Title

The influence of a menthol and ethanol soaked garment on human temperature regulation and perception during exercise and rest in warm, humid conditions.

Authors

[1]Gillis, D. Jason., [2]Barwood, M.J., [3]Newton P.S, [4]House, J.R., [4]Tipton, M.J.

Author affiliations

[1]Human Performance Laboratory, Department of Sport and Movement Science, Salem State University, Salem, MA, 01970, USA

[2]Dept. Sport, Exercise and Rehabilitation, Northumbria University, Newcastle-Upon-Tyne, U.K

[3] Human Performance Research and Development, Canadian Forces Morale and Welfare Services, Ottawa, Ontario, Canada

[4]Extreme Environments Laboratory, Department of Sport and Exercise Science, Portsmouth University, Portsmouth, PO1 2ER UK

Corresponding author

Jason Gillis, Ph.D.

Assistant Professor of Exercise Science

Email. jason.gillis@salemstate.edu

Telephone. +1.978.542.7421

Abstract

This study assessed whether donning a garment saturated with menthol and ethanol (M/E) can improve evaporative cooling and thermal perceptions versus water (W) or nothing (CON) during low intensity exercise and rest in warm, humid conditions often encountered in recreational/occupational settings. It was hypothesised there would be no difference in rectal (Tre) and skin (Tsk) temperature, infra-red thermal imagery of the chest/back, thermal comfort (TC) and rating of perceived exertion (RPE) between M/E, W and CON, but participants would feel cooler in M/E versus W or CON. **METHODS**: Six volunteers (mean [SD] 22 [4] years, 72.4 [7.4] kg and 173.6 [3.7] cm) completed (separate days) three, 60-minute tests in 30°C, 70%rh, in a balanced order. After 15-minutes of seated rest participants donned a dry (CON) or 80mL soaked (M/E, W) long sleeve shirt appropriate to their intervention. They then undertook 30-minutes of low intensity stepping at a rate of 12 steps/minute on a 22.5cm box, followed by 15-minutes of seated rest. Measurements included heart rate (HR), Tre, Tsk (chest/back/forearm), thermal imaging (back/chest), thermal sensation (TS), TC and RPE. Data were reported every fifth minute as they changed from baseline and the area under the curves were compared by condition using one-way repeated measures ANOVA, with an alpha level of 0.05. **RESULTS**: Tre differed by condition, with the largest heat storage response observed in M/E (p<0.05). Skin temperature at the chest/back/forearm, and thermal imaging of the chest all differed by condition, with the greatest rate of heat loss observed in W and M/E respectively (p<0.01). Thermal sensation differed by condition, with the coolest sensations observed in M/E (p<0.001). No other differences were observed. **CONCLUSIONS**: Both M/E and W enhanced evaporative cooling compared CON, but M/E causes cooler sensations and a heat storage response, both of which are likely mediated by menthol.

Key words

Menthol, ethanol, human, thermoregulation, thermal sensation, thermal comfort

1. Introduction

In warm, humid conditions, the thermal gradient between the skin and environment is reduced, along with the capacity for both dry and evaporative heat loss. These factors, along with an elevation in metabolic heat production from exercise, have long been known to reduce work capacity (Rowell *et al.,* 1966). Thermoreceptors located within the body convey information about the accumulation of thermal energy to higher brain structures, and when mean body temperature rises uncontrollably, the cumulative neuronal input is thought to contribute to an inhibitory signal that lowers power output to protect the organism from heat injury (Nybo, 2010). Lessening the inhibitory signal during exercise in the heat may enhance, or help to maintain work. Given the inhibitory signal seems to be accentuated by warm thermoreceptor activation (Tucker *et al.,* 2006; Schlader *et al.,* 2011a, 2011b), it might be attenuated by the cold receptor activation that follows chemical or thermal stimulation. The purpose of this study was to assess whether donning a garment saturated with menthol and ethanol (M/E) can improve evaporative cooling and thermal perceptions versus water (W) or nothing (CON) during low intensity exercise and rest in warm, humid conditions that may be encountered in a recreational or occupational setting.

There is a broad literature assessing the effectiveness of various cooling interventions (ice vests, water immersion) during exercise in the heat, many of which are impractical during an actual sporting or working scenario (Barwood *et al.,* 2009; Cheung, 2010a; Duffield, 2008). Wetting the skin with water is a simple cooling strategy that can enhance evaporative heat loss and lower skin temperature during exercise in warm, humid conditions (Bassett *et al.,* 1987), and it may also reduce perceptions of heat stress and the requirement for sweat production. In an effort to enhance evaporative heat loss and lessen warm sensations in the heat, some commercial companies have added menthol and ethanol to their water-based skin cooling products. Menthol is a chemical compound that activates the cold receptor TRPM8 (McKemy *et al.,* 2002; Peier *et al.,* 2002) and elicits cool sensations when applied to the skin of heat stressed humans (Barwood *et al.,* 2012; 2014; 2015; Gillis *et al.,* 2010; 2015; Lee *et al.,* 2012). But menthol also induces a heat storage response that is in part mediated by a reduction in cutaneous skin blood flow (Gillis *et al.,* 2015) and possibly a withdrawal of sudomotor function i.e. a delay in the onset of sweating, or a reduction in sweat rate. (Kounalakis *et al.,* 2010). Ethanol, on the other hand is an alcohol that vaporises more quickly than water or sweat, and has the potential to increase the rate of evaporative heat loss from the skin (Godts *et al.,* 2005).

The benefit of wetting the skin with a water-based solution containing ethanol and/or menthol compared to water alone, or nothing at all, is not clear. Mujika *et al.,* (2010) provided highly trained rowers with forearm sweatbands soaked in either a cooling solution containing ethanol, menthol and water, or water alone (no Control condition), during an indoor 2000 m self-paced time trial. The authors observed no significant difference in perceived exertion, time to finish, or pacing strategy between the interventions. The evaporative cooling capacity of this intervention was perhaps limited because the surface area exposed to the solution was small (forearms only) and the sweat bands created an additional barrier to evaporative heat loss between the skin and the environment. Also, the possible negative influence of the ethanol/menthol solution on thermoregulation could not be assessed because the self-paced study design did not control for metabolic heat production. The question raised herein could be answered by applying an ethanol/menthol solution over a larger surface area to allow for greater heat exchange. Replacing the cotton sweat band with a lightweight breathable fabric garment may also improve the vapour pressure gradient between the skin and the air and increase evaporative heat loss. The thermoregulatory and perceptual influence of this intervention should be assessed during fixed work-rate exercise to control metabolic heat production. Given the dearth of research assessing the influence of an ethanol and menthol-based solution in humans, initial research should induce a light to moderate cardiovascular and thermoregulatory challenge to ensure participant safety.

In addition to the evaporative cooling potential attributed to ethanol, menthol, which is also contained within some cooling solutions, '*elicits cold sensations at otherwise indifferent skin temperatures'* (Hensel 1981, p.32), but also give rise to heat storage due in part to a reduction in skin blood flow (Gillis *et al.,* 2015) and possibly a withdrawal of sudomotor function (Kounalakis *et al.,* 2010). It is difficult to predict whether the theoretical improvement in evaporative cooling imparted by ethanol will outweigh the potential heat storage induced by menthol, and whether thermal perception will improve, or be impaired as a result. It remains unclear whether wetting the skin with a menthol/ethanol/water-based cooling solution absorbed into breathable garments may provide effective short and long term improvements in evaporative cooling and thermal perceptions.

The primary aim of this study was to assess whether donning a shirt soaked with a water-based solution containing menthol and ethanol could improve evaporative cooling and thermal perceptions compared to a water-only soaked shirt, or nothing at all, during rest and exercise in a warm, humid environment. It was hypothesised that there would be no difference (null hypothesis) in deep body temperature, thermal comfort and rating of perceived exertion between the menthol/ethanol skin wetting (M/E), water skin wetting (W), and a dry condition (CON) during rest or exercise, but participants would feel cooler in M/E compared to either W or CON (alternative hypothesis).

2. Methods

2.1. Participants

This experiment received ethical approval from the BioSciences Research Ethics Committee at the University of Portsmouth. Six volunteer participants took part in this within-participant repeated-measures study design, with a mean (SD) age, mass and height of 22 (4) years, 72.4 (7.4) kg and 173.6 (3.7) cm respectively.

2.2. Experimental protocol

Participants completed three, 60-minute tests in warm, humid conditions (30 °C, 70 % rh). In order to safely assess the effectiveness of the ethanol/menthol solution in humans, a light to moderate cardiorespiratory and thermoregulatory challenge was chosen. Such activity may be comparable to that undertaken by recreational gym users, or those undertaking walking/hiking exercise for extended periods in warm, humid conditions. From an occupational perspective those working underground (i.e. mining) may also be exposed to warm conditions whilst completing moderate exercise for the duration of a shift. Each test began with 15-minutes of seated rest followed by a 30-minute period when participants engaged in low intensity stepping exercise at a rate of 12 steps per minute onto a 22.5 cm box, and ending with another 15-minutes of seated rest.

During each test participants were assigned in a balanced order to one of three different conditions consisting of long sleeve sports shirts (breathable 100 % polyester) soaked with either 80 mL of 0.2 % menthol + 20 % ethanol (M/E), 80 mL of water alone (W) or an un-soaked dry shirt serving as a Control (CON); otherwise participants wore shorts and trainers.

2.3. Measurements

Participants arrived at the laboratory, were weighed naked and equipped with a heart rate (HR) monitor (Team System Polar, UK). They then self-inserted a calibrated rectal thermistor (Grant Instruments, Cambridge Ltd., Royston, UK) 15 cm beyond their anal sphincter. Three calibrated skin thermistors (Grant Instruments, Cambridge, UK) were secured by single pieces of adhesive tape (Tegaderm™ Film, 3M, UK) at the right chest, left scapula and right forearm. An estimation of upper body mean skin temperature was obtained using a thermographic camera, which captured images of the back and upper torso/chest. The thermal imaging camera (A320 series, ThermaCAM™, FLIR systems, Kent, UK) captured images of shirtless participants in the infra-red spectral range of 7.5 µm to 13 µm, with a temperature range from minus 20 °C to 120 °C and an accuracy of 2 %. At 25 °C the camera had a sensitivity of 0.07 °C, and a focal plane array containing 320 x 240 pixels. Thermal images were analysed using proprietary software (Researcher 2.9, FLIR systems, Kent, UK), which allowed the user to select a region of interest *i.e.* chest/front torso (from the nipple line to the umbilicus), or back (from the shoulders to the height of the umbilicus), and obtain a mean surface temperature from that region. Skin and rectal temperatures were recorded on an electronic data logger (Squirrel 1000/1250 series, Grant Instruments, Cambridge, Ltd., Royston, UK) each minute during testing. Environmental wet-bulb globe temperature was measured and recorded every minute throughout the experiment (Grant Instruments, Cambridge, UK). Laminated paper scales for thermal sensation (TS) and thermal comfort (TC) (Zhang, 2003), rating of perceived exertion (RPE, Borg, 1982) were held in front of participants at minute 3, 13, 25, 35, 45 and 55 throughout the test to establish the perceptual responses.

2.4. Description the water and menthol/ethanol solutions

The ethanol/menthol solution was a proprietary blend made by Physicool Ltd™ (London, U.K.) and was composed of 0.2 % (16.8 mg) menthol, 20 % (16 mL) ethanol, combined with 64 mL of water; as menthol is not soluble in water, the ethanol suspended the menthol in solution. When applied on the upper body (excluding the hands, head and neck), which represents approximately 55 % of the total surface area (Yu *et al.,* 2010), this equated to 1.68 mg of menthol per 100 cm^2 surface area for the average male with a total body surface area of 1.76 m^2. The water-only condition used tap water. All solutions were stored at room temperature (approximately 20 ˚C) and transferred into the environmental chamber three hours before testing, where they remained until they were applied. The water or menthol/ethanol solutions were measured in a graduated cylinder to 64 mL and poured in a sealed waterproof pouch. Long sleeve breathable shirts were then placed in the sealed pouch and soaked with the intervention-specific liquid until all fluid was absorbed into the fabric. Participants then donned the shirts.

2.5. Statistical analyses

Dependent variables were reported in figures every fifth minute as they changed (Δ) from baseline. The area under the curve was calculated 1) as a simple measure to express differences between conditions as the data changed from baseline, 2) to avoid type II error associated with multiple time comparisons between conditions in an experiment with a limited sample size. The area under the curve was calculated for each participant and condition by summing all values obtained after participants donned the shirt i.e. from minute 5 to 60. The area under the curve values were then compared by condition using a one-way repeated measures ANOVA for parametric data or Friedman's ANOVA for non-parametric data. Bonferroni's multiple comparison test was used to follow up the direction of effect. Mean (SD) values were reported and the alpha level was set at 0.05. All statistical testing was performed using GraphPad Prism version 5.00 for Windows, (GraphPad Software, San Diego California USA). Post-hoc power analyses were conducted using G*power software.

3. Results

3.1. Environmental conditions

Environmental temperature and relative humidity (rh) did not differ ($p > 0.05$) between conditions. Mean (SD) dry, globe and wet bulb temperatures were 29.5 (0.1) °C, 29.6 (0.1) °C and 26.4 (0.6) °C respectively. Mean (SD) relative humidity was 68.5 (0.5) %.

3.2. Exercise intensity

During the first resting period, the overall group mean (SD) HR remained at 76.9 (10.4) beats · min^{-1}, but increased to 94.5 (9.0) beats · min^{-1} with the period of stepping exercise. Heart rate returned to 74.5 (8.7) beats · min^{-1} during the final resting period. No significant difference in HR area under the curve was observed by condition ($p > 0.05$). RPE remained stable ('very light') during each phase of stepping exercise across all conditions. Friedman's ANOVA showed no difference in RPE by spray group ($p > 0.05$). Median (range) RPE in CON, W and M/E averaged over the stepping phase were 8 (7 to 13), 8 (7 to 12) and 8 (6 to 16), respectively.

3.3. Thermometry

A one-way repeated measures ANOVA showed a significant difference by condition ($p = 0.0432$) in the area under the ΔT_{re} curve (Figure 1b). *Post-hoc* testing indicated that M/E caused a significantly greater mean (SD) heat storage response (2.8 [0.7] °C) than W (1.8 [0.6] °C) ($p < 0.05$). The average starting rectal temperature across all conditions was 37.09 (0.05) ˚C.

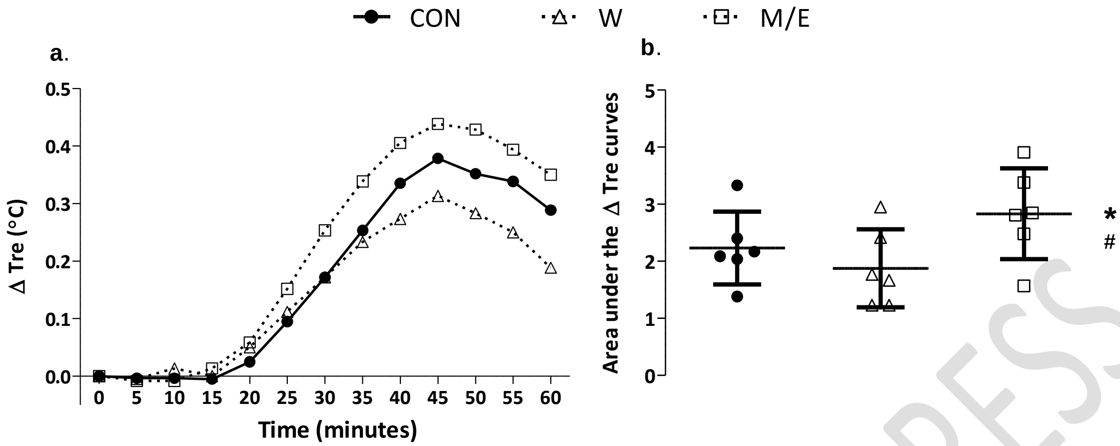

Fig. 1. Mean change in rectal temperature (ΔT_{re}) (a) and the area under the ΔT_{re} curve post-skin wetting by condition (b) during exercise and rest, by condition ($n = 6$). *Significant difference ($p < 0.05$) by condition. *Post-hoc* test: # Significant difference between W and M/E ($p < 0.05$).

A one-way repeated-measures ANOVA showed a difference by condition ($p = 0.0064$), and *post-hoc* testing showed significantly lower mean (SD) chest skin temperature (as indicated by the area under the curve; data not shown) 15.1 (9.2) °C in W, and 14.4 (9.3) °C in M/E, compared to CON (4.4 [5.3] °C) respectively ($p < 0.01$). A one-way repeated-measures ANOVA showed a difference by condition ($p = 0.0023$) (Figure 2b), and *post-hoc* testing showed significantly lower mean (SD) back skin temperature (as indicated by the area under the curve) of 12.0 (8.8) °C in W, and 12.9 (8.2) °C in M/E, compared to CON (5.7 [4.5] °C) respectively ($p < 0.01$).

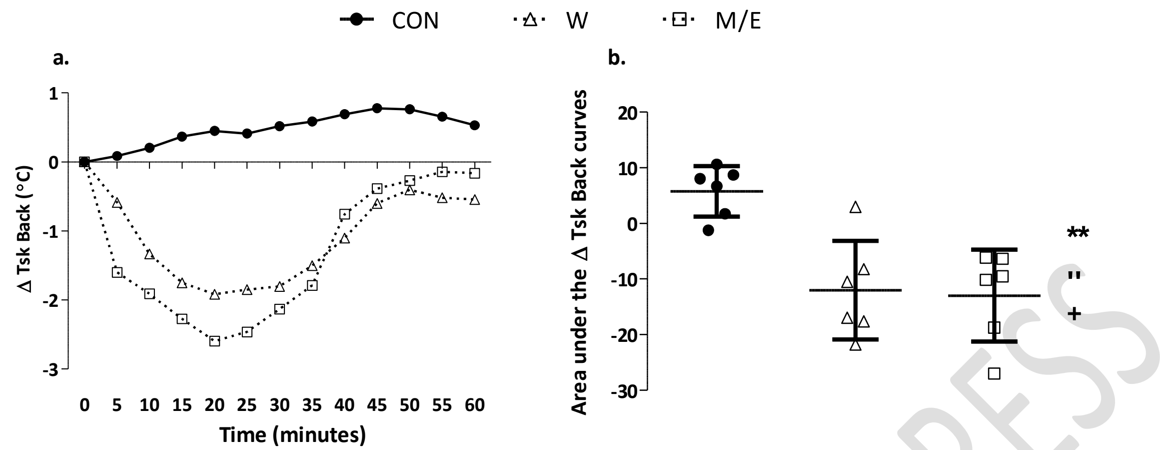

Fig. 2. Mean change in back skin temperature (ΔTsk$_{back}$) (a) and the area under the ΔTsk$_{back}$ curve post-skin wetting by condition (b) during exercise and rest, by condition (n = 6). **Significant difference ($p < 0.01$) by condition. *Post-hoc* test: Significant difference between CON and M/E (**"**, $p < 0.01$) and between CON and W (**+**, $p < 0.01$).

A one-way repeated-measures ANOVA showed a difference by spray group ($p = 0.0002$) (Figure 3b), and *post-hoc* testing showed a significantly lower mean (SD) forearm skin temperature (as indicated by the area under the curve) of 8.8 (4.7) °C in W, and 13.8 (5.5) °C in M/E, compared to CON (2.5 [2.3] °C) respectively ($p < 0.01$).

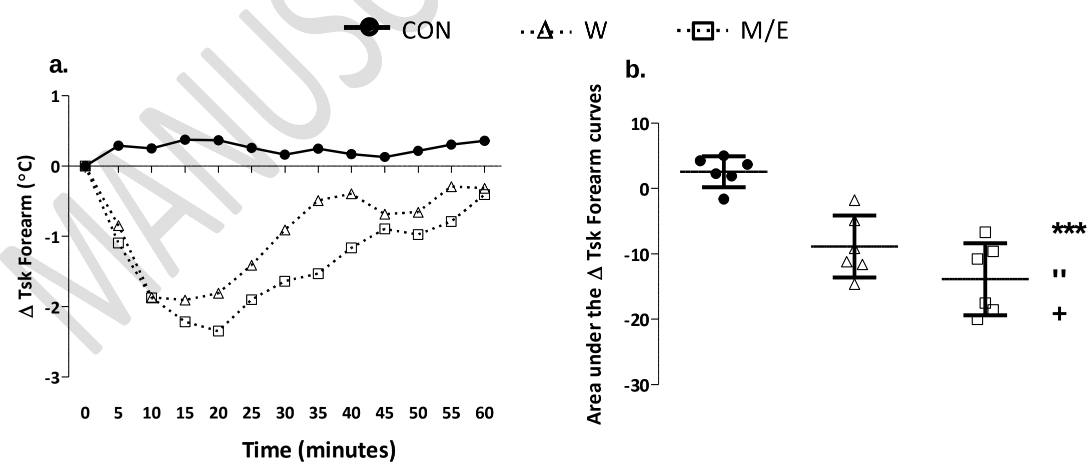

Fig. 3. Mean change in forearm skin temperature (ΔTsk$_{forearm}$) (a) and the area under the ΔTsk$_{forearm}$ curve post-skin wetting by condition (b) during exercise and rest, by condition (n = 6). ***Significant difference ($p < 0.0001$) by

condition. *Post-hoc* test: Significant difference between CON and M/E (**”,** $p < 0.001$) and between CON and W (**+,** $p < 0.01$).

3.4. Infra-red thermography

A one-way repeated-measures ANOVA showed a difference by spray group ($p < 0.0001$), and *post-hoc* testing showed a significant difference between CON and W ($p < 0.001$) and between CON and M/E ($p < 0.001$). Specifically, at the tenth minute front surface temperature was cooler in W (31.1 [0.3] °C) and M/E (30.3 [0.6] °C), compared to CON (33.8 [0.4] °C). At minute 50 front surface temperature was 32.6 (0.3) °C, 31.7 (0.6) °C, and 32.7 (0.8) °C in CON, W and M/W respectively (data not shown). No significant differences were observed in back torso surface temperature between conditions (data not shown).

3.5. Thermal perception

A one-way repeated-measures ANOVA showed a difference by spray group ($p = 0.0003$) (Figure 4b), and *post-hoc* testing showed a significant difference between CON and M/E ($p < 0.0001$) and between W and M/E ($p < 0.001$). At minute 13, participants in M/E felt ‘cool’ to ‘slightly cool’. Over the remainder of the experiment thermal sensation in M/E returned to ‘neutral’. Participants in CON and W felt ‘slight warm’ throughout the entire experiment (Fig 4a).

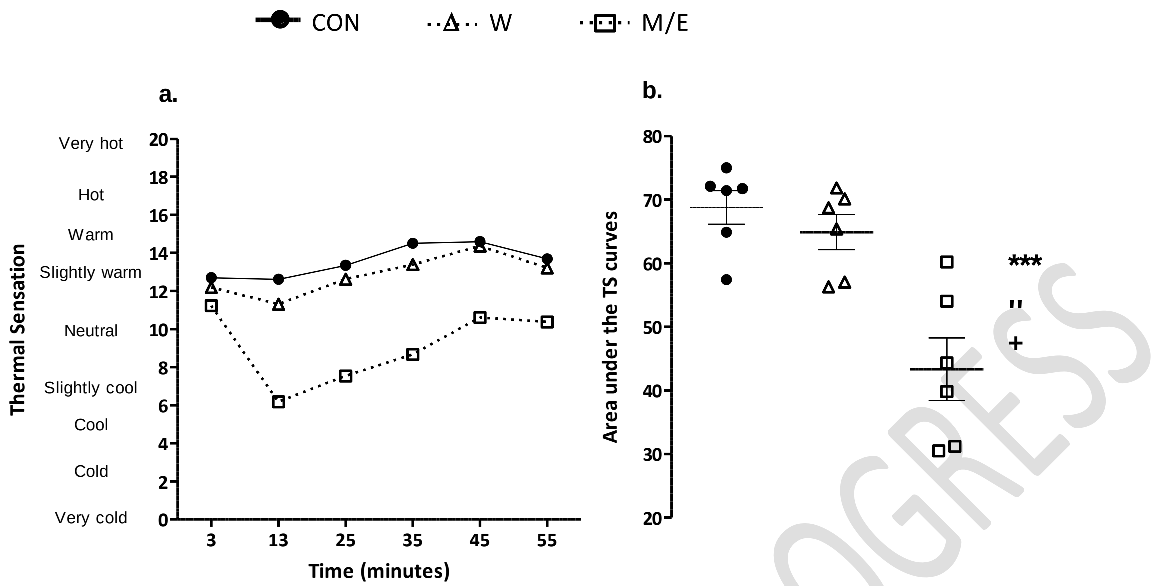

Fig. 4. Mean whole body thermal sensation (a) and the area under the thermal sensation curve post-skin wetting by condition (b) during exercise and rest, by condition ($n = 6$). ***Significant difference ($p < 0.0001$) by condition. *Post-hoc* test: Significant difference between CON and M/E (**”**, $p < 0.0001$) and between M/E and W (**+**, $p < 0.001$).

No significant differences were observed in whole body thermal comfort during rest and exercise between conditions. Thermal comfort remained between ‘just comfortable’ and ‘comfortable’ between conditions throughout the entire experiment (data not shown).

4. Discussion

A combined menthol, ethanol and water-soaked shirt was compared to a water-only soaked shirt and a dry shirt during exercise in warm, humid conditions to identify which intervention provided the greatest improvements in evaporative cooling and thermal perceptions.

The combination of stepping exercise and heat stress used in this study was sufficient to induce a light to moderate cardiovascular and thermoregulatory challenge. During exercise, M/E showed a greater increase in T_{re} and a lower skin temperature compared to CON. The inverse relationship between skin and deep body temperature in the M/E condition was most likely mediated by menthol and ethanol (Gillis *et al.,* 2010; Gillis *et al.,* 2015). The M/E condition contained 16.8 mg of menthol (0.2 % of 80 mL), this equated to 1.6 mg · 100 cm^{-2} of menthol spread over the upper body. Gillis *et al.,* (2010) observed a similar heat storage response with 2.1 mg · 100 cm^{-2} of menthol covering the same surface area, but not with 0.5 mg · 100 cm^{-2} of menthol. Indeed, a number of studies have reported a similar heat storage response, most probably mediated by cutaneous vasoconstriction and a withdrawal of sudomotor function, after applying menthol to heat stressed humans in doses larger than that used herein, and covering greater surface areas (Gillis *et al.,* 2010; 2015; Kounalakis *et al.,* 2010; Lee *et al.,* 2012). Although a menthol-mediated reduction in skin blood flow may contribute to a lower skin temperature, the rapid reduction in skin temperature observed in the M/E condition was most probably due to the action of ethanol. An 80 mL solution composed of 20 % ethanol (16 mL), 80 % water (64 mL), and menthol has the potential to remove 171.5 kilojoules (kJ) of thermal energy from the skin as it evaporates (14.7 kJ from ethanol and 156.8 kJ from water). Alternatively, 80 mL of water will remove 196.6 kJ, or 25 kJ more thermal energy than the 20 % ethanol + water solution. The ethanol component of the solution will evaporate more quickly than the water, and herein lays the enhanced cooling potential of the 20 % ethanol solution. Specifically, at an ambient temperature of 21 °C and 60 % rh, one gram of ethanol will store 920 joules of thermal energy and evaporate in just above five minutes (Godts et al., 2005). One gram of water, however, stores 2,450 joules, but takes 30 minutes to evaporate completely in the same environmental conditions (Godts et al., 2005).

Although ethanol appeared to cool the skin more than water alone or no skin wetting in the minutes immediately after application, its influence appeared to wear-off, such that by the 35th minute there was no visible difference in skin temperatures between M/E and W at the back and chest, suggesting that this period was too long to maximise the evaporative cooling potential of the ethanol-based solution. Although these findings suggest that the optimum application frequency of a similar ethanol-based solution would be every 20-minutes to 30-minutes, these data also indicate that water, which lowered the rate of rise in T_{re} compared to CON and M/E, provides comparable evaporative cooling to ethanol beyond 30-minutes. These findings are in contrast to Bassett *et al.,* (1987), who employed 120-minutes of treadmill running in similar conditions (29 °C, 66 % rh), and examined the physiological responses to repeated skin wetting (50 mL water spraying every 10-minutes). They found that although water spraying lowered skin temperature compared to a no-spray condition, it did not influence deep body temperature. As the intensity of exercise was greater in the study by Bassett *et al.,* (1987) (mean HR was 155 beats · min^{-1}) compared to the present study (mean HR was 95 beats · min^{-1}), sweat production likely differed; hence, the evaporative potential of the water application was perhaps greater than in the study by Bassett *et al.,* (1987). Notably, wetting the skin of treadmill runners already sweating (1 L · h^{-1}; Bassett *et al.,* 1987) is perhaps inefficient because any additional water will drip-off before it stores enough thermal energy to evaporate. Incidentally, each bead of dripped water will absorb some thermal energy as it runs off, which perhaps explains why Bassett *et al.,* (1987) observed lower skin temperatures. It seems that water spraying has the potential to enhance evaporative skin cooling when it is used on participants possessing a comparably low level of sweat production; or more generally, during lower intensity exercise, or in dry, hot or windy conditions. This is not to say that additional skin wetting would fail to enhance evaporative heat loss, it only means that some of the water and sweat will drip from the body without evaporating. It is not clear whether an ethanol-based solution will improve evaporative heat loss when used after participants have reached a plateau in sweat production.

That participants felt cooler in M/E compared to CON and W *after* the ethanol had evaporated suggests this effect was attributable to menthol. This assertion that is not new (Barwood *et al.,* 2012; 2014; 2015;

Gillis *et al.,* 2010, Gillis *et al.,* 2015; Lee *et al.,* 2012; Watson *et al.,* 1978; Green, 1992; Yosipovitch *et al.,* 1996; Wasner *et al.,* 2004; Namer *et al.,* 2005; Green & Schoen, 2007), and likely results from menthol-mediated activation of the cold receptor TRPM8 located in the terminals of sensory afferent neurons (McKemy *et al.,* 2002; Peier *et al.,* 2002). It is also noteworthy that although participants in the menthol and ethanol condition felt coolest, they also experienced the greatest heat storage response. Further investigation is required to explore the possible disassociation between perceived and actual body temperature as mediated by menthol. In any case, cool sensations in the M/E condition appeared to subside within 30-minutes, coinciding with the end of exercise and the evaporation of ethanol. But as a result, it is not clear whether the decay in thermal sensation over time follows from an habituation (Gillis *et al.,* 2015), absorption of menthol in the skin and its clearance into the blood (Martin *et al.,* 2004), or whether other factors interact to quicken its diminishment, such as the elevation in body temperature with exercise, or the subsequent increase in RPE.

Thermal comfort did not improve with thermal sensation. As exercise followed skin wetting in this study, TC may not have improved as a result of increasing perception of effort, or perhaps an elevation in deep body temperature accompanying exercise. It is interesting to note that the ethanol-mediated reduction in skin temperature and the menthol-mediated improvement in TS were not enough to sway TC in either direction. Furthermore, it is difficult to isolate factors that may have influenced TC in this study. Schlader *et al.,* (2009) highlighted the importance of skin temperature in thermal comfort. Yet in the present study, cooling the skin caused no change in TC. Perhaps the skin was cooled too quickly, and when combined with the added perceptual cooling influence of menthol, contributed to a negative allesthesial response (Cabanac, 1972). Frank *et al.,* (1999) meanwhile, have suggested that both deep body and skin temperature contribute equally, and individually, to TC. In this view, the increase in T_{re} observed during exercise would be expected to lower comfort, whilst the ethanol-mediated reduction in skin temperature should have enhanced it. The conflicting signals, when integrated in the somatosensory cortex, may have balanced, giving rise to the observation of no change in comfort. Similarly, Flouris and Cheung (2009) suggested that mean body temperature, combining deep body and skin temperature, likely drives TC: and although mean body temperature was not calculated in the present study, it probably would not have changed, as the menthol-mediated elevation in T_{re} would have been balanced by the ethanol induced reduction in skin temperature. Given that TC also did not change in this study, this lends some support to the notion that mean body temperature was an important modulator of TC.

Anecdotally, some participants described feelings of irritation after menthol and ethanol skin wetting; so it is possible that the sensation of irritation prevented a clear improvement in TC. Up to 50 % of primary neurons that respond to cold and menthol also have the noxious heat receptor TRPV1 (McKemy *et al.,* 2002); and Green (2004) has suggested that some of the neurons that have TRPM8 receptors may also project in the nociceptive pathway rather than, or along with the cold pathway. Alternatively, an increase in skin wettedness has been shown to reduce comfort (Fukazawa & Havenith, 2009), and wetting the upper body of participants may have thereby prevented an overall improvement in comfort. Lastly, menthol and ethanol skin wetting may have induced sensations that were 'too cold' (*i.e.* negative allesthesia); indeed, a warm stimulus is not always considered comfortable, nor is a cold stimulus always uncomfortable (Cabanac, 1972). That TC was not negatively altered following menthol and ethanol skin wetting raises the possibility of using a water-based menthol solution to improve thermal perceptions during exercise in the heat.

The methodology employed in this experiment may limit the generalizability of the findings. The combination of stepping exercise and heat stress used in this study was sufficient to induce a mild cardiovascular and thermoregulatory challenge, but it is not clear whether a similar response would be observed in more stressful situations. Moreover, the low intensity exercise protocol contributed to a low metabolic cost of work, which may have lessened the chances of observing a difference amongst conditions. As the menthol/ethanol intervention was targeted towards the upper body only, lower body skin temperature measurements were not obtained as they are less sensitive to detecting changes between conditions. Resultantly, calculations for mean skin and mean body temperature were not performed, which may hinder comparison with other research. Future work in this area may aim to assess sweat rate in order to separate the thermoregulatory influence of sweating versus evaporation of ethanol and/or water alone. Finally, although the difference in deep body temperature between conditions is perhaps more statistically than physiologically relevant, and borders the sensitivity of the rectal thermistor itself, it is noteworthy that this elevation was mediated by a small dose of menthol (16.8 mg, 0.2 %) working simultaneously in the presence of ethanol-enhanced skin cooling. This finding, and the research of others (Gillis, House & Tipton, 2010; Kounalakis et al., 2010), underscores the thermoregulatory potency of the chemical compound menthol, and emphasizes the need for further research assessing its physiological influence in humans.

Post-hoc analysis of effect size and observed power were calculated using G*Power software. The analysis indicated that measurements of thermal sensation (Power: 0.785, effect size: 0.69), IR chest skin temperature (Power: 0.99, effect size: 1.07), and forearm skin temperature (Power: 0.84, effect size: 0.74) were adequately powered to correctly reject the null hypotheses. Skin temperature measurements obtained at the back (Power: 0.48, effect size: 0.52) and chest (Power: 0.35, effect size: 0.45), and measurements of deep body temperature (Power: 0.17, effect size: 0.32) were underpowered. Given the effect sizes, approximately 8 to 15 participants would have been required to achieve 80 % power in the aforementioned dependent variables. Although the lowered statistical power raises the possibility that the null hypotheses for rectal, back and chest temperatures were incorrectly rejected in the present experiment, previous research using a comparable menthol dose has demonstrated a comparable elevation in rectal temperature (Gillis, House & Tipton, 2010; Gillis et al., 2015). Future research should however assess the influence of ethanol on skin temperature during exercise in order to confirm the measurements obtained in the present experiment.

Given these findings, the null hypothesis that T_{re} would not differ between conditions is rejected in favour of the alternative hypothesis that T_{re} is elevated during exercise following menthol/ethanol skin wetting. The null hypothesis that TC and RPE will not change after donning a menthol and ethanol saturated shirt is not rejected. The alternative hypothesis that donning a menthol and ethanol saturated shirt induces cooler sensations than a water saturated or dry shirt is supported.

5. Conclusions

It is concluded that both M/E and W enhance evaporative cooling compared CON, but M/E causes cooler sensations and a heat storage response, both of which are likely mediated by menthol. Future research might assess the efficacy of an ethanol-only solution on work capacity and performance in participants experiencing compensable and uncompensable heat gain, and possible undesirable implications arising from the menthol-mediated disassociation between actual and perceived body temperature.

Conflicts of interest

This research project was made possible by funding from UK Sport.

Acknowledgements

Special thanks to Dr. Scott Drawer and Dr. Nikolai Boehlke of the Research and Innovation team at UK Sport.

References

Barwood, M.J., Corbett, J., Thomas, K., Twentyman P. 2015. Relieving thermal discomfort. Effects of sprayed L-menthol on perception, performance and time trial cycling in the heat. Scand J Med Sci Sports. 25(Suppl 1), 211-8.

Barwood, M.J., Corbett, J., White, D.K. 2014. Spraying with 0.20% L-menthol does not enhance 5 km running performance in the heat in untrained runners. J Sports Med Phys Fitness. 54(5), 595-604.

Barwood, M.J., Corbett, J., White, D., James, J. 2012. Early change in perception is not a driver of anticipatory exercise pacing in the heat. Br J Sports Med. 46(13), 936-42.

Barwood, M.J., Davey, S., House, J.R., Tipton, M.J. 2009. Post-exercise cooling techniques in hot, humid conditions. Eur J Appl Physiol. 107(4), 385-96.

Bassett, D.R., Nagle, F.J., Mookerjee, S., Darr, K.C., Ng, A.V., Voss, S.G., Napp, J.P. 1987. Thermoregulatory response to skin wetting during prolonged treadmill running. Med Sci Sports Exerc. 19(1), 28-32.

Burton, A.C. 1935. Human calorimetry: the average temperature of the tissues of the body. J Nutr. 9(8), 261-280.

Cabanac, M., Massonnet, B., Belaiche, R. 1972. Preferred skin temperature as a function of internal and mean skin temperature. J Appl Physiol. 33(6), 699-703.

Cheung, S.S. 2010. Heat stress. In. Advanced Environmental Exercise Physiology. (p. 27-48). Champaign, IL. USA. Human Kinetics.

Duffield, R. 2008. Cooling interventions for the protection and recovery of exercise performance from exercise-induced heat stress. In. Marino, F.E. (Volume 53): Thermoregulation and Human Performance. Physiological and Biological Aspects Medicine and Sports Science. (pp. 89-103). Basel, Switzerland: Karger.

Flouris, A.D., Cheung, S.S. 2009. Human conscious response to thermal input is adjusted to changes in mean body temperature. Br J Sports Med. 43, 199-203.

Frank, S.M., Raja, S.N., Bulcao, C.F., Goldstein, D.S. 1999. Relative contribution of core and cutaneous temperatures to thermal comfort and autonomic responses in humans. J Appl Physiol. 86, 1588-1593.

Fukazawa, T., Havenith, G. 2009. Differences in comfort perception in relation to local and whole body skin wettedness. Eur J Appl Physiol. 106, 15-24.

Gillis, D.J., Weston N, House, J.R., & Tipton, M.J. 2015. Influence of repeated daily menthol exposure on human temperature regulation and perception. Physiol Behav, 139:511-8.

Gillis, D.J., House, J.R., Tipton, M.J. 2010. The influence of menthol on thermoregulation and perception during exercise in warm, humid conditions. Eur J Appl Physiol. 110, 609-618.

Godts, P., Dupont, D., Leclercq, D. 2005. Direct measurement of the latent heat of evaporation by flowmetric method. IEEE transactions on instrumentation and measurement. 54(6), 2364-2369.

Green, B.G. 2004. Temperature perception and nociception. J Neurobiol. 61, 12-29.

Green, B.G., Schoen, K.L. 2007. Thermal and nociceptive sensations from menthol and their suppression by dynamic contact. Behav Brain Res. 176, 284-291.

Green, B.G. 1992. The sensory effects of l-menthol on human skin. Somatosensory and Motor Research. 9, 235-44

Hensel, H. 1981. Thermoreception and Temperature Regulation. (p.81). New York, NY, USA. Academic Press.

Kounalakis, S., Botonis, P., Koskolou, M., Geladas, N. 2010. The effect of menthol application to the skin on sweating rate response during exercise in swimmers and controls. Eur J Appl Physiol. 109(2), 183-189.

Lee, J.Y., Kakao, K., Bakri, I., Tochihara, Y. 2012. Body regional influences of L-menthol application on the alleviation of heat strain while wearing firefighter's protective clothing. Eur J Appl Physiol. 112(6), 2171-83.

Martin, D., Valdez, J., Boren, J., Mayersohn, M. 2004. Dermal absorption of camphor, menthol and methyl salicylate in humans. J Clin Pharmacol. 44, 1151-57.

McKemy, D.D., Neuhausser, W.M., Julius, D. 2002. Identification of a cold receptor reveals a general role for TRP channels in thermosensation. Nature. 416, 52-58

Mujika, I., De Txabarri, R.G, Pyne, D. 2010. Effects of a new evaporative cooling solution during rowing in a warm environment. Int J Sports Physiol Perf. 5, 412-16.

Namer, B., Seifert, F., Handwerker, H.O., Maihofner, C. 2005. TRPA1 and TRPM8 activation in humans: effects of cinnamaldehyde and menthol. Neuroreport. 16, 955-59.

Nybo, L. 2010. Cycling in the heat: performance perspectives and cerebral challenges. Scand J Med Sci Sports. 20(suppl. 3), 71-79.

Peier, A.M., Moqrich, A., Hergarden, A.C., Reeve, A.J., Andersson, D.A., Story, G.M., et al., 2002. A TRP channel that senses cold stimuli and menthol. Cell. 108, 705-15.

Rowell, L.B., Marx, H.J., Bruce, R.A., Conn, R.D., Kusumi, F. 1966. Reductions in cardiac output, central blood volume, and stroke volume with thermal stress in normal men during exercise. J Clin Invest. 45(11), 1801-1816.

Tucker, R., Marle, T., Lambert, E.V., Noakes, T.D. 2006. The rate of heat storage mediates an anticipatory reduction in exercise intensity during cycling at a fixed rating of perceived exertion. J Physiol. 574(3), 905-915.

Schlader, Z.J., Simmons, S.E., Stannard, S.R., Mundel, T. 2011a. The independent roles of temperature and thermal perception in the control of human thermoregulatory behaviour. Phys Behav. 103, 217-224.

Schlader, Z.J., Simmons, S.E., Stannard, S.R., Mundel, T. 2011b. Skin temperature as a thermal controller of exercise intensity. Eur J Appl Physiol. 111(8), 1631-9.

Schlader, Z.J., Prange, H.D., Mickleborough, T.D., Stager, J.M. 2009. Characteristics of the control of human thermoregulatory behaviour. Phys Behav. 98, 557-562.

Wasner, G., Schattschneider, J., Binder, A., Baron, R. 2004. Topical menthol – a human model for cold pain by activation and sensitization of C nociceptors. Brain. 127, 1159-71.

Watson, H.R., Hems, R., Rowsell, D.G., Spring, D.J. 1978. New compounds with the menthol cooling effect. J Soc Cosmet Chem. 29, 185-200.

Yosipovitch, G., Szolar, C., Hui, X.Y., Maibach, H. 1996. Effect of topically applied menthol on thermal, pain and itch sensations and biophysical properties of the skin. Archives of Dermatological research. 288, 245-48.

Yu, C.Y., Lin, C.H., Yang, Y.H., 2010. Human body surface area database and estimation formula. Burns. 36, 616-629.

Zhang, H. 2003. Human Thermal Sensation and Comfort in Transient and Non-Uniform Thermal. Unpublished doctoral thesis. University of California, Berkeley, CA, USA.