Evidence of viscerally-mediated cold-defence thermoeffector responses in man

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KEY POINTS:

- Visceral thermoreceptors that modify thermoregulatory responses are widely accepted in animal, but not human, thermoregulation models.
- Recently, we have provided evidence of viscerally-mediated sweating alterations in humans during exercise brought about by warm and cool fluid ingestion.
- In this study, we characterize the modification of shivering and whole-body thermal sensation during cold stress following the administration of a graded thermal stimuli delivered to the stomach via 52°C, 37°C, 22°C and 7°C fluid ingestion.
- Despite no differences in core and skin temperature, 52°C fluid ingestion rapidly decreased shivering and sensations of cold compared to 37°C, whereas 22°C and 7°C fluid ingestion led to equivalent increases in these responses.

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- Warm and cold fluid ingestion independently modifies cold defence thermoeffector responses, supporting the presence of visceral thermoreceptors in humans. However, the cold-defence thermoeffector response patterns differed from previously identified hot-defence thermoeffectors.

ABSTRACT: Sudomotor activity is modified by both warm and cold fluid ingestion during heat stress, independently of differences in core and skin temperatures, suggesting independent viscerally-mediated modification of thermoeffectors. The purpose of the present study was to determine whether visceral thermoreceptors modify shivering responses to cold stress. Ten males (27±5y, 1.73±0.06m, 78.4±10.7kg) underwent whole-body cooling via 5°C water perfusion-suit, on four occasions, to induce a steady-state shivering response, at which point two aliquots of 1.5 ml/kg (SML) and 3.0 ml/kg (LRG), separated by 20-min, of either 7°C, 22°C, 37°C or 52°C water were ingested. Rectal, mean skin and mean body temperature (T_b), electromyographic activity (EMG), metabolic rate (M) and whole-body thermal sensation on a visual analogue scale (WBTS) ranging from 0mm [very cold] to 200mm [very hot] were all measured throughout. T_b was not different between all fluid temperatures following SML (7°C:35.7±0.5°C, 22°C:35.6±0.5°C, 37°C:35.5±0.4°C, 52°C:35.5±0.4°C; P=0.27) or LRG (7°C:35.3±0.6°C, 22°C:35.3±0.5°C, 37°C:35.2±0.5°C, 52°C:35.3±0.5°C; P=0.99) fluid ingestion. With SML ingestion, greater metabolic rate and cooler thermal sensations were observed with 7°C (M:179±55W, WBTS:29±21mm) compared to 52°C (M:164±34W, WBTS:51±28mm; all P<0.05) ingestion. With LRG ingestion, compared to shivering and thermal sensations with 37°C ingestion (M:215±47W, EMG:3.9±2.5%MVC, WBTS:33±2mm) values were different (all P<0.05) following 7°C (M:269±77W, EMG:5.5±0.9%MVC, WBTS:14±12mm), 22°C (M:270±86W, EMG:5.6±1.0%MVC, WBTS:18±19mm) and 52°C (M:179±34W, EMG:3.3±2.1%MVC, WBTS:53±28mm) ingestion. In conclusion, ingesting 52°C fluids decreased shivering and the sensation of coolness, whereas 22°C and 7°C fluids increased shivering and sensations of coolness to similar levels, independently of core and skin temperature.

List of abbreviations:

Small volume (SML)

Large volume (LRG)

Electromyographic activity (EMG)

Metabolic rate (M)

Whole-body thermal sensation (WBTS)

Mean body temperature (T_b)

Rectal temperature (T_{re})

Mean skin temperature (T_{sk})

Maximal voluntary contraction (MVC)

Heart rate (HR)

Mean arterial pressure (MAP)

INTRODUCTION

Traditionally in models of human thermoregulation, hot and cold defence responses are primarily driven by thermoreceptors within the hypothalamus and modified by afferent inputs from peripheral cutaneous thermoreceptors (Werner, 2010). Conversely in animal models of thermoregulation, multiple locations for peripheral thermoreceptors which modify thermoeffector responses have been identified, including: muscle (Jessen *et al.*, 1983), cutaneous (Minut-Sorokhtina & Glebova, 1976) and possibly abdominal (Cranston *et al.*, 1978) veins, spinal column (Rautenberg *et al.*, 1972), upper airway (Orani *et al.*, 1991), abdominal wall (Riedel, 1976), lower oesophagus (El Ouazzani & Mei, 1982), stomach (Rawson & Quick, 1972; El Ouazzani & Mei, 1982) and small intestine (Rawson & Quick, 1972). Additionally, several peripheral thermoreceptors responsible for modifying non-thermoregulatory responses, such as inducing bladder contractions, have been identified (Gardiner *et al.*, 2014).

Recently, we demonstrated warm and cold fluid administration via ingestion and direct delivery to the stomach via a nasogastric tube, but not mouth swilling, modified sudomotor responses in the heat, independently of differences in core and skin temperatures (Morris *et al.*, 2014). Later, these findings were expanded upon, as similar responses were observed with ice slurry ingestion, including the modification of vasomotor activity (Morris *et al.*, 2015). From a thermal

control perspective, the modification of sudomotor activity by both cold and warm fluid ingestion was likely the result of warm and cold visceral thermoreceptors influencing thermoeffector activity in a manner comparable to cutaneous thermoreceptors, as both spinal tract visceral and cutaneous thermoreceptors follow similar central circuitry pathways (Nakamura, 2011). Therefore, the ingestion of warm fluids would likely lead to excitation of warm-sensitive neurons in the hypothalamus (Nakamura & Morrison, 2010), whereas the ingestion of cool and cold water would excite inhibitory neurons within the hypothalamus which then project to the warm-sensitive neurons in the preoptic area of the hypothalamus (Nakamura & Morrison, 2011). In the present study, similar findings in the cold should be observed, whereby cold thermoeffector responses (i.e. shivering) should be increased by cold fluid ingestion stimulating cold thermoreceptors, leading to inhibition of warm-sensitive neurons in the hypothalamus whereas warm fluid ingestion would lead to excitation of warm-sensitive neurons and an inhibition of cold thermoeffector responses (Simon, 2006; Morrison, 2016; Tan *et al.*, 2016; Song *et al.*, 2016).

Previously, modifications to heat-defence thermoeffector responses (sudomotor and vasomotor) have been sufficient to negate the effects of ingesting aliquots of and 1.5°C, 10°C and 50°C fluids (Bain *et al.*, 2012), as well as ice slurry ingestion (Morris *et al.*, 2015). The adequacy of these responses to a variety of temperature stimuli suggests that not only do visceral thermoreceptors exist, but also possess sizeable representation and integration within the central nervous system, which is consistent with the large visceral thermal and sensory integration previously demonstrated in animals (Nakamura, 2011). Additionally, the administration of graded fluid temperatures to various areas of the gastrointestinal tract in humans has consistently elicited a perceptual response, but inconsistently elicited a thermally-mediated gastric tension response (Villanova *et al.*, 1997). This disparity between perceptual and physiological responses likely stems from the unique integration pathways that have been demonstrated between afferents evoking physiological and perceptual responses (Osaka, 2004; Nakamura & Morrison, 2008).

The aim of the present investigation was to determine whether ingestion of different fluid temperatures would modify cold-defence thermoeffectors (i.e. shivering), as represented by metabolic rate and electromyographic activity, as well as whole-body thermal sensation in the cold, independently of differences in core, skin, and mean body temperature. A secondary aim was to determine whether shivering would be proportionately modified relative to the thermal stimuli applied. It was hypothesized that compared to a thermoneutral fluid (37°C), ingesting hot (52°C) fluids would decrease shivering responses, while ingesting cool (22°C) and cold (7°C) fluids would increase shivering and that all modifications to shivering would occur independently of any differences in core, skin, or mean body temperature.

METHODS

Ethical Approval

The experimental protocol was approved by the University of Sydney Human Research Ethics Committee and was in accordance with the Declaration of Helsinki. Written informed consent was provided by all volunteers in the study prior to participating in any data collection. The participants also completed Physical Activity Readiness Questionnaires (PAR-Q) as well as American

Heart Association/American College of Sports Medicine Health/Fitness Facility Pre-participation Screening Questionnaires.

Participants

Ten healthy young men were recruited to participate in the experiment (age: 27 ± 5 y; body mass: 78.4 ± 10.7 kg, height: 1.73 ± 0.06 m). To be included in the study, participants had to be regarded as "healthy". Exclusion criteria for the study included being on prescription medication, being a smoker and having any currently diagnosed medical conditions including but not limited do: metabolic, cardiovascular, and respiratory disorders. The participants were instructed to refrain from consuming alcohol or partaking in any strenuous physical activity for 24 hours prior to testing, to refrain from consuming caffeine the day of testing, and to maintain a consistent routine (e.g. sleep schedules and diet) during the day before and the day of experimental sessions.

Study Design

In order to isolate the independent effect of visceral thermoreceptors on cold-defence thermoeffector responses, an open-loop study design was employed. This design consisted of maintaining core and skin temperature at a fixed point, while thermal stimuli were applied to the abdomen via fluid ingestion. This study design was based on previous investigations into regional cutaneous thermosensitivity in humans (Cotter & Taylor, 2005). Participants were cooled for 40 min prior to any fluid administration in order to achieve steady-state shivering, as well as relatively steady-state core and skin temperature. The duration required to achieve steady-state was determined through pilot testing. The cooling was performed by circulating 5°C water through a perfusion suit (One-piece Coretec, Delta Temax, Inc., Pembroke, ON, Canada). The temperature perfusing the suit was monitored continuously using a thermistor (TM400, Covidien, Mansfield, MA, USA) at the inlet of the suit. The water temperature used was selected to induce a moderate level of shivering [i.e. ~300 W or approximately half of the maximal shivering response (Haman *et al.*, 2005)]. Participants were in a semi-reclined position throughout the duration of the experiment, and underwent 15 min of rest in a thermoneutral room (22°C, 50%RH) before ambient temperature was lowered to 15°C, 50%RH when cooling began.

In order to assess how shivering and whole-body thermal sensation responses would respond to an array of thermal stimuli, four fluid temperatures: cold (7°C), cool (22°C), thermoneutral (37°C), and hot (52°C); and two volumes: small (1.5 ml/kg of body mass; SML) and large (3.0 ml/kg of body mass; LRG) were used. The resultant range of ingested fluid volumes was between 100-150 ml for the SML aliquot and 200-300 ml for the LRG aliquot. These volumes were selected so that the larger of the two volumes was not sufficiently large to affect rectal temperature via local cooling (which in pilot testing began to occur with aliquots of 3.5 ml/kg) and for the small volume to be half the size of the large volume. The participants were instructed to ingest the SML aliquot in under 30 s and the LRG aliquot in under 1 min, as had been done in earlier studies (Bain *et al.*, 2012; Morris *et al.*, 2014, 2015). The temperatures were specifically selected in order to administer thermal stimuli of similar magnitudes but opposite direction, relative to an approximate core body temperature of 37°C (i.e. 52°C = 37°C +15°C; 22°C = 37°C-15°C) and to administer approximately double the negative thermal stimuli on the cold side (i.e. 22°C = 37°C-15°C; 7°C = 37°C-30°C). Additionally, by administering fluids of precisely double the volume, we were able to

assess whether the modifications of the responses would be twice as large. The above was accomplished through four experimental trials, each consisting of a SML and LRG ingestion of the same temperature fluid at 40 and 60 min of cooling, respectively. This order of ingestion was maintained for all trials as the response time for the SML ingestion was shorter than the LRG ingestion and we required the metabolic responses to return to 37°C trial levels prior to the administration of the LRG fluid. Conversely, the order of temperature ingestion between trials was performed in a counterbalanced manner, using an incomplete Latin square design.

Measurements

Metabolic rate: Metabolic data was continuously measured throughout rest and cooling using a Quark CPET metabolic cart (Cosmed, Rome, Italy). Subjects donned a rubber face mask and were asked to ensure a proper seal between the face and the mask had been formed by covering the inspiration/expiration opening and exhaling, verifying no air could escape from the edges of the mask. During the trial, participants were asked to breathe normally. Metabolic rate (M) was calculated from minute-average values for oxygen consumption in litres per minute, and the respiratory exchange ratio (Nishi, 1981), and reported in watts.

Electromyography (EMG): Shivering EMG signals were recorded simultaneously from eight muscle sites located on the left side of the body: upper trapezius, latissimus dorsi, pectoralis major, rectus abdominis, vastus lateralis, rectus femoris, vastus medialis, and gastrocnemius. These muscles were selected as they have been used in previous investigations into shivering using similar protocols (Haman *et al.*, 2004*a*, 2004*b*) and have been demonstrated to be responsible for >90% of metabolic heat production during cold exposure (Bell *et al.*, 1992). The skin was prepared with alcohol and an abrasive gel to reduce skin impedance. Two surface electrodes (100 series, Covidien,Mansfield, MA, USA) were placed over each measurement site, two cm apart and parallel to the orientation of the muscle fibers (Basmajian & Blumenstein, 1980). Inter-electrode resistances were always ensured to be <10 kΩ. EMG signals were amplified and filtered (EMG 100B/C BIOPAC Systems Inc., California, USA; gain 100-1000, bandpass filtered between 10 and 500 Hz) before transferring to a PC with a 16 bit analog to digital converter (MP150, BIOPAC Systems Inc., California, USA) at a sample rate of 2000 Hz using AcqKnowledge software (v3.9.0, BIOPAC Systems Inc., California, USA).

Six isometric maximal voluntary contraction (MVC) tests were performed on each testing day at the completion of the experiment while the participants were still being cooled, to ensure skin temperature was the same during the tests as it was during fluid ingestion. The MVC tests were based on those recommended in the literature (Boettcher *et al.*, 2008; Rutherford *et al.*, 2011; Ginn *et al.*, 2011): 1) palm press at shoulder adduction at 90°, 2) shoulder extension at 30° abduction, 3) knee extension at 15°, 4) knee flexion at 15°, 5) plantar flexion in a neutral position, and 6) dorsiflexion at 15° plantar flexion. Each test lasted 5 s: 1 s to reach maximum, 3 s sustained maximum; and gradual release over the final 1 s with a minimum rest interval of 30 s between repetitions. Strong verbal encouragement was provided throughout MVC testing. EMG signals were high pass filtered (10 Hz, dual pass 4th order Butterworth), rectified, and low pass filtered (4 Hz, dual pass 4th order Butterworth) using (Matlab 2014b, The Math Works, Natick, MA). The MVC for each muscle was taken as the maximum during these rectified and filtered signals and was used to normalize the EMG signals for that muscle during the trials. The values from the 8 sites were then combined into a non-weighted average to represent the mean EMG activity as %MVC. Finally, we

considered both lower levels of EMG activity from increases in tonal muscle contraction and low intensity shivering as well as higher levels of EMG activity from bursting muscle contraction as shivering, as both forms of muscle contraction contribute to metabolic thermogenic responses in the cold (Haman *et al.*, 2004*a*, 2004*b*).

Thermometry: Rectal (T_{re}) temperature was measured with general purpose paediatric thermistor probe (TM400, Covidien, Mansfield, MA, USA) inserted to a depth of 15 cm past the anal sphincter. Skin temperature (T_{sk}) was measured using thermistors integrated into heat flow sensors (2252 Ohms, Concept Engineering, Old Saybrook, CT, USA) secured to shaved and cleaned skin with double-sided adhesive discs and surgical tape (Transpore, 3M, London, ON, Canada) at four sites. Mean T_{sk} was expressed as an average of the four sites using the weighting of 30% chest, 30% arm, 20% thigh and 20% calf (Ramanathan, 1964). As previous thermoregulatory research in humans has demonstrated that core temperature has approximately nine to ten times the influence on thermoeffector responses as skin temperature (Nadel *et al.*, 1971; Gisolfi & Wenger, 1984; Simon *et al.*, 1986), mean body temperature (T_b) was estimated as a forcing function for thermoeffector responses using a weighting of $0.9 \times T_{re}$ and $0.1 \times T_{sk}$. Thermometric measurements were displayed in real-time using LabView (v7.0, National Instruments, Austin, TX, USA) at a sampling rate of 5 seconds.

Whole-body thermal sensation: Participants reported their whole-body thermal sensation (WBTS) on a hand-scored 200 mm visual analogue scale (anchor points: Very cold [0 mm] and Very hot [200 mm]; middle point: Neutral [100 mm]). These measurements were taken exactly 5 min before and after fluid ingestion.

Cardiovascular measurements: Heart rate (HR) was recorded at 5-s intervals throughout the trial using a Polar RS400X coded transmitter and stored with a Polar Advantage interface and Polar Precision Performance software (Polar Electro Oy, Kempele, Finland). Blood pressure was measured using an automatic blood pressure cuff (M10-IT, Omron, Hoofddorp, Netherlands) and expressed as mean arterial pressure (MAP).

Water temperature: The temperature of the aliquots of water to be ingested for the 22°C, 37°C, and 52°C trials was carefully maintained in a hydrostatic controlled water bath (Polyscience – DA05A, Niles, IL, USA) until two minutes before each time of ingestion, at which point the correct volume was measured, the temperature verified and thereafter the aliquot was returned to the water bath to maintain temperature until immediately prior to ingestion. The temperature of the 7°C aliquot was attained by cooling water to ~1.5°C in a thermos with ice and then combining the 1.5°C with warm water until the aliquot was at 7°C, 1 min prior to ingestion. The temperature of the water was measured using a factory-calibrated glass thermometer (Durac Plus, Blue Spirit, precision thermometer, Cole-Parmer) with a certified range between -1°C and +100°C with an accuracy of ±0.1°C and precisely recorded immediately prior to ingestion.

Statistical analysis

All data are expressed as a mean with standard deviation. In order to ensure an adequate sample size for the experiment, a power calculation using G*Power 3 software (Heinrich-Heine-Universität Düsseldorf, Germany (Faul *et al.*, 2007)) was performed employing an α of 0.05, a β of 0.20, and an effect size of 1.0, calculated from the smallest significant difference between metabolic rate during low and moderate shivering (Haman *et al.*, 2005). A sample size of 10 individuals was determined for sufficient statistical power.

Thermometric (suit temperature, T_{re}, T_{sk} and T_b), physiological (M, EMG, MAP and HR) and perceptual (WBTS) responses were compared using the 10-min and 15-min post-ingestion averages for the SML and LRG ingestions, respectively. Additionally, in order to isolate the effect of the fluid ingestion on thermoeffector response, a second comparison was made whereby all responses were standardised to the 37°C condition by subtracting the 37°C values from all other trials as well as subtracting the 2 min pre-ingestion averages from all values in order to remove any within-trial pre-ingestion differences. For WBTS and MAP, data were standardized to the 5 min pre-ingestion averages. Accordingly, all responses and change in responses following fluid ingestion, relative to the 37°C trial, for thermometric, physiological and perceptual data were analysed using a two-way repeated measures ANOVA employing the independent variables of fluid temperature (absolute comparison, 4 levels: 7°C, 22°C, 37°C, 52°C; relative to 37°C comparison, 3 levels: 7°C, 22°C, 52°C) and ingestion size (2 levels: SML, LRG). Additionally, in order to ascertain the mean latency and duration of the shivering response following ingestion, M and EMG values from the 37°C trial were subtracted from those of the 7°C, 22°C and 52°C trials, and the change in thermoeffector responses were compared for 22 min post-ingestion, relative to the 1-min average immediately prior to ingestion. These data were assessed using a two-way repeated measures ANOVA, employing the independent variables of post-ingestion time (0 to 22 min) and fluid temperature (3 levels: 7°C, 22°C, 52°C).

When significant main effects or interactions were found, individual differences were assessed using independent Student's *t*-tests, while maintaining a fixed probability (5%) of making a type I error by using a Holm-Bonferroni correction.

RESULTS

Suit water temperature

The water temperature entering the suit was the same temperature between trials (7°C: 4.6±0.2°C, 22°C: 4.4±0.6°C, 37°C: 4.7±0.5°C, 52°C: 4.5±0.3°C; P=0.37).

Core and skin temperatures

The 1-min average data for all thermometry data is displayed in Figure 1. Fluid temperature had no effect on T_{re} (P=0.47), T_{sk} (P=0.71), or T_b (P=0.46).

Metabolic rate

The 1-min average data for M are displayed in Figure 1 and the change in M following fluid ingestion, relative to the 37°C trial, are displayed in Figure 2. Both fluid temperature and fluid size affected M (P<0.01). Following the SML ingestion, M was greater in the 7°C compared to 52°C trial, (P=0.05) but no other differences were found (P>0.05). Following LRG ingestion, M was greater in the 7°C and 22°C compared to both the 37°C and 52°C trials (P<0.05). Additionally, M was smaller in the 52°C compared to the 37°C trial (P<0.05) and not different between the 7°C and 22°C trials (P=0.98). The same trends in the data were observed after accounting for pre-ingestion differences and relative to the 37°C values (Figure 2).

The 1-min averages for M, after accounting for pre-ingestion differences and relative to the 37°C values (Figure 5), were not different after the SML ingestion (P=0.35) but differed after the LRG ingestion (P<0.001). Specifically, M was greater than baseline after 4 min and 5 min with the 22°C

and 7°C trials, respectively, and remained lower until 20 min post-ingestion for both the 22°C and 7°C trials. In the 52°C trial, M was greater than baseline after 2 min and remained greater until 15 min post-ingestion.

Electromyographic activity

The 1-min average data for EMG are displayed in Figure 1 and the change in EMG following fluid ingestion, relative to the 37°C trial, are displayed in Figure 2. Both fluid temperature and fluid size affected EMG (P=0.04). Following the SML ingestion, EMG was greater in the 7°C compared to 52°C trial, (P<0.01) but no other differences were found (P>0.05). Following LRG ingestion, EMG was greater in the 7°C and 22°C compared to both the 37°C and 52°C trials (P<0.05). Additionally, EMG was lower in the 52°C compared to the 7°C trial (P<0.05) and not different between the 7°C and 22°C trials (P=0.60).

The 1-min averages for EMG, after accounting for pre-ingestion differences and relative to the 37°C values (Figure 5), were not different after the SML ingestion (P=0.24) but differed with the LRG ingestion (P=0.05). Specifically, EMG was greater than baseline after 1 min and 3 min with the 22°C and 7°C trials, respectively, and remained lower until 21 min and 20 min post-ingestion for the 22°C and 7°C trials, respectively. In the 52°C trial, EMG was greater than baseline after 1 min and remained greater until 9 min post-ingestion.

Whole-body thermal sensation

The change in thermal sensation data following fluid ingestion, relative to the 37°C trial, are displayed in Figure 3. Both fluid temperature and fluid size affected WBTS (P<0.01). Following the SML ingestion, WBTS was lower (colder) in the 7°C compared to 52°C trial, (P<0.01) but no other differences were found (P>0.05). Following LRG ingestion, WBTS was lower (colder) in the 7°C and 22°C compared to both the 37°C and 52°C trials (P<0.05). Additionally, WBTS was greater (warmer) in the 52°C compared to the 7°C trial (P<0.05) and not different between the 7°C and 22°C trials (P=0.45).

Heart rate and blood pressure

The 1-min average data for HR is displayed in Figure 1. HR was different between fluid temperatures and time points (P<0.001). No differences in HR were observed before (P>0.05) or after SML ingestion (P>0.05). No differences existed in advance of the LRG ingestion (P>0.05). Following the LRG ingestion, HR was lower in the 52°C compared to the 22°C (P<0.01) and 7°C (P<0.01) trials.

Mean arterial pressure (MAP) data are displayed in Figure 4. MAP was not affected by fluid ingestion (P=0.41) nor was there an interaction between time and fluid temperature (P=0.30).

DISCUSSION

Collectively, the findings from the present study demonstrate that the previously identified visceral thermoreceptors, which modify heat-defence thermoeffector responses (i.e. sweating), similarly alter cold-defence thermoeffector responses (i.e. shivering). Specifically, compared to 37°C fluid ingestion, 7°C and 22°C fluid ingestion independently increased shivering, whereas 52°C fluid ingestion independently decreased shivering, as both M and EMG were different between trials

without any measured differences in T_{re}, T_{sk}, or T_b at any point in time; thereby supporting the concept of visceral thermoreceptors independently modifying thermoeffector responses in humans. Following the LRG ingestion, the thermoeffector responses in the 7°C and 22°C trials were similar, even though the 7°C stimulus was twice as cold, relative to body temperature, as the 22°C stimulus. Whole-body thermal sensation followed similar response-patterns to the thermoeffector responses. Collectively, these findings demonstrate both cold defence thermoeffector responses and thermal sensation are modified by visceral thermoreceptors.

Thermoeffector responses were different between trials despite no differences in T_{re}, T_{sk}, or T_b. These findings add to the growing body of evidence in the literature concerning human visceral thermoreception. The response time observed in EMG data following cold/hot fluid ingestion (Figure 5) aligns with our previous observation of changes in heat-defence thermoeffector responses within one minute of cold/warm fluid ingestion (Morris *et al.*, 2014, 2015). These findings support the notion that the thermoreceptors responsible for modifying thermoeffector responses reside either in the stomach or small intestine, as stimulation of both locations elicited thermoeffector responses in the ewe (Rawson & Quick, 1972). However, due to the rapidity of the response, the location of the receptors is most likely in the stomach. Indeed the rapid EMG response time is a strong counterargument to other proposed mechanisms for altering thermoeffector responses, specifically hypothalamic cooling through heat transfer between the blood, bodily tissues, and ingested fluid (Siegel & Laursen, 2012).

Both M and EMG responses following large 22°C and 52°C ingestion increased and decreased, respectively, to equal but opposite levels, whereas 7°C and 22°C ingestion resulted in almost identical changes in thermoeffector responses, even though the 7°C stimuli were twice as cold, relative to body temperature, as the 22°C stimuli. We consider these findings to be the greatest evidence to date of visceral thermoreceptors in humans. Were the modification of thermoeffector responses due to subtle changes in blood temperature, which in turn affected brain temperature as has been previously proposed (Siegel & Laursen, 2012), 7°C ingestion should have elicited proportionately greater defence shivering responses due to greater cooling of the blood. As maximal shivering does not occur until a core temperature 34-35°C (Castellani *et al.*, 2006), no upper limit should have been met. Rather, as some research suggests cold fluids have been shown to dramatically increase gastric emptying time compared to cool and hot fluids (Ritschel & Erni, 1977) and as the stomach and duodenum, but not other segments of the small and large intestine, affect thermoeffector responses (Rawson & Quick, 1972), it is possible the cold water passed from the thermosensitive area to the non-thermosensitive area before all heat exchange occurred.

Contrary to our initial expectations, thermoeffector responses in the LRG trials were more than twice the size of those in the SML trial. These findings may be due to the small ingested fluid mass with the SML ingestion, as a greater relative proportion of the ingested thermal stimuli could have been lost to oral tissues, which we have previously demonstrated do not possess thermoeffector-modifying thermoreceptors capable of modifying thermoeffector responses during heat stress (Morris *et al.*, 2014). Supporting this idea, 1.5°C fluid ingestion in that study reduced oesophageal temperature for ~7 min (unpublished data), whereas ingestion of 4°C fluid of a similar volume reduced intragastric temperature for ~25 min (Sun *et al.*, 1988), indicating that while some heat transfer occurs in the mouth and oesophagus, most occurs in the stomach. However, a similar response has not yet been investigated during cold stress and therefore future studies that

simultaneously measure tissue temperature at several locations along the gastroinitestinal tract with fluid ingestion, particularly during cold stress, are warranted.

In the present study, blood pressure greatly increased and HR decreased following the onset of cooling, which is the typical cold exposure response in humans, as cutaneous blood vessels undergo maximal vasoconstriction, elevating blood pressure and the resulting baroreflex slows HR (Budd & Warhaft, 1966; Collins *et al.*, 1985). Following fluid ingestion, blood pressure was unaffected whereas HR changed in a similar pattern as M and EMG. These findings are likely because the level of cooling employed in this study is beyond the point of maximal vasoconstriction which has been previously identified (Cannon & Keatinge, 1960) and was subsequently confirmed using laser Doppler velocimetry during pilot testing. Therefore blood pressure would be unaffected by changes in cutaneous vasomotor activity, whereas HR would be modified to meet the oxygen demands of the shivering muscles. Conversely in mice, cold administration causes an immediate increase in HR and brown adipose tissue activation (Nakamura & Morrison, 2011). While brown adipose tissue activity was not investigated in the present study, it is not a prominent cold defence thermoeffector in man, with maximal heat production values ranging from 2 to 20 W (Yoneshiro *et al.*, 2011). However, as activation of shivering and non-shivering thermogenesis occurs through the same circuitry pathways (Nakamura & Morrison, 2011) it is quite possible brown adipose tissue activity would be affected by hot and cold fluid ingestion. This information could be of interest for studies in animal species that rely more heavily on brown adipose tissue in the cold, as well as researchers interested in brown adipose tissue activation for potential weight loss in humans (Chechi *et al.*, 2014).

The participants' WBTS responses (Figure 4) demonstrated a similar response pattern to that of the physiological measures in that the participants felt similarly cold following 22°C and 7°C ingestion, rather than reflect the magnitude of the ingested thermal stimulus. Furthermore, while the participants' local thermal sensation was not formally recorded at either the mouth or the abdomen, the participants' were able to differentiate between the 22°C and 7°C fluids during ingestion and remarked upon this difference during testing. This discrepancy in perceptual responses is consistent with previous observations that local cutaneous thermal sensation operates independently from whole-body thermal sensation (Chatonnet & Cabanac, 1965; Cotter & Taylor, 2005). Additionally physiological and perceptual/behavioural responses are similarly uncoupled, as mouth swilling cold fluids mildly benefits exercise performance and thermal comfort in the heat (Burdon *et al.*, 2013), but does not affect sweating (Morris *et al.*, 2014), whereas cold fluid administration directly to the stomach does affect sweating (Morris *et al.*, 2014). Therefore it appears that WBTS and physiological responses share a similar thermal forcing function, comprised of cutaneous, visceral and hypothalamic thermal afferents.

Perspectives

The viscera are innervated by both spinal and vagal thermoafferents and while the spinal thermoreceptor circuitry and how it contributes to thermoregulation is well known (Nakamura, 2011), much less is known about the vagal thermoreceptor circuitry and how it contributes to thermoregulation. Generally, vagal afferents are involved in reflex control of gastrointestinal function, primarily eliciting chemical secretions and changes in motility (Andrews & Sanger, 2002). Some research suggests a role for vagal thermoreceptors in modifying febrile responses (Romanovsky *et al.*, 1997) as well as metabolism and core temperature during starvation (Székely *et*

al., 1997). Additionally, one study demonstrated stimulation of vagal thermoreceptors located in the oesophagus changed respiration rate in cats (El Ouazzani & Mei, 1982). Conversely, other research has demonstrated no electrical response to cooling of vagal nerves in the abdomen (Gupta *et al.*, 1979) as well as spinal but not vagal thermoreceptor stimulation causing modifications to thermoeffector responses (Rawson & Quick, 1972; Riedel, 1976). Therefore, more work is needed to identify the exact thermoregulatory circuitries and their independent effects on thermoregulation of vagal afferents.

Previously, core body thermoreceptors have been identified in the spinal cord and hypothalamus (Simon, 2006). The present findings give evidence for core body thermoreceptors that reside outside the central nervous system which provide temperature feedback for an area which contains multiple organs essential to life. Additionally, we believe visceral thermoreceptors act as auxiliary thermoreceptors, similar to cutaneous thermoreceptors, whereby the thermoreceptors function as an early response system to environmental thermal stress (Romanovsky, 2014). Indeed, the temperature of ingested foods, and particularly fluids, plays an important role for wild animals, especially for those in cold environments (Wilson & Culik, 1991; Berteaux, 2000). Considering humans did not evolve in thermally controlled environments and would periodically need to ingest non-thermoneutral foods and fluids, the ability to detect and defend against ingested substances which could threaten thermal homeostasis would likely be beneficial. As the mouth is densely innervated with thermoreceptors yet evokes no physiological responses with hot and cold stimuli (Morris *et al.*, 2014), it is likely the function of oral thermoreceptors to guard against fluids of noxious temperatures. Conversely, visceral thermoreceptors likely function to account for warm and cold food and fluids in advance of core temperature being altered. The finding that thermoeffectors are only modified by temperature stimuli applied to the stomach and duodenum, but not other areas of the gastrointestinal tract, supports this proposition as warm and cold food and fluids would likely equilibrate with body temperature prior to reaching these tissues (Rawson & Quick, 1972).

Limitations

In the present study mean skin temperature was comprised of four skin temperature sites, whereas as typically more sites are employed under cold environmental conditions (Parsons, 2003). However, suit temperature was not different between trials and fluid temperature explained a mere 0.03% of the variation observed in skin temperature, therefore it is highly unlikely difference in skin temperature existed were not detectable. Additionally, in order to minimize the duration of cold exposure for the participants, the small aliquot was always administered first, as pilot testing demonstrated that modifications to the thermoeffector responses always returned to baseline in less than 20 min. Because of this timing, some water from the first aliquot, albeit of insufficient volume and temperature to alter thermoregulatory responses, may have remained in the stomach when the second aliquot was administered. Another potential concern is that while sudomotor and vasomotor effectors are sympathetically innervated, muscles are somatically innervated and therefore the shivering responses could be altered by voluntary contractions. However, the central circuitry responsible for modifying shivering thermogenesis via somatomotor innervation parallel those for autonomic thermogenesis (Nakamura & Morrison, 2011). As such, any conscious modification of muscle activity would likely be overridden by the central demand to attain thermal balance; akin to an individual attempting to hold their breath. Finally, as modifications in metabolic rate were of primary interest, the level of cooling was selected to elicit moderate shivering and in

doing so elicited maximum vasoconstriction (Cannon & Keatinge, 1960), which was confirmed during pilot study and therefore not measured in the main study.

CONCLUSION

In support of previous work from our laboratory, cold defence thermoeffector responses are modified following 50°C, 22°C and 7°C fluid ingestion, independently of any changes in core or skin temperature, supporting the notion of thermoeffector-modifying visceral thermoreceptors. While 22°C and 52°C fluids respectively increased and decreased shivering responses to equal but opposite levels, shivering responses were the same between the 22°C and 7°C fluid ingestion. Additionally, thermoeffector responses did not respond proportionately to manipulations of fluid volume. Finally, whole-body thermal sensation responded similarly to the thermoeffector responses. Collectively these results reaffirm the concept of thermoeffector-modifying visceral thermoreceptors in man and provide novel evidence that these visceral thermoreceptors can contribute to thermoregulatory function.

Competing interests

The authors report no competing interests.

Author contributions

All experimental testing was performed at the Thermal Ergonomics Laboratory, Faculty of Health Sciences, University of Sydney, Australia. N.M., D.F., M.H. and O.J. contributed to the conception and design of the work; to interpretation and data analysis; and to drafting and revising the work critically for important intellectual content. N.M. and D.F. also performed the data acquisition. All authors approved the final version of the manuscript; and agreed to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. All persons designated as authors qualify for authorship, and all those who qualify for authorship are listed.

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REFERENCES

Andrews PLR & Sanger GJ (2002). Abdominal vagal afferent neurones: an important target for the treatment of gastrointestinal dysfunction. *Curr Opin Pharmacol* **2,** 650–656.

Bain AR, Lesperance NC & Jay O (2012). Body heat storage during physical activity is lower with hot fluid ingestion under conditions that permit full evaporation. *Acta Physiol Oxf Engl* **206,** 98–108.

Basmajian JV & Blumenstein R (1980). *Electrode placement in EMG biofeedback*. Williams & Wilkins.

Bell DG, Tikuisis P & Jacobs I (1992). Relative intensity of muscular contraction during shivering. *J Appl Physiol Bethesda Md 1985* **72,** 2336–2342.

Berteaux D (2000). Energetic Cost of Heating Ingested Food in Mammalian Herbivores. *J Mammal* **81,** 683–690.

Boettcher CE, Ginn KA & Cathers I (2008). Standard maximum isometric voluntary contraction tests for normalizing shoulder muscle EMG. *J Orthop Res Off Publ Orthop Res Soc* **26,** 1591–1597.

Budd GM & Warhaft N (1966). Body temperature, shivering, blood pressure and heart rate during a standard cold stress in Australia and Antarctica. *J Physiol* **186,** 216–232.

Burdon CA, Hoon MW, Johnson NA, Chapman PG & O'Connor HT (2013). The effect of ice slushy ingestion and mouthwash on thermoregulation and endurance performance in the heat. *Int J Sport Nutr Exerc Metab* **23,** 458–469.

Cannon P & Keatinge WR (1960). The metabolic rate and heat loss of fat and thin men in heat balance in cold and warm water. *J Physiol* **154,** 329–344.

Castellani JW, Young AJ, Ducharme MB, Giesbrecht GG, Glickman E, Sallis RE & American College of Sports Medicine (2006). American College of Sports Medicine position stand: prevention of cold injuries during exercise. *Med Sci Sports Exerc* **38,** 2012–2029.

Chatonnet J & Cabanac M (1965). The perception of thermal comfort. *Int J Biometeorol* **9,** 183–193.

Chechi K, Nedergaard J & Richard D (2014). Brown adipose tissue as an anti-obesity tissue in humans. *Obes Rev Off J Int Assoc Study Obes* **15,** 92–106.

Collins KJ, Easton JC, Belfield-Smith H, Exton-Smith AN & Pluck RA (1985). Effects of age on body temperature and blood pressure in cold environments. *Clin Sci* **69,** 465–470.

Cotter JD & Taylor NAS (2005). The distribution of cutaneous sudomotor and alliesthesial thermosensitivity in mildly heat-stressed humans: an open-loop approach. *J Physiol* **565,** 335–345.

Cranston WI, Hellon RF & Townsend Y (1978). Thermal stimulation of intra-abdominal veins in conscious rabbits. *J Physiol* **277,** 49–52.

El Ouazzani T & Mei N (1982). Electrophysiologic properties and role of the vagal thermoreceptors of lower esophagus and stomach of cat. *Gastroenterology* **83,** 995–1001.

Faul F, Erdfelder E, Lang A-G & Buchner A (2007). G*Power 3: A flexible statistical power analysis program for the social, behavioral, and biomedical sciences. *Behav Res Methods* **39,** 175–191.

Gardiner JC, Kirkup AJ, Curry J, Humphreys S, O'Regan P, Postlethwaite M, Young KC, Kitching L, Ethell BT, Winpenny D & McMurray G (2014). The role of TRPM8 in the Guinea-pig bladder-cooling reflex investigated using a novel TRPM8 antagonist. *Eur J Pharmacol* **740,** 398–409.

Ginn KA, Halaki M & Cathers I (2011). Revision of the Shoulder Normalization Tests is required to include rhomboid major and teres major. *J Orthop Res Off Publ Orthop Res Soc* **29,** 1846–1849.

Gisolfi CV & Wenger CB (1984). Temperature regulation during exercise: old concepts, new ideas. *Exerc Sport Sci Rev* **12,** 339–372.

Gupta BN, Nier K & Hensel H (1979). Cold-sensitive afferents from the abdomen. *Pflüg Arch Eur J Physiol* **380,** 203–204.

Haman F, Legault SR, Rakobowchuk M, Ducharme MB & Weber J-M (2004*a*). Effects of carbohydrate availability on sustained shivering II. Relating muscle recruitment to fuel selection. *J Appl Physiol Bethesda Md 1985* **96,** 41–49.

Haman F, Legault SR & Weber J-M (2004*b*). Fuel selection during intense shivering in humans: EMG pattern reflects carbohydrate oxidation. *J Physiol* **556,** 305–313.

Haman F, Péronnet F, Kenny GP, Massicotte D, Lavoie C & Weber J-M (2005). Partitioning oxidative fuels during cold exposure in humans: muscle glycogen becomes dominant as shivering intensifies. *J Physiol* **566,** 247–256.

Jessen C, Feistkorn G & Nagel A (1983). Temperature sensitivity of skeletal muscle in the conscious goat. *J Appl Physiol* **54,** 880–886.

Minut-Sorokhtina OP & Glebova NF (1976). On the Vascular Component of the Peripheral Cold Reception. In *Progress in Brain Research*, ed. Ilyinsky AI and OB, Somatosensory and Visceral Receptor MechanismsProceedings of an International Symposium held in Leningrad, U.S.S.R., pp. 119–127. Elsevier. Available at: http://www.sciencedirect.com/science/article/pii/S0079612308643447 [Accessed November 6, 2015].

Morris NB, Bain AR, Cramer MN & Jay O (2014). Evidence that transient changes in sudomotor output with cold and warm fluid ingestion are independently modulated by abdominal, but not oral thermoreceptors. *J Appl Physiol Bethesda Md 1985*; DOI: 10.1152/japplphysiol.01059.2013.

Morris NB, Coombs G & Jay O (2015). Ice Slurry Ingestion Leads to a Lower Net Heat Loss during Exercise in the Heat. *Med Sci Sports Exerc*; DOI: 10.1249/MSS.0000000000000746.

Morrison SF (2016). Central neural control of thermoregulation and brown adipose tissue. *Auton Neurosci* **196,** 14–24.

Nadel ER, Bullard RW & Stolwijk JA (1971). Importance of skin temperature in the regulation of sweating. *J Appl Physiol* **31,** 80–87.

Nakamura K (2011). Central circuitries for body temperature regulation and fever. *Am J Physiol - Regul Integr Comp Physiol* **301,** R1207–R1228.

Nakamura K & Morrison SF (2008). A thermosensory pathway that controls body temperature. *Nat Neurosci* **11,** 62–71.

Nakamura K & Morrison SF (2010). A thermosensory pathway mediating heat-defense responses. *Proc Natl Acad Sci U S A* **107,** 8848–8853.

Nakamura K & Morrison SF (2011). Central efferent pathways for cold-defensive and febrile shivering. *J Physiol* **589,** 3641–3658.

Nishi Y (1981). Measurement of thermal balance in man. *Bioeng Therm Physiol Comf*29–39.

Orani GP, Anderson JW, Sant'Ambrogio G & Sant'Ambrogio FB (1991). Upper airway cooling and l-menthol reduce ventilation in the guinea pig. *J Appl Physiol Bethesda Md 1985* **70,** 2080–2086.

Osaka T (2004). Thermogenesis elicited by skin cooling in anaesthetized rats: lack of contribution of the cerebral cortex. *J Physiol* **555,** 503–513.

Parsons K (2003). *Human Thermal Environments*, Second Edition. Taylor & Francis Inc, New York, NY.

Ramanathan NL (1964). A new weighting system for mean surface temperature of the human body. *J Appl Physiol* **19,** 531–533.

Rautenberg W, Necker R & May B (1972). Thermoregulatory responses of the pigeon to changes of the brain and the spinal cord temperatures. *Pflüg Arch* **338,** 31–42.

Rawson RO & Quick KP (1972). Localization of intra-abdominal thermoreceptors in the ewe. *J Physiol* **222,** 665–667.

Riedel W (1976). Warm receptors in the dorsal abdominal wall of the rabbit. *Pflüg Arch Eur J Physiol* **361,** 205–206.

Ritschel WA & Erni W (1977). The influence of temperature of ingested fluid on stomach emptying time. *Int J Clin Pharmacol Biopharm* **15,** 172–175.

Romanovsky AA (2014). Skin temperature: its role in thermoregulation. *Acta Physiol Oxf Engl* **210,** 498–507.

Romanovsky AA, Simons CT, Szekely M & Kulchitsky VA (1997). The vagus nerve in the thermoregulatory response to systemic inflammation. *Am J Physiol - Regul Integr Comp Physiol* **273,** R407–R413.

Rutherford DJ, Hubley-Kozey CL & Stanish WD (2011). Maximal voluntary isometric contraction exercises: A methodological investigation in moderate knee osteoarthritis. *J Electromyogr Kinesiol* **21,** 154–160.

Siegel R & Laursen PB (2012). Keeping your cool: possible mechanisms for enhanced exercise performance in the heat with internal cooling methods. *Sports Med Auckl NZ* **42,** 89–98.

Simon E (2006). Deep-body thermosensitivity: Another look.: A tribute to Harold Theodore (Ted) Hammel (1921–2005). *J Therm Biol* **31,** 4–18.

Simon E, Pierau FK & Taylor DC (1986). Central and peripheral thermal control of effectors in homeothermic temperature regulation. *Physiol Rev* **66,** 235–300.

Song K, Wang H, Kamm GB, Pohle J, Reis F de C, Heppenstall P, Wende H & Siemens J (2016). The TRPM2 channel is a hypothalamic heat sensor that limits fever and can drive hypothermia. *Science* **353,** 1393–1398.

Sun WM, Houghton LA, Read NW, Grundy DG & Johnson AG (1988). Effect of meal temperature on gastric emptying of liquids in man. *Gut* **29,** 302–305.

Székely M, Szelényi Z & Kis A (1997). Fasting and re-feeding: alterations in resting metabolic rate and body temperature. *Therm Physiol*235–238.

Tan CL, Cooke EK, Leib DE, Lin Y-C, Daly GE, Zimmerman CA & Knight ZA (2016). Warm-Sensitive Neurons that Control Body Temperature. *Cell* **167,** 47–59.e15.

Villanova N, Azpiroz F & Malagelada JR (1997). Perception and gut reflexes induced by stimulation of gastrointestinal thermoreceptors in humans. *J Physiol* **502,** 215–222.

Werner J (2010). System properties, feedback control and effector coordination of human temperature regulation. *Eur J Appl Physiol* **109,** 13–25.

Wilson RP & Culik BM (1991). The cost of a hot meal: facultative specific dynamic action may ensure temperature homeostasis in post-ingestive endotherms. *Comp Biochem Physiol A* **100,** 151–154.

Yoneshiro T, Aita S, Matsushita M, Kameya T, Nakada K, Kawai Y & Saito M (2011). Brown adipose tissue, whole-body energy expenditure, and thermogenesis in healthy adult men. *Obes Silver Spring Md* **19,** 13–16.

LIST OF FIGURES

Figure 1. One-minute averages (±SD), in clockwise order starting from top left for: rectal temperature (T_{re}), metabolic rate (M), electromyographic activity (EMG), heart rate (HR), mean body temperature (T_b) and skin temperature (T_{sk}). The coloured lines represent the 7°C (blue), 22°C (green), 37°C (orange) and 52°C (red) trials, respectively. The grey area denotes rest before cooling and the solid lines denote fluid ingestion. Statistical analyses are absent from the graph for clarity. See text for the interpretation of the data.

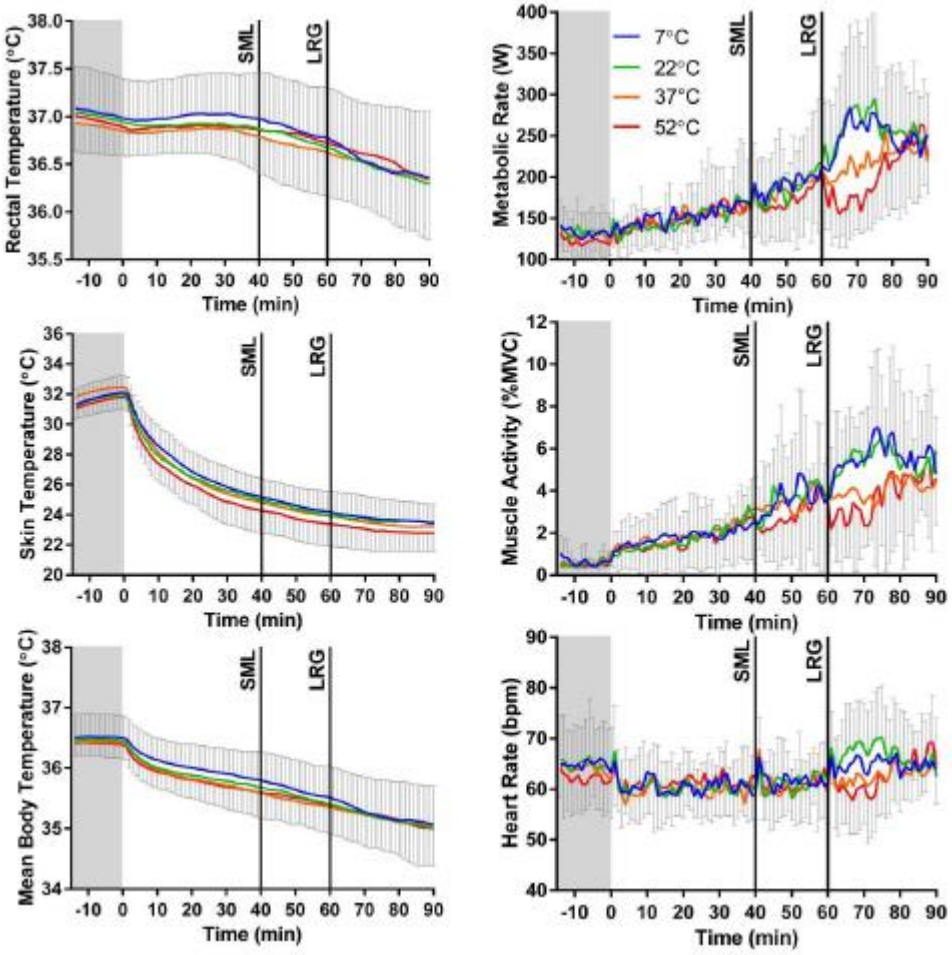

Figure 2. Mean change in cold-defence physiological responses (±SD), from 2 min before to 10 min (SML) or 15 min (LRG) after fluid ingestion, relative to the 37°C trial, for metabolic rate (M) and electromyographic activity (EMG). The coloured bars represent the 7°C (blue), 22°C (green) and 52°C (red) trials, respectively. Where * denotes 7°C or 22°C > 52°C (P<0.05).

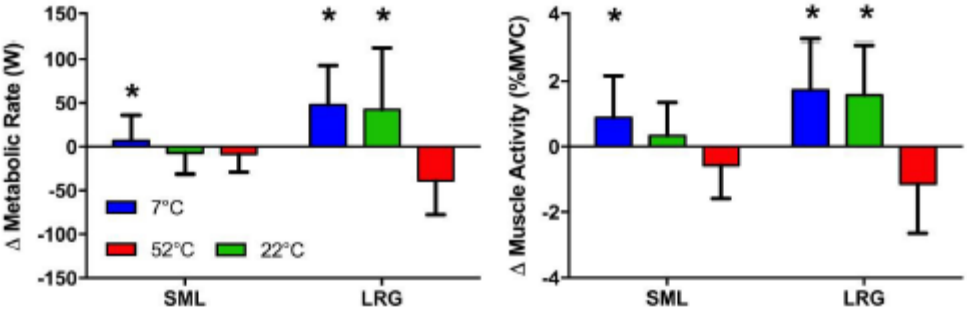

Figure 3. Mean perceptual responses (±SD), left side panels represent whole-body thermal sensation (WBTS) 5 min before and 5 min after fluid ingestion and right side panels represent the change in WBTS from pre to post ingestion, relative to the 37°C trial. The coloured bars represent the 7°C (blue), 22°C (green) and 52°C (red) trials, respectively. Where # denotes 7°C or 22°C < 52°C (P<0.05).

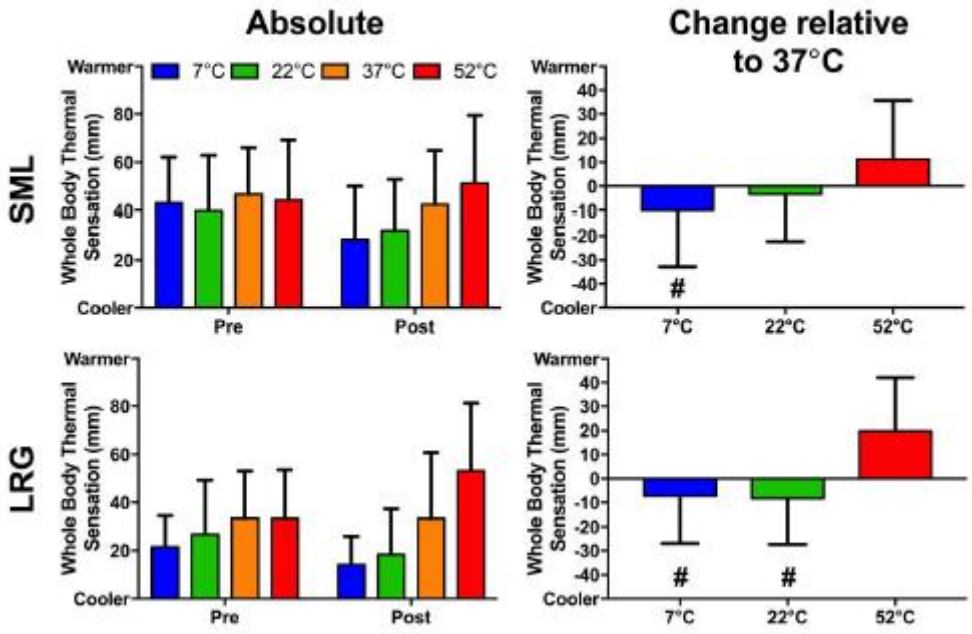

Figure 4. Mean arterial pressure (MAP) (±SD). The coloured lines represent the 7°C (blue), 22°C (green), 37°C (orange) and 52°C (red) trials, respectively. The grey area denotes rest before cooling and the solid lines denote fluid ingestion.

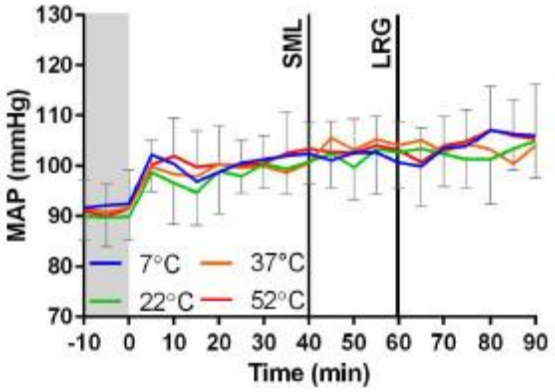

Figure 5. Change in thermoeffector responses (±SD) from baseline (i.e. relative to the 1 min average prior to ingestion, standardized to the 37°C condition in order to account for any differences in responses prior to ingestion). The top panels depict the changes following the 1.5 ml/kg (SML) ingestion and the bottom panels depict the changes following the 3.0 ml/kg (LRG) ingestion. Left columns illustrate change in metabolic rate (M) and right columns illustrate change in electromyographic activity (EMG). The coloured lines represent the 7°C (blue), 22°C (green) and 52°C (red) trials, respectively. Dashed (7°C), black (22°C) and dotted (52°C) are significantly different to 37°C ($P<0.05$).