afferent fibres via
376 exercise-induced analgesia (EIA) (Koltyn, 2000), therefore transmission of thermal sensory information
377 may be reduced. Limited research exists investigating sex-specific differences in EIA with only one study

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

380 Thermo-behavioural adjustments in work-load during the trials, enabled changes in perceived exertion to
381 be observed using the fixed-RPE protocol. Males voluntarily adopted a higher power output (~6.5%) after
382 rinsing with L-menthol which supported our previous observations (~4%) (Flood et al., 2017). However, in
383 females, no difference in power output (>2%) following L-menthol-rinsing was observed. This was not
384 anticipated in our initial hypothesis. That a lowering of thermal sensation was reported across both sexes
385 in the early stages of the exercise trial suggests that the effectiveness of L-menthol in inducing non-thermal
386 cooling cannot explain this discrepancy. Despite no significant changes, exercise time was extended in
387 males (~4%) and females (~6 %). It is also unclear how L-menthol may have extended exercise performance
388 in females compared to control conditions when any change in thermal sensation had dissipated beyond
389 ~40 % of the exercise trial. Females are reported use thermal behaviour (such as modification of work-
390 load) to a greater extent than males during exercise (Vargas et al., 2019) which may suggest that a more
391 conservative pacing strategy was adopted. We have also previously proposed that L-menthol could act as
392 a potential distractor to moderately uncomfortable stimuli, such as exercise in a hot environment,
393 irrespective of its cooling properties (Jeffries et al., 2018), which is possible. It should be noted that exercise
394 duration is an arbitrary measure of performance when using a fixed-RPE protocol and should be carefully
395 interpreted. In females, it is possible that by not increasing power output, despite reporting a reduction in
396 thermal sensation, exercise duration could be extended by consciously adopting a more conservative
397 pacing strategy. If we approximate energy utilised (work done) by multiplying exercise duration (s) by
398 power (J/s) across participants, in the L-menthol trial total work done was increased by 7% in males and
399 8% in females relative to the control trial. Therefore, the pacing strategy adopted by males in the L-menthol
400 condition was inherently more aggressive by selecting a higher power output and yet this did not extend
401 total work done beyond the more conservative strategy adopted by females. Typically, males exhibit
402 different self-pacing strategies when compared to females. In repeated sprint study designs, males self-
403 pace at higher exercise intensities, achieve higher total work and show greater power decrements than
404 women, despite comparable cardiovascular strain (Billaut and Bishop, 2012; Panissa et al., 2016). The noted
405 increase in fatigue is likely to be a consequence of their greater absolute initial sprint performance, rather
406 than a sex-specific difference in fatigue (Billaut and Bishop, 2012). Behaviourally, in the case of decision
407 making, males also appear to adopt a higher risk strategy based on physical fitness, or an alteration in
408 motivation to perform exercise, compared to females (Deaner et al., 2015). When examining competition
409 data, sex differences in marathon pacing in non-elites was larger for males in the 2007 Chicago marathon
410 which was hot (27 °C) when compared to the 2009 Chicago marathon which was cool (3 °C), illustrating the
411 greater propensity for a risky pacing strategy, despite unfavourable environmental conditions (Deaner et
412 al., 2015).

413 Our findings refute our initial hypothesis that greater thermal sensitivities in females (Gerrett et al., 2014,
414 2015) and greater sensitivity to cold stimuli when compared to males (Gerrett et al., 2015) would
415 potentiate L-menthol's effect. The oral cavity is one of the most densely innervated parts of the body in
416 terms of peripheral receptors (Haggard and de Boer, 2014). Mouth-rinsing with L-menthol activates
417 peripheral TRPM8 thermoreceptors on the oral mucosa transmitting information via the trigeminal system
418 which mediates sensations such as burning, cooling and tingling (Laska et al., 1997). Despite reported sex-
419 related differences in chemosensation, examination of irritants, including menthol, have failed to report
420 sex-specific differences in trigeminal sensitivity (Ohla and Lundström, 2013). Psychophysical tests have
421 identified that these differences may be due to differing cognitive appraisal between the sexes, therefore
422 altering subjective perception (Lundström et al., 2005; Ohla and Lundström, 2013). However, in this study
423 females reported a smaller reduction in thermal sensation following L-menthol mouth-rinsing, than males,
424 which then dissipated. In thermally challenging environments, females tend to be more sensitive to warm
425 stimuli than males and perceive a thermal stimulus to be hotter (Gerrett et al., 2014, 2015). Therefore the
426 hot environment may have been perceived as a greater thermal threat thereby reducing or de-prioritising
427 L-menthol's perceived cooling properties. At present it is clear that further research is required to
428 understand these sex differences in behavioural thermoregulation.

429 Although not a primary aim of this study we administered L-menthol in a thermally neutral solution ~32
430 °C. This was important to experimentally establish L-menthol's efficacy when oral cooling facilitated by the
431 delivery solution was removed. We and others have administered L-menthol in oral rinses between 19-23
432 °C, in males (Flood et al., 2017; Mündel and Jones, 2010; Riera et al., 2014; Stevens et al., 2015, 2016) and
433 one study at ~40 °C (Gibson et al., 2019). Perceived sensation of cold in the oral cavity reaches zero at ~32
434 °C (the cold threshold) with perception beginning to shift to warmth as liquid temperature increases above
435 ~35 °C (the warmth threshold), despite oral temperature being (~36 °C) (Green, 1986). Indeed L-menthol
436 solutions below oral temperature have been suggested to feel cooler than water of the same temperature
437 (Green, 1985). These have been demonstrated to potentiate exercise performance in hot humid conditions
438 following neutral (23 °C), cold (3 °C) and ice-slushy (-1 °C) beverage ingestion during a 20-km time-trial
439 (Riera et al., 2014). Therefore it was important to achieve oral temperature neutrality and therefore
440 solutions were administered at ~32 °C to isolate L-menthol's true non-thermal cooling properties. We can
441 confirm, as previously discussed, in males L-menthol was equally effective in enhancing exercise
442 performance in the heat when delivered at ~32 °C when compared to our previous study ~19 °C using
443 identical protocols and ambient conditions albeit a different participant group (Flood et al., 2017).
444 Unfortunately we are unable to make the same comparisons in females.

445

446 *Limitations*

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

464 *Conclusion*

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

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618 **Figure legends**

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

628

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

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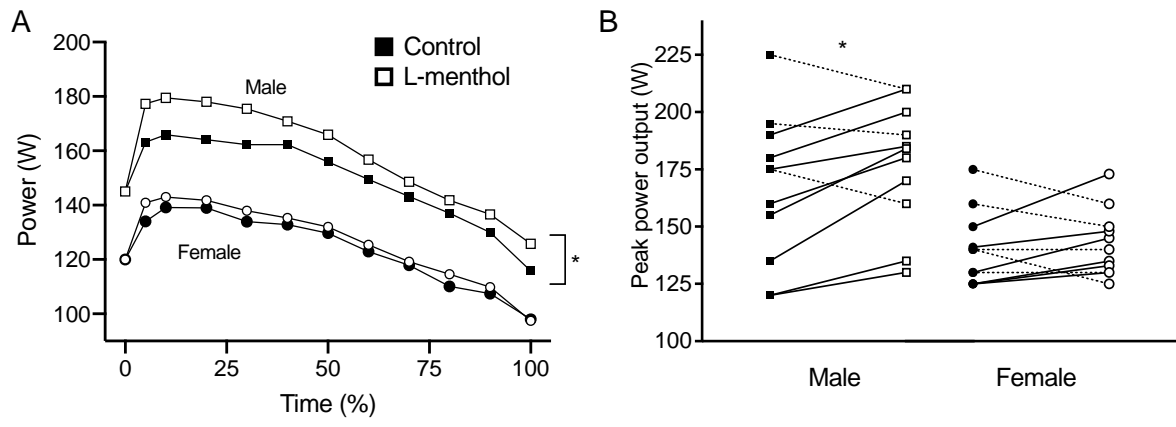
634 Figure 3. A. Core temperature during the fixed-RPE trial for males and B. females. C. Heart rate during the
635 fixed-RPE trial for males and D. females. Conditions are indicated by colour, L-menthol (white) and control
636 (black) and sex indicated on figures. All data are shown as mean \pm SD,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

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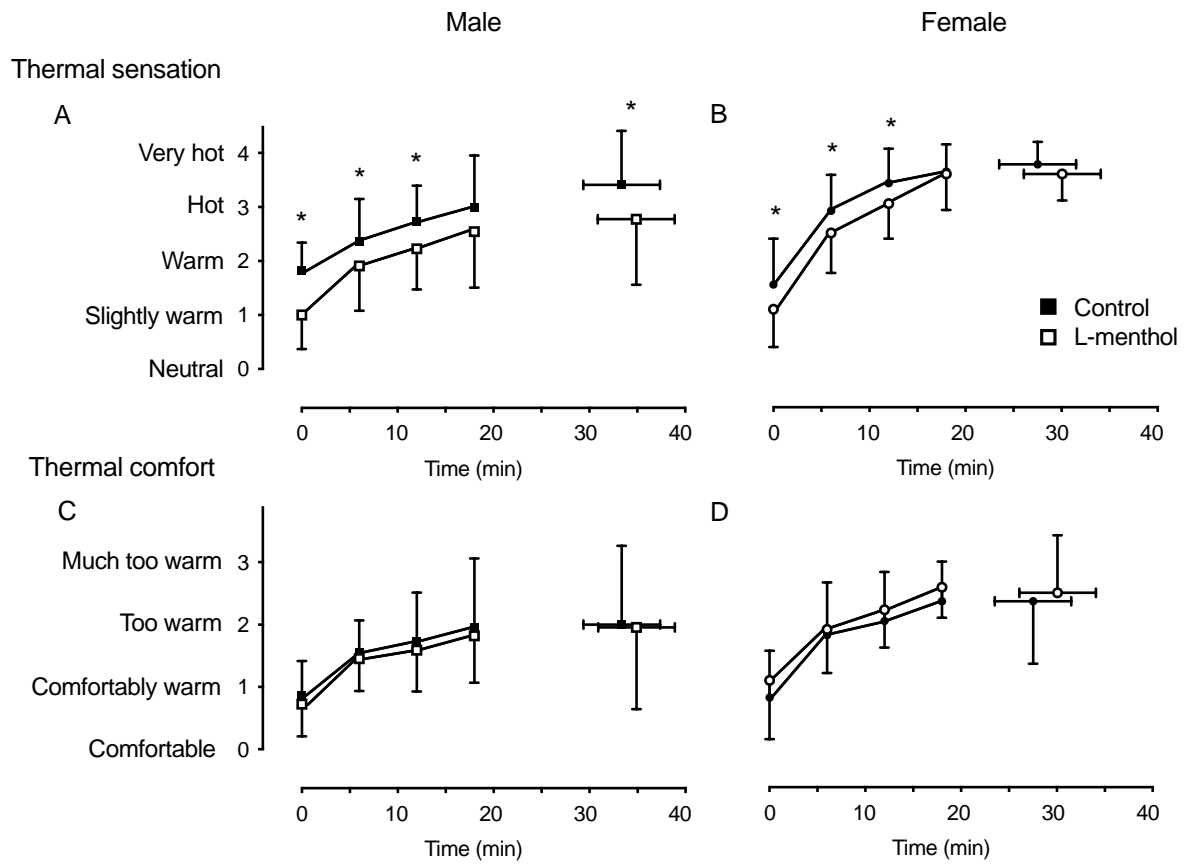
639 Declarations of interest: none
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641 Funding: Authors received no funding for this study
642

643 Figure 1
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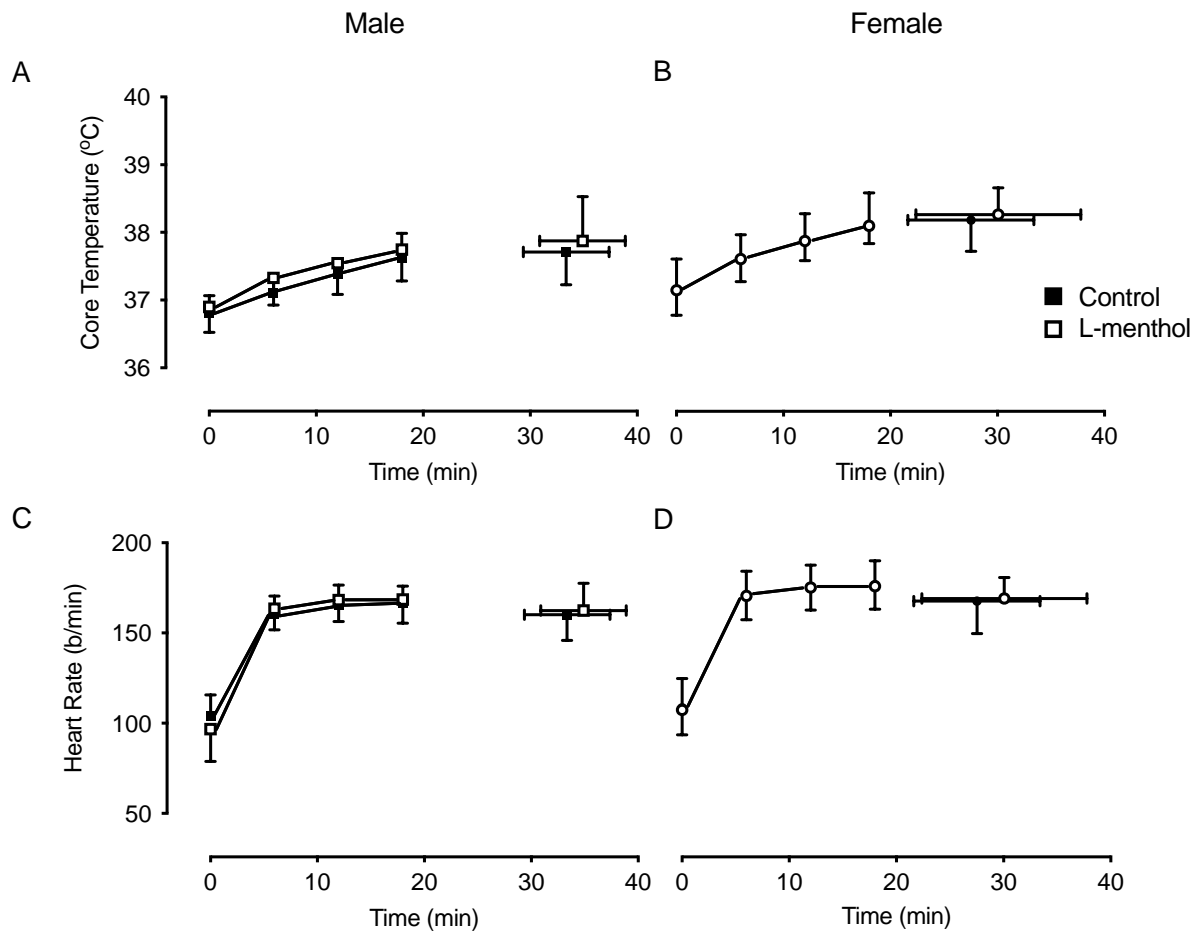
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