Do peripheral thermoreceptors in the abdomen modify human sudomotor responses?

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B.Sc., University of Ottawa, 2011

Thesis submitted to the
Faculty of Graduate and Postdoctoral Studies
In partial fulfillment of the requirements
For the degree of Master of Science - Human Kinetics

School of Human Kinetics
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ACKNOWLEDGEMENTS

First and foremost I would like to thank Dr. Ollie Jay for his ongoing support and for the many opportunities he has given me throughout my master's degree. The amount of help and support I was given was more than what was normally required by a supervisor and I thank him for that.

Secondly I would like to thank the other students I have worked with during the last three years for help with data collection and general learning: Anthony Bain, Yannick Molgat-Seon, Tomasz Deren, Nicole Lesperance, Sheila Dervis, Jovana Smoljanic, Zuzana Novak, Nick Ravanelli, and Geoff Coombs. I would like to specifically thank Matthew Cramer with whom I've spent many hours discussing ideas and who has helped me to become a better researcher.

I would like to thank my friends and family who supported me throughout my degree and who were understanding when I didn't give them the time they deserved. A special thanks to Renée who let me get away with many late nights at the office and cancelled dinner plans.

I would like to thank my thesis committee members, Dr. Anthony Carlsen and Dr. Glen Kenny, for taking their time to read and critique my thesis, as it has helped improve the quality of my work, while taking them away from their busy schedules.

Finally I would like to thank all the participants who volunteered for the studies contained in this thesis. Specifically, I would like to thank Jacques Leblanc who had volunteered for many studies in the Thermal Ergonomics Laboratory and who sadly passed away on July 21st, 2013. He was a fierce friend and he will be missed.

THESIS ABSTRACT

Previous research has demonstrated that ingesting fluid of different temperatures results in different whole-body sweat losses (WBSL) and transient changes in local sweat rate (LSR) without any parallel differences in core or skin temperatures. The purpose of this thesis was to determine the potential location and relative contribution of gastrointestinal thermoreceptors that modify sudomotor activity. Eight participants cycled for 75 min while cold (1.5°C) and warm (50°C) water was either swilled in the mouth, or delivered directly to the stomach bypassing the mouth using a nasogastric tube, after 15, 30 and 45-min of exercise. Mouth-swilling warm or cold water did not alter sudomotor output, however delivering warm or cold water directly into the stomach led to a temperature-dependent change in sudomotor output, despite similar core and skin temperatures. These data indicate that thermoreceptors independently modulating sudomotor output probably reside within the abdominal area, but not the mouth.

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CHAPTER I: INTRODUCTION

1.1 Introduction

Presently, multiple studies (3, 30, 52) demonstrate the ingestion of fluids of different temperatures during exercise elicits different whole-body sweat losses (WBSL), with the ingestion of cooler fluids leading to lower WBSL values and warm fluids leading to a greater WBSL. Additionally, these differences in WBSL occur without concomitant differences in either core or skin temperatures (3, 30, 52). As core and skin temperatures are generally accepted as the primary governors of the sudomotor response (34, 48), it should be expected that alterations in either rectal, tympanic, or skin temperatures should occur either prior to, or in parallel with, the fluid temperature-dependent changes in sudomotor activity. It follows that a thermoreceptor input from tissues other than the hypothalamus and skin must therefore be responsible for the changes in sudomotor activity observed with fluid ingestion.

Recent unpublished data from our laboratory (Figure 1) demonstrate ingesting hot and cold fluids (i.e. 50 and 1.5ºC) transiently increase or decrease local sweat rate in comparison to a thermoneutral fluid temperature (i.e. 37ºC). Additionally, similar alterations in local sweat rate are observed across multiple locations, indicative of a centrally, as opposed to a peripherally, mediated responses (i.e. the changes are due to differences in central nervous system stimulation in opposition to differences at the level of the sweat gland) (33, 34). Moreover, these changes in local sudomotor activity occur independently of changes in rectal, tympanic, skin, or mean body temperatures (Figure 2). Taken together, these data suggest the existence of thermoreceptors located elsewhere in the body other than in the brain or skin that can mediate thermoeffector responses. As the fluids were ingested, the most likely area for these thermoreceptors is somewhere along the gastrointestinal tract.

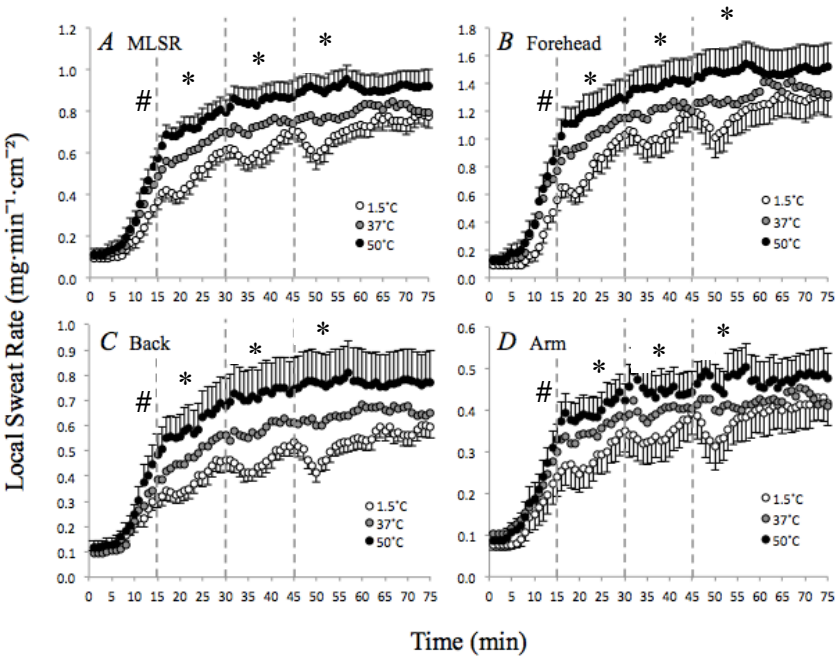

Figure 1. (Unpublished Thermal Ergonomics Laboratory data) Mean values (SE) for local sweat rate (LSR) during the ingestion of 1.5 (open circles), 37 (grey circles), and 50°C (black circles) water at three locations: the forehead (panel B), upper back (panel C), forearm (panel D), and the combined mean value from each site (panel A). Dashed lines indicate time of fluid ingestion. # denotes LSR is significantly lower ($p<0.05$) in 1.5 compared to 50ºC trial. * denotes LSR in 1.5 and 50ºC is significantly lower and higher, respectively, compared to 37ºC trial. n=12.

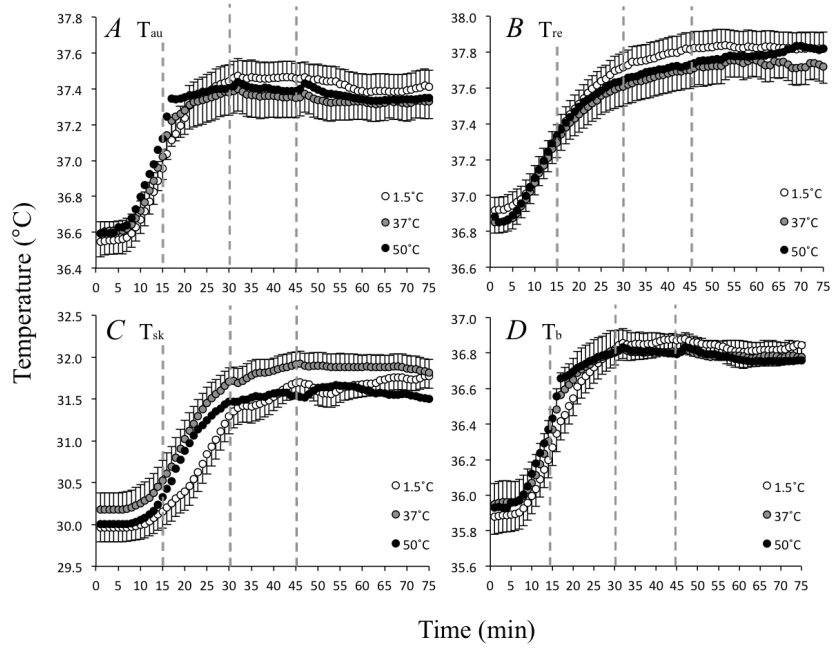

Figure 2. (Unpublished Thermal Ergonomics Laboratory data) Mean temperature (SE) for rectal temperature (T_{re}) (panel A), aural canal temperature (T_{au}) (panel B), skin temperature (T_{sk}) (panel C) and mean body temperature (T_b) (panel D) during ingestion of 1.5 (open circles), 37 (grey circles), and 50°C (black circles) water. Dashed lines indicate time of fluid ingestion. n=12.

1.2 Rationale and statement of the problem

The ingestion of fluids of different temperatures during exercise results in fluid temperature-dependent differences in WBSL without parallel differences in either core (i.e. tympanic and rectal) or skin temperatures. Recent unpublished data from our laboratory demonstrated that local changes in sweat rate in response to fluids of different temperatures are transient, responding to heat stimuli in less than 1-min and cold stimuli in less than 2-min. Additionally these changes occurred with no concomitant changes in core (rectal and tympanic) or skin temperatures, thereby suggesting thermoreceptors located elsewhere are responsible for the observed changes. The location of these thermoreceptors (probably somewhere within the gastrointestinal tract) is presently unclear. Therefore the purpose of this study was to assess whether thermoreceptors in the mouth and/or in the stomach (or abdominal) region independently mediate changes in local sweat rate (LSR) during exercise.

1.3 Hypothesis

It was hypothesized that hot (50°C) and cold (1.5°C) fluids administered directly into the stomach via a nasogastric tube, thereby bypassing the mouth, would elicit changes in LSR but when hot and cold fluid were held in the mouth, little or no effect on LSR would be observed. This was hypothesised because thermoreceptors in the mouth are primarily somatic sensory neurons, which elicit behavioural thermoeffector responses whereas abdominal thermoreceptors are primarily autonomic sensory neurons, which elicit physiological thermoeffector responses.

1.4 Objectives

The primary objective of this thesis was to determine whether independently stimulating thermoreceptors in the mouth or in the abdomen, via nasogastric tube, could cause changes in sudomotor activity independently from changes in core and skin temperature temperature.

1.5 Relevance

Mechanistically, the results from this thesis are the first data to determine the existence of thermoreceptors in the abdomen capable of modifying sudomotor activity in humans. The results from this thesis contribute to the characterization of the sudomotor thermal reflex observed following ingestion of beverages differing in temperature. Manipulation of this reflex could contribute to the maintenance of hydration status during activities that threaten thermal homeostasis including sport, military activities, daily activities in hot environments, and during heat waves.

1.6 Delimitations and limitations

Exercise was used to induce a thermal load on the participants during the trials, which could have introduced variability in to the LSR measurements as other factors that affect sudomotor activity could have been altered (i.e. baroreceptors and metaboreceptors). Furthermore it is impossible to measure hypothalamic temperature in conscious exercising humans. To account for these issues, future studies in this field could be done with passive heating in order to decrease some of the variability introduced through exercise and to better examine the effect water ingestion has on cardiovascular and cutaneous blood flow measurements. Additionally, to confirm no changes in hypothalamic temperature were present,

similar studies could be performed in animals, where direct measurement of hypothalamic temperature is possible.

CHAPTER II: REVIEW OF THE LITERATURE

2.1 Temperature regulation

Humans are homeotherms, meaning they maintain a relatively stable body temperature of ~37ºC despite relatively large changes in environmental conditions. This body temperature ensures enzymatic reactions occur are at a near-optimal level at all times (39). As such, the human body has evolved an intricate thermoregulatory system comprising of sensory afferents, a central integrator system, and efferent-effector responses (49). As the sensory afferents and central integrator system are the primary focus of this thesis, they are discussed in greater depth below. The efferent-effector responses in humans include vasoconstriction, piloerection, shivering thermogenesis, and non-shivering thermogenesis during cold stress, and vasodilatation and sweating during heat stress (45). The remainder of this literature review focuses primarily on the physiological responses to heat stress.

Physiological responses to heat alter temperature gradients and the water vapour pressure gradient between the skin surface and the surrounding environment in order to facilitate enhanced heat dissipation. Specifically, vasodilation causes a greater outflow of blood from metabolically active organs and muscles to the peripheral cutaneous tissue, thereby increasing skin temperature. Depending upon environmental conditions, elevations in skin temperature allow for greater heat loss or lower heat gain from the environment, through conduction, convection, and radiation. Additionally, sweating occurs, which causes a large amount of heat to be liberated through the phase change from liquid to vapour at the skin surface. The rate of evaporative heat loss is consequently determined by the water vapour pressure gradient between the skin and the ambient environment (15).

Thermoregulatory control models

Conceptually, attaining and maintaining thermal balance is typically thought to occur by some combination of a closed-loop or feedback system and an open-loop or feedforward system. Additionally, some researchers propose an intermediary auxiliary feedback system in addition to, or in place of, the typical hypothalamus controlled model (51). An overview of these different types of systems can be found in Figure 3.

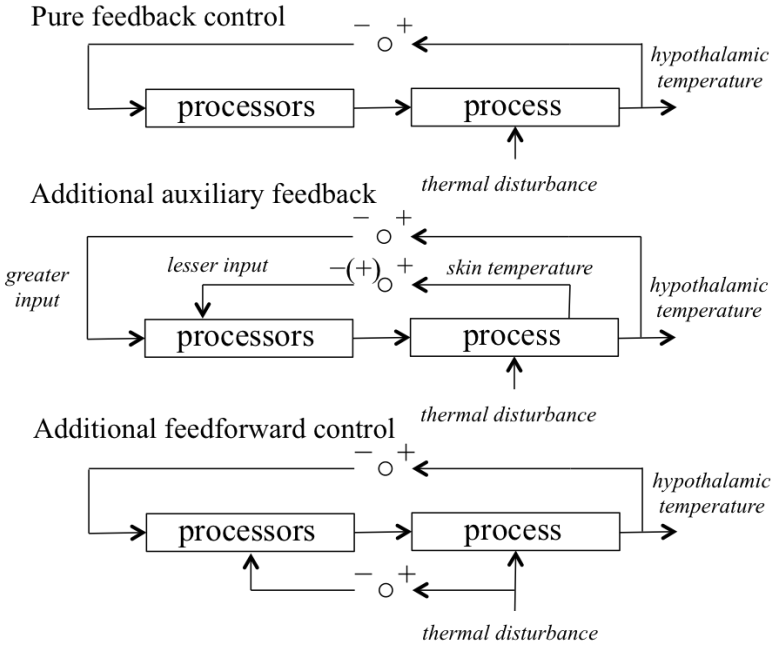

Figure 3. (Original figure based upon Werner 2010 (51)) Different examples of thermoregulatory control models.
A. Pure feedback, in this model, the preoptic area (POA) regulates all physiological responses based on cerebral artery blood temperature.
B. Additional auxiliary feedback, in this model, the POA is the primary regulator for physiological responses, with modification from skin temperature, though other models include visceral and spinal cord temperatures.
C. Additional feedforward control, in this model skin thermoreceptors elicit thermoregulatory responses (behavioural and physiological) prior to any change in core (i.e. POA) temperature.

Feedforward control system

The feedforward system is dictated by peripheral thermoreceptors in the skin. The primary purpose of this loop is to detect environmental temperatures that could threaten thermal homeostasis and elicit responses in order to defend core temperature in advance of undue thermal stress (26). As such, the primary effector response of the feedforward system is to elicit behavioural adjustments in order to preserve thermal status. Behavioural adjustments include adding or removing clothing, seeking a more thermally pleasant environment such as shade on a hot sunny day, or increasing movement in the cold (47).

Behavioural responses are typically far more effective in preserving thermal homeostasis than physiological responses and more energy efficient since physiological responses to thermal stressors are either metabolically costly (shivering) or consume vital body-water stores (sweating). Feedforward control can also modify physiological response by near-instantaneous modifications in sudomotor activity (33, 34), although it should be noted these modifications are most responsive when the thermoeffectors are primed (i.e. sweat rate will change most rapidly once someone is already sweating) (33). These modifications are believed to be a result of altering hypothalamic thermoreffector signalling (described in detail below).

Feedback control system

The feedback system typically refers to the manner by which the hypothalamus regulates core body temperature. In this model, the hypothalamus uses an actual measurement of core temperature by the influx of blood from the anterior cerebral arteries and adjusts the body's physiological thermoeffector mechanisms (i.e. sweating and vasodilatation) appropriately in order to establish thermal homeostasis (6). Activation of thermoeffectors via the hypothalamus typically occurs in parallel with elevated rate of metabolic heat production, fever, or in hot or cold environments where the peripherally monitored feedforward system has failed to prevent either heat gains or heat losses to the environment that change core temperature .

Auxiliary feedback system

An auxiliary feedback loop describes the hypothalamus as the primary controller receiving auxiliary input from peripheral skin thermoreceptors, which modifies the central hypothalamic feedback signal to account for the temperature status of peripheral tissues (51). The relative modification of effector output according to peripheral thermoreceptor activity is reflected by the "core" and "shell" weightings typically employed (e.g. 0.9/0.1) when using mean

body temperature as a forcing function for sudomotor and vasomotor activity (48, 51). The level of control this auxiliary system can impose upon thermoeffector responses is debated. Some researchers consider this system as only one aspect of a larger thermoregulatory system (32, 37), while others believe that the auxiliary system is responsible for all physiological response to a thermal stressor and that the feedforward control system only affects behavioural responses (51). Contrary to the auxiliary or even typical feedback model, other researchers (28, 29) state that all thermoregulatory responses are either feedforward or that independent body regions are controlled separately without a main central organizer. These views, however, are not widely accepted (37, 51).

In addition to the typical skin and hypothalamus thermoafferents, a recent model based on data from anatomical and physiological studies performed on rats and other animals suggests additional thermoreceptors contribute to thermoafferent signalling (37). In this model, the hypothalamus is described as the control centre for all physiological responses to thermal stress with a feedforward system functioning in parallel to an auxiliary feedback system. In addition to thermal afferents from the skin, thermal inputs from the spinal cord sensitive to the temperature of blood returning from the periphery, and thermoafferents from the viscera inform the hypothalamus on the thermal state of the entire body (37). However due to the invasiveness of these measures, spinal and visceral thermoafferents to date have only been demonstrated in rats and not in humans (37).

Role of the nervous system in thermoregulation

No matter the type of thermoregulatory system in use, for any thermoeffector response to occur a thermoafferent signal indicating a threatening thermal load must be projected to the central integrator, which relays a signal to the appropriate thermoeffectors via thermoefferent

neurons. Figure 4 depicts different potential thermoafferent pathways to the central integrator and different thermoefferent pathways to thermoeffectors.

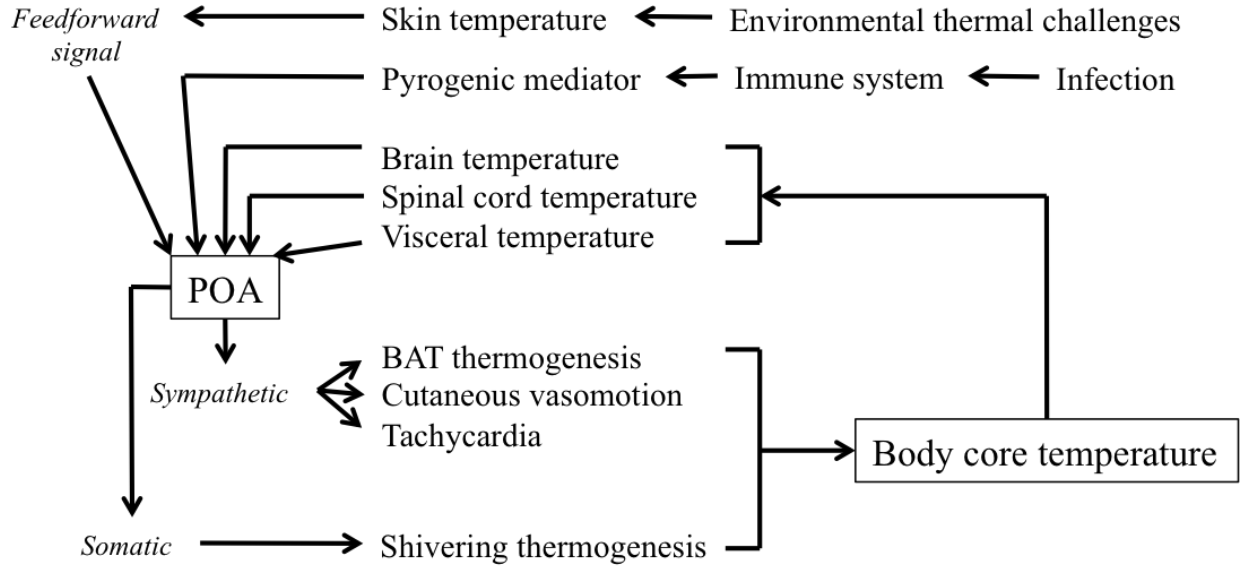

Figure 4. (Original figure based upon Nakamura 2011 (37)) Thermoregulatory control model based on anatomical and physiological neurology experiments on animals. Although this model illustrates the contributing factors to feedforward and feedback control mechanisms in response to cold stress, the same pathways would be used for heat stress, but with different end-result thermoeffectors (i.e. vasodilation and sweating instead of thermogenesis and vasoconstriction). Using control-theory terms, POA thermoregulatory control would act like a traditional feedback system, thermal afferents from the spinal cord, viscera, and local brain temperature would act as auxiliary modifiers to the feedback system, and the response-modifying effect of skin thermoreceptors would act like a feedforward system.

Central thermosensors

Brain temperature is sensed by warm-sensitive neurons in the preoptic area (POA) of the hypothalamus. It is hypothesized that warm- and not cold-sensitive neurons reside in the POA due to the fact that 1) humans are much closer to the upper temperature limit for survival, therefore heat sensitivity is beneficial for thermal safety 2) humans are endothermic, therefore heat sensitivity is beneficial for maintaining an optimal body heat content for enzymatic productivity (45). These neurons function using a tonic discharge process in thermoneutral conditions. Cooling of the POA activates cold defense mechanisms (brown adipose tissue thermogenesis, shivering thermogenesis) (22, 23), and heating the POA activates heat defensive

mechanisms (vasodilatation and sweating) (27, 28). The physiological responses evoked by changes in POA temperature have led researchers to conclude that hypothalamic temperature dictates physiological responses (45).

In addition to closely monitoring core temperature through changes in local blood temperature, in rats it has been demonstrated that the POA indirectly receives information from cutaneous and visceral thermoreceptors (9, 18). The tonic discharge of warm-sensitive thermoreceptors in the POA is down-regulated during cooling and accelerated during warming, and can be further modified by changes in skin temperature (35, 36). Moreover, it is possible that thermal afferent signals arising from cutaneous thermoreceptors are conducted to integration cells in the spinal cord, which then relays information to the POA and modulates whole-body thermoafferent signals (19). On the other hand, recent studies demonstrate that temperature sensitive transmembrane receptor potential (TRP) channels ending in the spinal column may influence tonic thermal signals in the POA (4, 50). Through this mechanism, afferent signals related to the temperature of blood returning from peripheral tissues are relayed to the brain, allowing alterations in thermoregulatory responses that account for disturbances in peripheral temperature. As such, the primary roles of the central thermosensation mechanism are to 1) establish a basal tone for the efferent pathways, 2) enhance thermoregulatory responses to compensate for environmental conditions that threaten thermal homeostasis once feedforward regulation has proven inadequate, and 3) respond to detrimental central warming due to increased metabolism (37).

Cutaneous thermosensors

Contrary to the functions of thermoreceptors in the POA, cutaneous thermoreceptors detect environmental changes that could threaten thermal homeostasis, be it hot or cold. As such,

both warm and cool thermoreceptors reside in skin (2, 11). Type C nerve fibres travel up the lateral spinothalamic tract and terminate at either the thalamus or the lateral parabrachial nucleus (LPB) (8, 31). Thermal stimuli carried by cutaneous thermoreceptors terminating in the thalamus are then transmitted to the primary somatosensory cortex which mediates thermal perception and allows humans to distinguish between hot and cold sensations (10, 12). These thermal perceptions are responsible for the powerful behavioural responses exhibited by humans to maintain core temperature but are not responsible for the feedforward physiological responses in response to extreme environmental temperatures (35, 38, 45). Conversely, the thermal stimuli carried by cutaneous thermoreceptors terminating in the LPB are relayed to the POA, which modifies the tonic discharge signal created in the POA through direct connections from the LPB to the median preoptic nucleus of the POA (35, 36). This effect on the POA has been observed in response to both hot and cold stimuli (35, 36).

Visceral thermosensors

Both hot and cold peripheral thermoreceptors have been found in the abdomens of many different species of birds and mammals but thus far not in humans (14, 20, 43, 44). These thermoreceptors are type C nerve fibres that are part of the vagus and splanchnic nerve bundles that ascend up the spinothalamic branches of the spinal cord. These visceral thermoafferents are comprised of the same type of nerve fibre as cutaneous thermoreceptors, and similarly relay thermal information to the LPB, the same segment of the brain thought to modulate the tonic signalling from the POA that elicits feedforward thermoregulatory responses (46). Despite these thermal afferents exhibiting similar excitation patterns as cutaneous thermoreceptors (20, 44), how thermal afferent information from these areas translate to whole-body thermoefferent responses is currently unknown (36).

It has been hypothesized that the large quantity of thermal afferents in the viscera of birds and small mammals help prevent the failure of critical homeostatic temperature-sensitive functions, despite a high local metabolic rate (32). This hypothesis seems reasonable since other highly metabollically active tissues, such as muscle, are highly innervated with thermoreceptors (45). Furthermore, at high core temperatures (i.e. 40ºC) an increase in permeability of the intestinal barrier can eventually lead to endotoxemia, a fairly common cause of illness and death due to hyperthermia in many homeothermic species including humans (5, 17, 21).

Oral thermosensation

A high density of thermoreceptors are located on the tongue and lips (46). These thermoreceptors project thermal information along the trigeminal nerve to the thalamus, which relays stimuli to the somatosensory cortex. Unlike cutaneous and visceral thermoreceptors, these fibres likely terminate mostly in the thalamus, as it has been shown that activity in the thalamus is highly representative of the activity of the oral thermoreceptors (41, 42). This large thalamic signal relay is translated to relatively large representation of the lips and tongue in the somatosensory cortex (40). Moreover, trigeminal thermoreceptor fibres have been shown to terminate in the LPB, though in smaller quantities than those that terminate in the thalamus (7). However, to date stimulation of oral-region thermoreceptors have not been shown to modify vasomotor or sudomotor activity in humans or animals. From an evolutionary standpoint, greater somatosensory representation would be beneficial as oral thermal homeostasis is not essential for life, though thermal homeostasis of the abdominal organs inferior to the mouth is. Therefore, inducing physiological responses is secondary to evoking a behavioural response to limit the ingestion of thermally threatening water or food.

2.2 Summary

The three parts comprising the human thermoregulatory control system are the thermal afferents, central integrator system and efferent thermoeffectors. Conceptually, these parts work together in a feedforward, feedback, or auxiliary feedback system to maintain core temperature. In the traditional feedback model, warm-sensitive thermoreceptors in the POA of the hypothalamus are responsible for setting a thermoregulatory basal tone signal, which speeds up or slows down when the POA is warmed or cooled. In doing so, the POA elicits physiological responses for when core temperature is being threatened. In the auxiliary feedback model developed using animal data only, additional feedback from spinal and visceral thermoreceptors project to the POA, so while the primary forcing function of physiological control is still POA temperature, additional peripheral thermal stimuli also contribute to describing the thermal state of the entire body. The feedforward system is the first line of defense against environmental thermal stress, which is picked up by thermoreceptors in the skin, and elicits behavioural and physiological responses prior to any change in core temperature, in order to protect thermal homeostasis. Behavioural responses occur as a result of thermosensory information projecting to the somatosensory cortex, while physiological responses occur due to thermosensory information being projected to the LPB. This signal relay modifies the basal tone signal of the hypothalamus through direct connections between the LPB and POA. In addition to cutaneous thermoreceptors projecting to the LPB, oral and abdominal viscera thermoreceptors have been shown in animals to also project to the LPB. To date the effect of oral and abdominal thermoafferents on thermoeffector responses has not been demonstrated in either animals or humans.

CHAPTER III: METHODS AND RESULTS

3.1 Methods

Ethical approval

The experimental protocol was approved by the University of Ottawa Research Ethics Board, and was therefore in accordance with the *Declaration of Helsinki.* Completed Physical Activity Readiness Questionnaires (PAR-Q) forms and written informed consent were obtained from all the volunteers who participated in the study prior to experimentation.

Participants

Using the LSR data from the 1.5°C and 50°C trials from the study mentioned in the introduction (Figure 1), a power calculation was performed using the calculated effect size of 1.25, an α of 0.05, a β of 0.2 which determined that eight participants were required for a sufficient level of statistical power. Therefore, eight non-heat acclimated participants (mean age: 22±3 y, body mass; 73.4±7.1 kg, VO_{2peak}: 52.8±5.4 $mL \cdot min^{-1} \cdot kg^{-1}$) were recruited. Participants did not consume caffeine or alcohol nor partake in any strenuous exercise 24 h prior to testing. They were asked to maintain a consistent routine (e.g. sleep schedules) and consume a similar diet during the day before and day of the experimental sessions. To the best of their knowledge, all participants were free from cardiovascular and metabolic health disorders before consenting to the study.

Protocol

Preliminary session. The participants attended a preliminary session in which total body mass, height, and peak oxygen consumption were measured. Peak oxygen consumption (VO_{2peak}) was measured using an upright cycle ergometer protocol consisting of a two minute warm up at 40 W followed by cycling at 100 W for the third minute with a 20 W increase every minute

thereafter until physical exhaustion. This protocol was based upon recommendations from the Canadian Society of Exercise Physiology (13).

Experimental sessions. Upon arrival to the laboratory, subjects were asked to provide a urine sample, which was analyzed for urine specific gravity (USG) using a spectrometer (Reichert TS 400, Depew, NY) to ensure that all participants were euhydrated prior to each experimental session. Participants were required to have a urine specific gravity below 1.020 (1) prior to commencing a trial. Mean pre-exercise USG values were 1.014±0.004. The participants then cycled for 75 min at 50% VO_{2peak}. The order in which the trials were performed was determined using an incomplete Latin Square design and each trial was separated by at least 48 h, but by no more than one week. A mechanical fan placed 1.25 m in front of the participants produced a mean whole body air velocity of 0.75 $m \cdot s^{-1}$, measured using a hot wire anemometer (Omega Engineering, Stamford, CT, USA). Participants were semi-nude, wearing a standardized clothing ensemble in all experimental trials consisting of only light running shorts, socks and shoes. All within-subject experimental sessions were completed at the same time of the day to avoid the influence of circadian variation. The ambient air temperature and relative humidity was selected to be similar to that of previous studies investigating whole-body sudomotor responses in order to best replicate the previously observed response (3, 30). The ambient environmental conditions were similar between all participants (23.7±1.3°C, 32±10% RH) and within participant sessions (±0.3ºC and ±5% RH).

Participants completed four experimental trials in which aliquots of exactly 3.2 $mL \cdot kg^{-1}$ of 1.5°C or 50°C water were either swilled in the mouth (SW trials) using 4 equal volume aliquots of water for 15 s at a time for a total swill time of 1-min per administration time point, or delivered directly into the stomach (NG trials) via a nasogastric tube (Ref# 54-8042, MED-

RX, Oakville, Canada) after 15, 30, and 45 min of exercise (equating to a group average of total water consumed per trial of 940±90 mL). This volume of fluid was selected to standardize for body mass while providing similar volumes to previous studies (3, 30).

Measurements

Water temperature. In the 1.5ºC trials, the water was poured into an insulated thermos with ice, which was then placed in a refrigerator 2 h prior to the experimental trials and left until 2 min before the ingestion of the water. In the 50ºC trials, the water was warmed using a hydrostatic controlled water bath (Polyscience – DA05A, Niles, IL, USA). The temperature of the water before ingestion was measured using a glass thermometer (Durac Plus, Blue Spirit, precision thermometer, Cole-Palmer), that was factory-calibrated, with a certified range between -1°C and +51°C with an accuracy of ±0.1°C. Fluid temperatures did not deviate more than 0.5°C from 1.5°C or 50°C for any participant.

Thermometry. Rectal temperature (T_{re}) was measured using a pediatric thermocouple probe (Mon-a-therm General Purpose Temperature Probe, Mallinckrodt Medical, St. Louis, MO, USA) inserted to a minimum of 12 cm past the anal sphincter. Aural canal temperature (T_{au}) was measured using a tympanic thermocouple probe (Mon-a-therm Tympanic, Mallinckrodt Medical, St. Louis, MO, USA) placed in the aural canal until resting near the tympanic membrane. The tympanic probe was held in position and isolated from the external environment with large amounts of cotton, which was held in place with surgical tape and an ear defender.

Skin temperature was measured at eight points over the right side of the body using thermistors integrated into heat flow sensors (2252 Ohms, Concept Engineering, Old Saybrook, CT, USA). The probes were attached using double-sided adhesive discs and surgical tape (Transpore, 3M, London, ON, Canada). Mean skin temperature (T_{sk}) was estimated using a

weighted average with the following regional proportions: forehead 7%, chest 17.5%, hand 5%, thigh 19%, scapula 17.5%, calf 20%, shoulder 7%, triceps 7% (24). All thermometry data were collected using a National Instruments data acquisition module (model NI cDAQ-9172) at a sampling rate of 5 s. Data were simultaneously displayed and recorded in spreadsheet format on a personal computer (Dell Inspirion 545) with LabVIEW 2009 software (National Instruments, TX, USA). Mean body temperature (T_b) was estimated using a weighting of $0.9 \times T_{core}$ (calculated separately both T_{au} and T_{re}) and $0.1 \times T_{sk}$ (16, 33).

Sudomotor measurements. Local sweat rate (LSR) was measured using a 4.0 cm² ventilated capsule placed on the left side of the forehead opposite the thermocouple, the right anterior forearm approximately 6 cm distal to the antecubital fossa, and the upper left back over the trapezius muscle mid-way between the neck and the acromion process. Anhydrous compressed air was passed through each capsule over the skin surface at a rate of ~1.8 $L \cdot min^{-1}$. Flow rate for each capsule was measured using an Omega FMA-A2307 flow rate monitor (Omega Engineering, Stamford, CT). Vapor content of the effluent air was measured using a 473 precision dew point mirror (RH Systems, Albuquerque, NM, USA) on the anterior forearm and two capacitance hygrometers (Series HMT333, Vaisala, Helsinki, Finland) for the forehead and upper back. All three hygrometers yielded values accurate to 0.035 $mg \cdot cm^{-2} \cdot min^{-1}$ and were factory calibrated. Values for LSR were calculated using the exact flow rate and the difference in water content between effluent and influent air. This value was normalized for the skin surface area under the capsule and expressed in $mg \cdot min^{-1} \cdot cm^{-2}$.

Whole-body sweat loss (WBSL) was measured using the change in total body mass during the trial to the nearest gram by weighing the participants using a platform scale (Combics 2, Sartorius, Mississauga, ON) immediately prior to exercise and upon the completion of

exercise. Values for WBSL were then corrected for respiratory mass loss, metabolic mass loss, saliva loss and weight gain through fluid ingestion (see reference (3) for equations).

Statistical analysis

In order to assess the LSR and thermometry data (T_{re}, T_{au}, T_{sk} and T_b), seven-minute averages from five time points (minutes 9-15, 17-23, 32-38, 47-53 and 69-75), corresponding with the last 7 minutes of the pre-drink phase, the seven minutes following drink ingestion during exercise, and 7 minutes of the no drinking phase, were analyzed in both studies. Thermometry and LSR data during the pre-drink and no-drink time periods were analyzed using a student's t-test. For the three time points during exercise, thermometry data were analyzed using a 2-way repeated measures ANOVA employing the independent variables of exercise time and fluid temperature. The LSR data was analyzed using a 3-way repeated measures ANOVA, employing the independent variables of exercise time, fluid temperature, and measurement site. The NG and SW trials were analyzed separately. The influence of fluid temperature on WBSL was analyzed using a one-way repeated measures ANOVA. The effect size of each ANOVA was calculated and reported as eta-squared values (μ^2).

When significant main effects or interactions were found, independent 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. The effect size of each t-test was calculated reported as Cohen's d (d). All analyses were performed using the statistical software package SPSS 21.0 for Windows (SPSS, Chicago, IL, USA).

3.2 Results

Whole-body sweat loss in the SW trials was similar (P=0.444, d=0.08) between 1.5°C fluid (693±92 g) and 50°C fluid (685±97 g). In the NG trials, WBSL was greater (P=0.024, d=1.18) with 50°C fluid (745±106 g) in comparison to 1.5°C fluid (630±89 g).

A comparison of LSR data between 1.5°C and 50°C fluid during mouth swilling (SW), and with the ingestion of fluid through a nasogastric tube (NG) are illustrated in Figures 4 and 5 respectively. In the SW trials, no differences were observed in LSR between 1.5°C and 50°C fluids at any point throughout exercise (P=0.738, μ^2=0.003) irrespective of when the fluids were swilled (P=0.668, μ^2<0.001) or LSR measurement site (P=0.630, μ^2<0.001). On the other hand, when fluid was delivered directly into the stomach via nasogastric tube, lower LSR values were observed at all sites with 1.5°C fluid after the first, second and third ingestion (P<0.001, μ^2=0.131) which occurred after 15, 30 and 45-min of exercise respectively. Specifically, the mean difference in LSR with 1.5°C and 50°C fluid ingestion was 0.20±0.20 mg·min^{-1}·cm^{-2} (p=0.012, d=0.264). The influence of fluid temperature was the same (P=0.573, μ^2<0.001) for all three fluid ingestions during exercise (i.e. after 15, 30 and 45-min of exercise respectively), and was the same irrespective of LSR measurement site (P=0.650, μ^2<0.001). After 75-min of exercise, which was 30-min after the last fluid ingestion, LSR was not different between 1.5°C and 50°C fluids at all measurement sites (P=0.118, d=0.138).

Rectal temperature (SW: P=0.444, μ^2=0.005; NG: P=0.561, μ^2=0.008), T_{au} (SW: P=0.844, μ^2=0.018; NG: P=0.737, μ^2<0.001) and T_{sk} (SW: P=0.430, μ^2=0.015; NG: P=0.598, μ^2=0.013) were similar between 1.5°C and 50°C fluids throughout exercise both during the SW trials and the NG trials. Similarly, T_b using either T_{re} (SW: P=0.471, μ^2=0.012; NG: P=0.485, μ^2=0.013) or T_{au} (SW: P=0.681, μ^2=0.020; NG: P=0.612, μ^2=0.001) as a representation of the

body "core" yielded similar values throughout exercise during both the NG and SW trials (Figure 6).

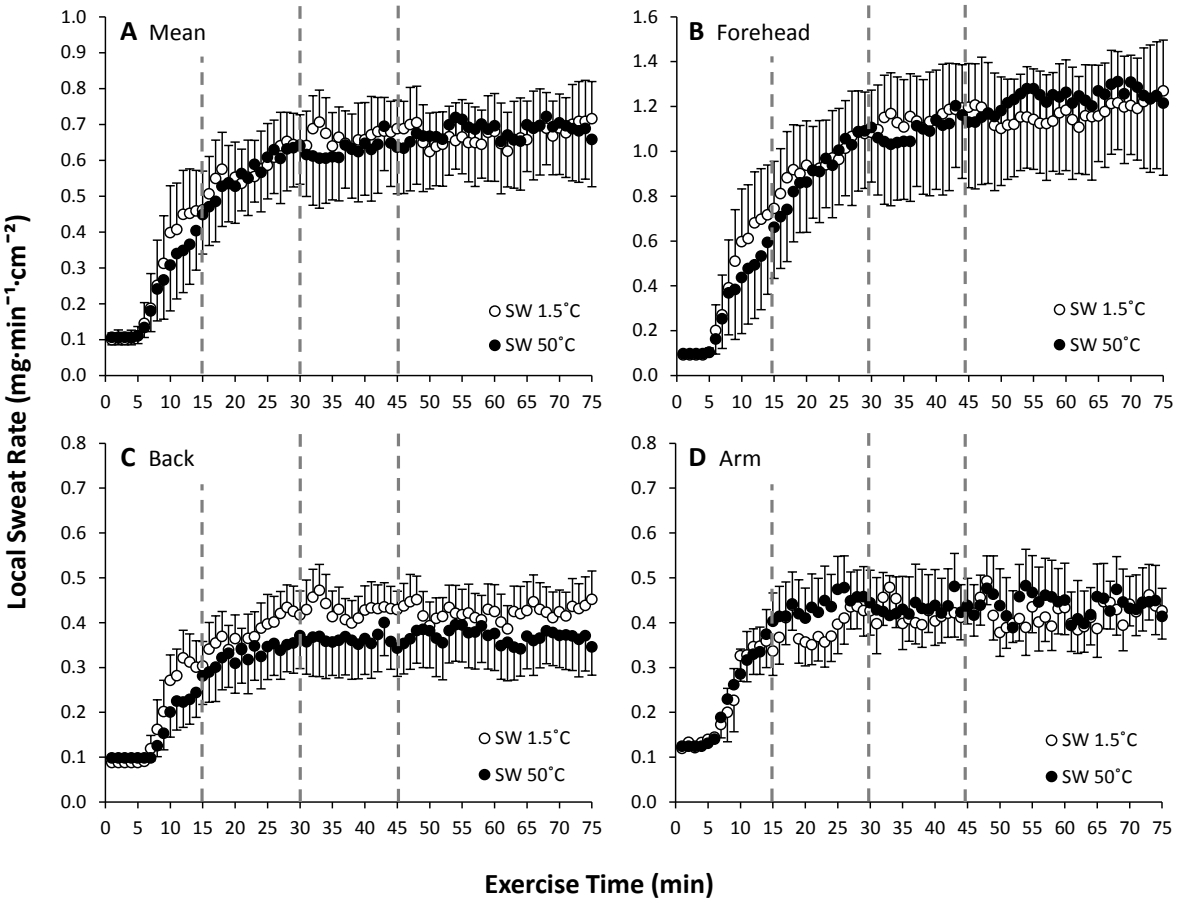

Figure 4. Mean local sweat rate (LSR) after mouth-swilling (SW trials) 1.5°C (open circles) and 50°C (black circles) fluid during exercise. Dashed lines denote when mouth-swills were administered. Values given are the grand mean (Panel A) of the following three locations: forehead (Panel B), upper back (Panel C), and forearm (Panel D). Error bars indicate standard error.

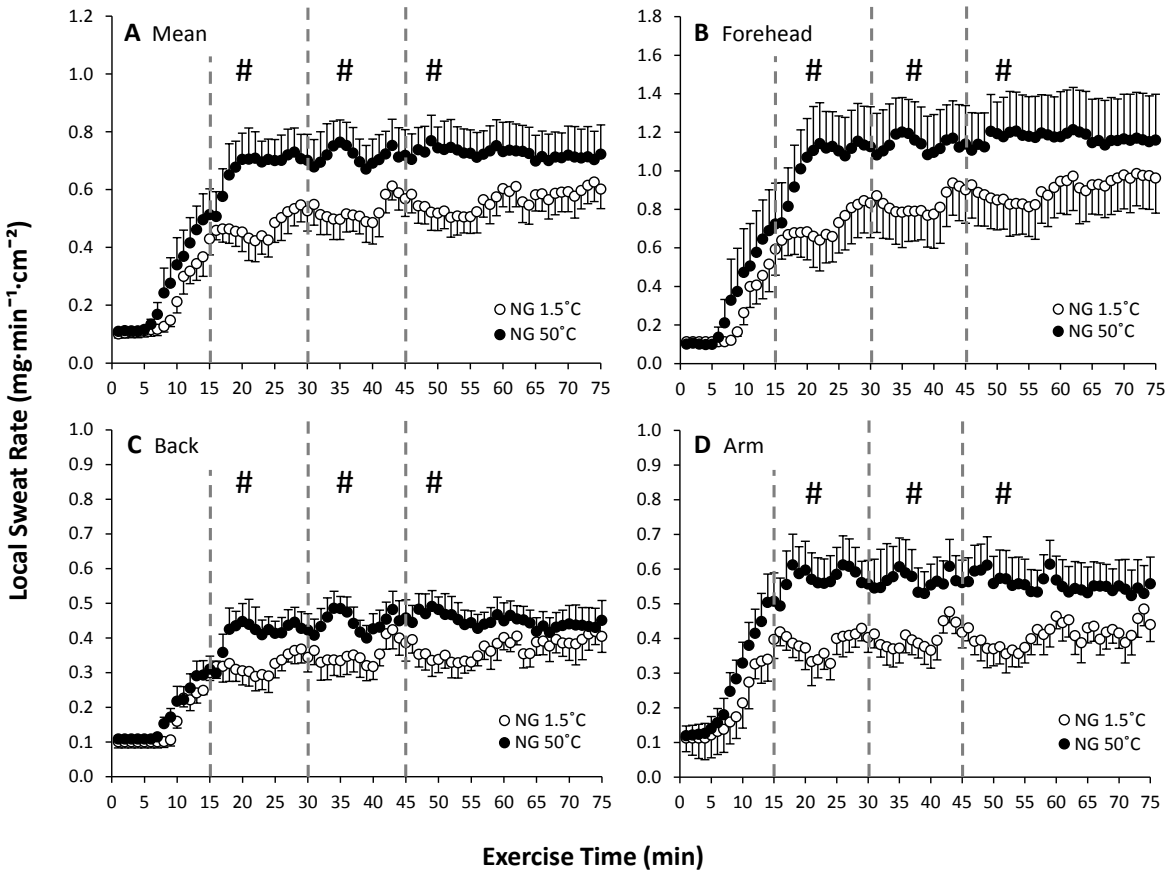

Figure 5. Absolute local sweat rate (LSR) after the ingestion of 1.5°C (open circles) and 50°C (black circles) fluid through a nasogastric tube (NG trials) during exercise. Dashed lines denote when fluids were ingested. Values given are the mean (Panel A) of the following three locations: forehead (Panel B), upper back (Panel C), and forearm (Panel D). # denotes where 1.5°C<50°C. Error bars indicate standard error.

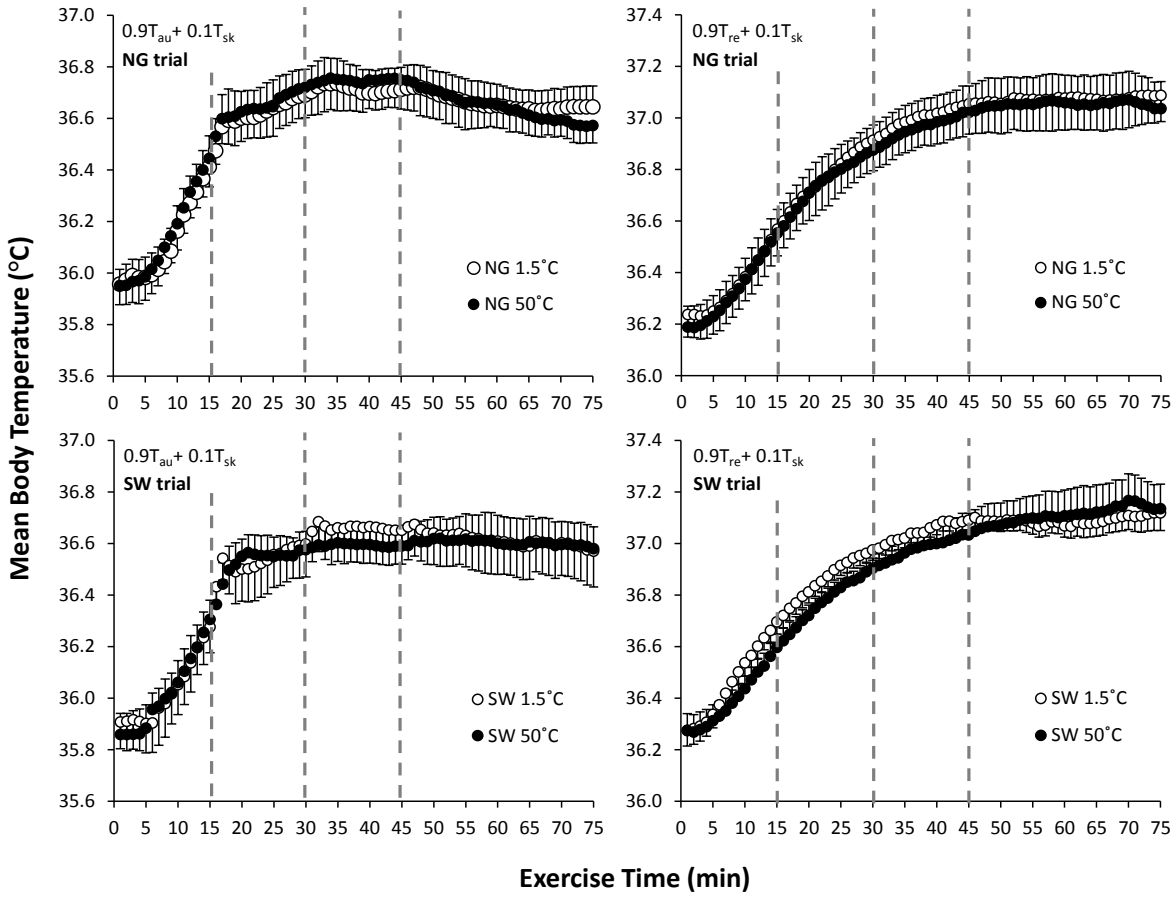

Figure 6. Mean body temperatures using a 0.9/0.1 weighting of "core" to "skin" temperatures using aural canal temperature (T_{au}) (left panels) and rectal temperature (T_{re}) (right panels) as an indication of the body "core", with 1.5°C (open circles) and 50°C (black circles) fluid during the NG trials (top row) and SW trials (bottom row). Dashed lines denote when fluids were ingested/administered. Error bars indicate standard error.

PART IV: THESIS DISCUSSION

4.1 Thesis discussion

Previous research has demonstrated ingesting water of different temperatures alters whole-body sweat losses (WBSL) independently of core and skin temperatures. The mechanism responsible for these alterations in sweat output, however, had not been previously investigated. Additionally, recent unpublished data from the Thermal Ergonomics Laboratory, which is presented in study 1 of the publishable manuscript (please see appendix), demonstrated differences in local sweat rate (LSR) following the ingestion of hot or cold water. Specifically, the changes in LSR were near immediate and transient and were not paralleled by changes in core or skin temperatures. Therefore, the purpose of this thesis was to identify the location of the thermoreceptors along the gastrointestinal tract responsible for altering sudomotor activity. This study was accomplished by measuring LSR (on the forehead, upper back and forearm), WBSL, core temperature (esophagus, rectum, and aural canal), and skin temperature at eight different sites during 75 min of cycling at 50% VO_{2max} while water of 1.5˚C and 50˚C was either swilled in the mouth only, or introduced directly into the stomach using a nasogastric tube. From the results of this thesis, it appears the previously observed changes in sudomotor activity (WBSL and LSR) were a temperature-mediated reflex response due to the stimulation of thermoreceptors in the abdominal area.

The changes in LSR following the ingestion of a cold or warm fluid occur within 1-2 min of ingestion, first demonstrated by earlier data from our laboratory and confirmed by the thesis study. The speed of the response is the first indicator that the changes in LSR were due to a thermally mediated reflex, as blood flow to the intestinal region is low during exercise, while it is high in the active muscles. Therefore small amounts of blood with altered temperature from the ingested fluids would mix with large amounts of blood returning from the rest of the body

(including active musculature) and the resulting net temperature change of the blood due to the ingested fluid would be minimal. Although a comparison to a 37ºC control trial was not possible in study 2, changes in LSR due to ingesting 1.5ºC and 50ºC water were comparable between the nasogastric tube and normal ingestion trials, whereas no changes or differences were observed in the LSR traces in the mouth-swill trials at any time point.

In addition to the rapidity of the changes in LSR following the drink ingestion, changes in LSR were observed at three different locations on the body (head, back and arm), indicating that this response was centrally mediated, rather than a local response. Mean arterial pressure (MAP) was also not measured in the present study, and it is possible that the ingestion of 1.5°C fluid could have elicited a pressor response, which in turn could have influenced LSR. However, MAP-mediated changes in LSR with cold water ingestion in the present study seem very unlikely. A pressor response with oral water ingestion is only observed in individuals with autonomic failure and to a lesser extent the elderly, but not in healthy young participants (25); and even when this pressor response is observed, it is not fluid temperature-dependent (25).

Furthermore, no differences were observed in core or skin temperature at any time point between trials. Although esophageal temperatures could not be compared throughout the trial due to the effect of the water on the probe, aural canal temperature was measured. This measurement has been shown to respond rapidly to temperature changes and indeed some researchers have demonstrated this measurement is the best low-invasive indicator surrogate for hypothalamic temperature. Despite these benefits, it is impossible to say for certain there were no changes in hypothalamic temperature that mediated the changes sudomotor activity.

4.2 Thesis conclusion

The present findings demonstrate for the first time changes in sudomotor activity during exercise resulting from the ingestion of non-thermoneutral fluids are due to a thermally mediated reflex caused by the stimulation of abdominal, but not oral thermoreceptors. This information is important to the field of thermoregulation, as previous thermoregulatory models in humans only presently consider thermal input from cutaneous and hypothalamic thermoreceptors. Additionally, by knowing the cause of this reflex, researchers can work to maximize the potentially beneficial outcomes of this response to help improve heat dissipation and hydration strategies.

Future studies need to be performed to further characterize and exploit this response, specifically, whether this response can be modified and maximized by altering the quantity, frequency, or intensity of the thermal load given. Also, whether an opposite response could occur has yet to be demonstrated (e.g. whether the shivering of an individual placed in a cold environment could be modified by ingesting fluids of different temperatures).

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CHAPTER VI: APPENDICES

APPENDIX A: PUBLISHABLE ARTICLE

Evidence that transient changes in sudomotor output with cold and warm fluid ingestion are independently modulated by abdominal, but not oral thermoreceptors

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Submitted for review in *Journal of Applied Physiology* (JAPPL-01059-2013) on 18/09/13

Running head: Sudomotor activity during cold and warm fluid ingestion

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Abstract

Two studies were performed to i) characterize changes in local sweat rate (LSR) following fluid ingestion of different temperatures during exercise, and ii) identify the potential location of thermoreceptors along the gastrointestinal tract that independently modify sudomotor activity. In study one, 12 males cycled at 50% VO_{2peak} for 75-min, while ingesting 3.2 ml·kg^{-1} of 1.5°C, 37°C, or 50ºC fluid, 5-min before exercise, and after 15, 30, and 45-min of exercise. In study two, 8 males cycled at 50% VO_{2peak} for 75-min while 3.2 ml·kg^{-1} of 1.5°C or 50ºC fluid was either delivered directly into the stomach via a nasogastric tube (NG trials), or mouth-swilled only (SW trials), after 15, 30, and 45-min of exercise. Rectal (T_{re}), aural canal (T_{au}) and mean skin temperature (T_{sk}), and LSR on the forehead, upper-back, and forearm were measured. In study 1, T_{re}, T_{au} and T_{sk} were identical between trials, but after each ingestion LSR was significantly suppressed at all sites with 1.5°C fluid and elevated with 50°C fluid, compared to 37°C ($P<0.001$). The peak difference in mean LSR between 1.5°C and 50°C fluid after ingestion was 0.29±0.06 mg·min^{-1}·cm^{-2}. In study 2, LSR was similar between 1.5°C and 50ºC fluids with mouth-swilling ($P=0.738$), but lower at all sites with 1.5°C fluid in the NG trials ($P<0.001$) despite no concurrent differences in T_{re}, T_{au} and T_{sk}. These data demonstrate i) LSR is transiently altered by cold and warm fluid ingestion despite similar core and skin temperatures; ii) thermoreceptors independently modulating sudomotor output probably reside within the abdominal area, but not the mouth.

Keywords: Body temperatures, Exercise, Fluid intake, Sweating, Thermoregulation

INTRODUCTION

To date, several studies (1, 14, 29) have reported that the ingestion of cold and warm fluids during exercise leads to large fluid temperature-dependent differences in whole-body sweat loss. The dynamic response of local sweating following the ingestion of fluids of different temperatures during exercise has never been characterized, however a notable consistency among all these previous studies is that different whole-body sweat losses were observed between fluid temperatures despite similar core and skin temperatures. As core temperature has been long-accepted as the primary stimulus for changes in sudomotor activity, with additional modifications from skin temperature (2, 17), different skin and/or core temperatures would be expected in advance of any notable divergence in local sweat rate following the ingestion of cold and warm fluids. It follows that thermoreceptors capable of independently modulating sudomotor activity may exist somewhere along the gastrointestinal tract.

One study in sheep identified thermoreceptors in the abdominal wall and small intestine that elicit autonomic thermoeffector responses (21). Several others have studied thermoreception in the mouth (19, 20), esophagus (7) and stomach (10) of birds and mammals by measuring the electrical current produced by thermoafferents, but no attendant thermoeffector responses were assessed in these studies. No study has thus far identified the presence of any thermoreceptors along the gastrointestinal tract of humans that modulate sudomotor activity. An oropharyngeal reflex mechanism modifying sudomotor output that is sensitive to hydration status but without any thermal component has been demonstrated (13, 27). Furthermore, Villanova *et al.* (28) assessed changes in thermal perception and gastric contractility following the ingestion of fluids of different temperatures, but thermoeffector responses were not reported. Indeed, in a recent review on thermoreception and subsequent thermoeffector responses, it was noted that for both

humans and animals "how abdominal thermal information contributes to thermoregulatory functions is mostly unknown" (18).

The aim of the present investigation was to explore the mechanism responsible for the different whole-body sweat losses previously reported following the ingestion of fluids of different temperatures during exercise. To this end, two studies were conducted. In the first study, the dynamic local sudomotor response was characterized at three different skin regions following the serial ingestion of cold (1.5°C), thermoneutral (37°C), and warm (50ºC) fluids during exercise. In the second study, the potential location of thermoreceptors along the gastrointestinal tract that independently modulate sudomotor activity was investigated by directing cold (1.5°C) or warm (50°C) fluids either directly into the stomach via a nasogastric (NG) tube or only into the mouth by swilling (SW). It was hypothesized that an immediate, but transient change in local sweat rate (LSR) that is directly dependent upon fluid temperature would be observed at all skin sites with fluid ingestion in the first study despite no differences in core or skin temperatures. In the second study, it was hypothesized that fluid temperature-dependent differences in LSR would be observed in the NG trials, but not the SW trials, indicating that thermoreceptors residing in the abdominal area of humans may independently modify sudomotor output.

METHODS

Ethical approval

The experimental protocol was approved by the University of Ottawa Research Ethics Board, and was therefore in accordance with the *Declaration of Helsinki.* Completed Physical Activity Readiness Questionnaires (PAR-Q) forms and written informed consent were obtained from all the volunteers who participated in the study prior to experimentation.

Participants

In study 1, twelve non-heat acclimated males (mean age: 23±3 y, body mass; 73.9±7.7 kg, VO_{2peak}: 53.9±5.4 $mL \cdot min^{-1} \cdot kg^{-1}$) were recruited. For Study 2, eight non-heat acclimated participants (mean age: 22±3 y, body mass; 73.4±7.1 kg, VO_{2peak}: 52.8±5.4 $mL \cdot min^{-1} \cdot kg^{-1}$) were recruited. Participants did not consume caffeine or alcohol nor partake in any strenuous exercise 24 h prior to testing. They were asked to maintain a consistent routine (e.g. sleep schedules) and consume a similar diet during the day before and day of the experimental sessions. To the best of their knowledge, all participants were free from cardiovascular and metabolic health disorders before consenting to the study.

Protocol

Preliminary session. In both studies, participants attended a preliminary session in which total body mass, height, and peak oxygen consumption were measured. Peak oxygen consumption (VO_{2peak}) was measured using an upright cycle ergometer protocol consisting of a two minute warm up at 40 W followed by cycling at 100 W for the third minute with a 20 W increase every minute thereafter until physical exhaustion. This protocol was based upon recommendations from the Canadian Society of Exercise Physiology (6).

Experimental sessions. In both studies, upon arrival to the laboratory, subjects were asked to provide a urine sample, which was analyzed for urine specific gravity (USG) using a spectrometer (Reichert TS 400, Depew, NY) to ensure that all participants were euhydrated prior to each experimental session. Participants were required to have a urine specific gravity below 1.020 (5) prior to commencing a trial. Mean pre-exercise USG values were 1.014±0.004. The participants then cycled for 75 min at 50% VO_{2peak}. The order in which the trials were performed was determined using an incomplete Latin Square design and each trial was separated by at least

48 h, but by no more than one week. A mechanical fan placed 1.25 m in front of the participants produced a mean whole body air velocity of 0.75 $m \cdot s^{-1}$, measured using a hot wire anemometer (Omega Engineering, Stamford, CT, USA). Participants were semi-nude, wearing a standardized clothing ensemble in all experimental trials consisting of only light running shorts, socks and shoes. All within-subject experimental sessions were completed at the same time of the day to avoid the influence of circadian variation. The ambient air temperature and relative humidity was similar between all participants (23.7±1.3°C, 32±10% RH) and within participant sessions (±0.3ºC and ±5% RH).

In study 1, participants undertook three experimental trials (one trial per fluid temperature), in which they ingested four aliquots of exactly 3.2 $mL \cdot kg^{-1}$ of 1.5°C, 37°C or 50°C water at 5 min before exercise and after 15, 30, and 45 min of exercise (equating to a group average of total water consumed per trial of 945±100 mL). This volume of fluid was selected to standardize for body mass while providing similar volumes to previous studies (1, 14). In study 2, participants completed four experimental trials in which aliquots of exactly 3.2 $mL \cdot kg^{-1}$ of 1.5°C or 50°C water were either swilled in the mouth (SW trials) using 4 equal volume aliquots of water for 15 s at a time for a total swill time of 1-min per administration time point, or delivered directly into the stomach (NG trials) via a nasogastric tube (Ref# 54-8042, MED-RX, Oakville, Canada) after 15, 30, and 45 min of exercise (equating to a group average of total water consumed per trial of 940±90 mL).

Measurements

Water temperature. In the 1.5ºC trials, the water was poured into an insulated thermos with ice, which was then placed in a refrigerator 2 h prior to the experimental trials and left until 2 min before the ingestion of the water. In the 37ºC and 50ºC trials, the water was warmed using a

hydrostatic controlled water bath (Polyscience – DA05A, Niles, IL, USA). The temperature of the water before ingestion was measured using a glass thermometer (Durac Plus, Blue Spirit, precision thermometer, Cole-Palmer), that was factory-calibrated, with a certified range between -1°C and +51°C with an accuracy of ±0.1°C. Fluid temperatures did not deviate more than 0.5°C from 1.5°C, 37°C or 50°C for any participant.

Thermometry. Rectal temperature (T_{re}) was measured using a pediatric thermocouple probe (Mon-a-therm General Purpose Temperature Probe, Mallinckrodt Medical, St. Louis, MO, USA) inserted to a minimum of 12 cm past the anal sphincter. In study 1, esophageal temperature (T_{es}) was measured by placing a pediatric thermocouple probe 40 cm past the participant's nostril and into the esophagus. Aural canal temperature (T_{au}) was measured using a tympanic thermocouple probe (Mon-a-therm Tympanic, Mallinckrodt Medical, St. Louis, MO, USA) placed in the aural canal until resting near the tympanic membrane. The tympanic probe was held in position and isolated from the external environment with large amounts of cotton, which was held in place with surgical tape and an ear defender. Esophageal temperature was used to ensure T_{au} values were equal to or greater than T_{es} prior to the start of exercise, thus verifying that the T_{au} probe had been sufficiently insulated (15). T_{es}, however, could not be used for the analysis of data during the exercise period due to the influence of fluid ingestion on T_{es} values.

Skin temperature was measured at eight points over the right side of the body using thermistors integrated into heat flow sensors (2252 Ohms, Concept Engineering, Old Saybrook, CT, USA). The probes were attached using double-sided adhesive discs and surgical tape (Transpore, 3M, London, ON, Canada). Mean skin temperature (T_{sk}) was estimated using a weighted average with the following regional proportions: forehead 7%, chest 17.5%, hand 5%,

thigh 19%, scapula 17.5%, calf 20%, shoulder 7%, triceps 7% (11). All thermometry data were collected using a National Instruments data acquisition module (model NI cDAQ-9172) at a sampling rate of 5 s. Data were simultaneously displayed and recorded in spreadsheet format on a personal computer (Dell Inspirion 545) with LabVIEW 2009 software (National Instruments, TX, USA). Mean body temperature (T_b) was estimated using a weighting of $0.9 \times T_{core}$ (calculated separately both T_{au} and T_{re}) and $0.1 \times T_{sk}$ (9, 16).

Sudomotor measurements. Local sweat rate (LSR) was measured using a 4.0 cm² ventilated capsule placed on the left side of the forehead opposite the thermocouple, the right anterior forearm approximately 6 cm distal to the antecubital fossa, and the upper left back over the trapezius muscle mid-way between the neck and the acromion process. Anhydrous compressed air was passed through each capsule over the skin surface at a rate of ~1.8 $L \cdot min^{-1}$. Flow rate for each capsule was measured using an Omega FMA-A2307 flow rate monitor (Omega Engineering, Stamford, CT). Vapor content of the effluent air was measured using a 473 precision dew point mirror (RH Systems, Albuquerque, NM, USA) on the anterior forearm and two capacitance hygrometers (Series HMT333, Vaisala, Helsinki, Finland) for the forehead and upper back. All three hygrometers yielded values accurate to 0.035 $mg \cdot cm^{-2} \cdot min^{-1}$ and were factory calibrated. Values for LSR were calculated using the exact flow rate and the difference in water content between effluent and influent air. This value was normalized for the skin surface area under the capsule and expressed in $mg \cdot min^{-1} \cdot cm^{-2}$.

Whole-body sweat loss (WBSL) was measured using the change in total body mass during the trial to the nearest gram by weighing the participants using a platform scale (Combics 2, Sartorius, Mississauga, ON) immediately prior to exercise and upon the completion of exercise.

Values for WBSL were then corrected for respiratory mass loss, metabolic mass loss, saliva loss and weight gain through fluid ingestion (see reference (1) for equations).

Statistical analysis

The trials in both studies were divided into early (0 to 15-min), mid (15 to 60-min), and late (60 to 75-min) stages of exercise. Seven-minute averages of the LSR and thermometry data (T_{re}, T_{au}, T_{sk} and T_b) were analyzed in both studies using a repeated measures ANOVA employing the independent variables of exercise time and fluid temperature. An additional independent variable of measurement site was used for analyzing the LSR data. In study 2, the NG and SW trials were analyzed separately. The relative influence of cold (1.5°C) and warm (50°C) fluid on LSR in study 1 was also isolated by calculating the change in LSR from the 1-min mean before each ingestion, and then expressing the difference relative to the response with 37°C fluid. These data were assessed using a repeated measures ANOVA with the independent variables of exercise time, fluid temperature and LSR measurement site. The influence of fluid temperature on WBSL was analyzed using a one-way repeated measures ANOVA.

When significant main effects or interactions were found, independent 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

Study 1

Whole-body sweat loss (WBSL) in the 37°C fluid trial was 767±113 g. In comparison, the WBSL of 671±89 g with 1.5°C fluid ingestion was lower (P=0.001), and the WBSL of 815±121 g with 50ºC fluid ingestion was greater (P=0.001).

The absolute local sweat rate (LSR) data with the serial ingestion of 1.5°C, 37°C and 50°C fluid during exercise are illustrated in Figure 1. After 15-min of exercise, which followed the pre-exercise fluid ingestion but preceded the first fluid ingestion during exercise, LSR was lower with 1.5ºC fluid compared to 50ºC fluid (P=0.001) at all LSR measurement sites. Following the first, second and third fluid ingestion during exercise (i.e. after 15, 30 and 45-min of exercise respectively), a fluid temperature-dependent change in LSR was observed leading to significantly lower absolute LSR values with 1.5ºC fluid, and significantly greater absolute LSR values with 50ºC fluid, compared to 37ºC fluid (all $P<0.05$). This influence of fluid temperature was the same for all three fluid ingestions during exercise (P=0.364) and was not different between LSR measurement sites (P=0.298). After 75-min of exercise, which was 30-min after the last fluid ingestion, absolute LSR was not different between fluid temperatures (P=0.251) irrespective of measurement site (P=0.498).

After accounting for any changes in absolute LSR during the thermoneutral (37°C) fluid trial, the change in LSR relative to the mean value 1-min prior to each ingestion, was significantly different between 1.5ºC and 50°C fluid (P=0.003). The changes in LSR after ingestion relative to the 37°C trial were negative with 1.5°C fluid and positive with 50°C fluid (Figure 2). The changes in mean LSR relative to those seen with 37°C fluid were different between 1.5°C and 50°C fluid, 2-min after ingestion following the first and second drink during exercise, and 1-min after ingestion for the third drink. These LSR differences were evident for 6, 9 and 11-min after the first, second and third drink respectively (Figure 2).

Despite the observed differences in LSR, rectal temperature (T_{re}) (P=0.304), aural canal temperature (T_{au}) (P=0.254) and mean skin temperature (T_{sk}) (P=0.082) were not different between fluid temperatures at any time throughout exercise. Likewise, mean body temperature

using T_{re} (P=0.879) or T_{au} (P=0.773) (Figure 3) as a representation of the body "core" were similar between fluid temperatures throughout exercise.

Study 2

Whole-body sweat loss in the SW trials was similar (P=0.444) between 1.5°C fluid (693±92 g) and 50°C fluid (685±97 g). In the NG trials, WBSL was greater (P=0.024) with 50°C fluid (745±106 g) in comparison to 1.5°C fluid (630±89 g).

A comparison of LSR data between 1.5°C and 50°C fluid during mouth swilling (SW), and with the ingestion of fluid through a nasogastric tube (NG) are illustrated in Figures 4 and 5 respectively. In the SW trials, no differences were observed in LSR between 1.5°C and 50°C fluids at any point throughout exercise (P=0.738) irrespective of when the fluids were swilled (P=0.668) or LSR measurement site (P=0.630). On the other hand, when fluid was delivered directly into the stomach via nasogastric tube, lower LSR values were observed at all sites with 1.5°C fluid after the first, second and third ingestion (P<0.001) which occurred after 15, 30 and 45-min of exercise respectively. After 75-min of exercise, which was 30-min after the last fluid ingestion, LSR was not between 1.5°C and 50°C fluids at all measurement sites (P=0.118). The influence of fluid temperature was the same (P=0.573) for all three fluid ingestions during exercise (i.e. after 15, 30 and 45-min of exercise respectively), and was the same irrespective of LSR measurement site (P=0.650).

Rectal temperature (SW: P=0.444; NG: P=0.561), T_{au} (SW: P=0.844; NG: P=0.737) and T_{sk} (SW: P=0.430; NG: P=0.598) were similar between 1.5°C and 50°C fluids throughout exercise both during the SW trials and the NG trials. Similarly, T_b using either T_{re} (SW: P=0.471;

NG: P=0.485) or T_{au} (SW: P=0.681; NG: P=0.612) as a representation of the body "core" yielded similar values throughout exercise during both the NG and SW trials (Figure 6).

DISCUSSION

To the best of our knowledge, the present study is the first to demonstrate that transient fluid-temperature dependent changes in local sudomotor activity across the body surface occur immediately following the ingestion of fluids of different temperatures during exercise (Figures 1 and 2). In parallel, no differences in core or skin temperatures were observed between the three fluid temperatures (Figure 3) suggesting that thermoreceptors residing somewhere along the gastrointestinal tract were likely responsible for independently modifying sudomotor activity. In study 2, the potential location of these thermoreceptors was investigated by administering warm (50°C) and cold (1.5°C) fluid either into the mouth area only (SW trials), or directly into the stomach area bypassing the mouth and esophagus using a nasogastric tube (NG trials). Fluid temperature-dependent differences in local sweat rate (LSR) were observed in the NG trials (Figure 5), but similar sweat rates were found between 1.5°C and 50°C fluids in the SW trials (Figure 4), again despite no differences in core and skin temperatures between 1.5°C and 50°C fluid trials in the NG or SW trials (Figure 6). The findings of the second study suggest that thermoreceptors residing in the abdominal area, but not the mouth, can independently alter sudomotor output.

The different LSR values observed between fluid temperatures in both studies are clear evidence of a thermally-mediated response. In study 1, deviations in local sweating were minimal with the ingestion of a thermoneutral (37°C) fluid, and a large fluid temperature-dependent divergence in LSR was evident with the ingestion of a cold (1.5°C) and warm (50°C) fluids, even when accounting for any changes in LSR in the thermoneutral (37°C fluid) trial

(Figure 2). Non-thermal modifiers of the central drive for sweating have been previously shown with fluid ingestion. The act of drinking temporarily inhibits the osmoregulatory inhibition of sudomotor activity in dehydrated subjects, with the ingestion of a small (~4 $mL \cdot kg^{-1}$ of body weight) aliquot of 38°C fluid eliciting an immediate rise in sweating (13, 24, 27). However, when subjects are euhydrated, or iso-osmotic, this non-thermal reflex sweating response is abolished (27). In the present study, euhydration was verified prior to the commencement of all trials, and large differences in LSR were observed between 1.5°C and 50°C fluids when the fluid bypassed the mouth altogether in the NG trials.

The sweating response was similar on the torso (upper back), a peripheral limb (forearm) and the head (forehead), indicating that the modification of sudomotor activity with fluid temperature also appears to be a systemic, rather than a localized response. Additionally, large differences in WBSL were also observed between fluid temperatures. From study 1, a cumulative effect of fluid temperature on absolute LSR values was evident due to the pre-exercise ingestion of 1.5°C and 50°C fluid, and successive ingestions during exercise separated by 15-min. However, when accounting for any differences in absolute LSR prior to each ingestion, significant suppressions and elevations in sudomotor activity were observed with 1.5°C and 50°C fluids respectively relative to 37°C fluid (Figure 2). In order to remove any potential confounding effect of the pre-exercise fluid ingestion in study 1, there was no fluid ingestion before exercise in study 2. LSR values were almost identical prior to the first fluid ingestion during exercise in both the SW and NG trials in study 2, however following the first ingestion, significant fluid temperature-dependent differences in LSR were observed in the NG trials, similar to those observed in study 1.

In both study 1 and study 2, the observed differences in sudomotor activity between 1.5°C and 50°C fluids occurred without any parallel differences in core temperature measured in the rectum and aural canal, or mean skin temperature measured at eight different sites. Both core temperature measurement sites have purported limitations. Rectal temperature exhibits a temporal lag relative to pulmonary artery, esophageal, and aural canal temperature measurements (8, 25), however no differences in T_{re} between fluid temperatures were observed following fluid ingestion at any time point. Additionally, T_{au} measurements have been criticized in the past for being overly influenced by ambient temperatures (30), however these errors can be minimized if the probe is properly insulated (3, 8). Moreover, T_{au} measurements were validated in the present study prior to exercise by ensuring that values were equal to or greater than esophageal temperature (T_{es}) (15).

Given the difficulty of directly measuring brain temperature in awake, exercising humans, we cannot be certain that undetected changes in hypothalamic temperature did not occur with the ingestion of cold and warm fluids in both study 1 and 2. However, blood flow to the stomach region drops from ~1.6 $L \cdot min^{-1}$ at rest to ~0.45 $L \cdot min^{-1}$ during exercise at ~50% VO_{2max} (22, 23). This relatively small amount of blood flow around the stomach region, compared with the relatively large amount of blood returning from the active muscles and skin, would likely minimize any temperature changes of the blood reaching the hypothalamus. On the other hand, local heating or cooling of the hypothalamic region may occur when fluid is either held in the mouth or as it passes down the esophagus (via direct heat exchange with blood in the carotid arteries) (26). This notion, however, is not supported by the data in study 2. There were no fluid temperature-related differences in LSR in the SW trials, and when fluid was delivered directly to

the stomach (thereby bypassing the mouth and esophagus) in the NG trials, large differences in LSR were observed between 1.5°C and 50°C fluids.

While it can be conclusively stated that the thermoreceptors responsible for the present sudomotor response are not located in the mouth, the exact location, or combined locations, is less certain. The nasogastric tube employed in the NG trials was not perfectly insulated and some minor heat transfer with the nasopharynx and esophagus probably occurred. The role of any thermoreceptors residing in the nasopharynx on the observed LSR response seems unlikely since similar changes in sweating were observed between the NG trials and study 1 when no stimulation of any nasopharyngeal thermoreceptors could have possibly occurred. Moreover, because the nasogastric tube delivered fluids directly to the bottom of the esophagus, cooling/heating of any potential esophageal thermoreceptors would have been much less in the NG trials than with standard drinking in study 1. Therefore the similar LSR responses between trials also do not seem to support the role of thermoreceptors in the esophagus. It follows that thermoreceptors residing in or around the stomach seem most likely responsible for the fluid temperature-dependent changes in sudomotor output.

A gastric tension thermoreflex caused by stimulation of hot and cold receptors in the stomach and small intestine has been demonstrated in humans (28). Similarly to the present study, the changes in gastric tension occurred within 2-min of thermal stimulation. Additionally, electrophysiological and immuno-histochemical studies in cats and mice have demonstrated the existence of temperature-sensitive neurons in the stomach (7, 31). Whether the reflexes observed in the present study were due solely to stimulation of thermoreceptors in the stomach is unclear, as thermoreceptors in the small intestine and abdominal wall, but not the liver, are known to elicit thermoeffector responses (i.e. panting and shivering) in sheep (21). As the sudomotor

response in the present study as well as the gastric tension reflex in the study by Villanova (28) appeared 2-min post-stimulation, it is possible this was the time required for a sufficient transfer of heat from the stomach to influence thermoreceptors present in the abdominal wall and small intestine.

Perspectives

The reduction in sudomotor activity with cold fluid ingestion could potentially be exploited to ease the logistical burden of individuals required to carry their own fluid supply, particularly considering that similar core temperatures are observed irrespective of fluid temperature. Furthermore, hydration status for a given ingested volume of fluid could potentially be better maintained with colder fluid temperatures, especially under conditions that elicit low levels of evaporative efficiency (e.g. hot/humid and/or high clothing insulation), since inefficient sweat losses (i.e. dripping sweat) may be reduced without altering whole-body evaporation. From a mechanistic point of view, further evidence of abdominal thermoreceptors independently modulating thermoeffector responses could be obtained by assessing physiological thermoregulatory responses following the ingestion of warm and cool fluids during exposure to the cold.

Limitations

Since participants were exercising, skin temperatures could not be fixed in either study. While there were no differences in skin temperature between trials in either study 1 or 2, future studies should consider a passive heating protocol to clamp core and skin temperatures at different levels while fluids are delivered to the stomach at different rates. Furthermore, skin blood flow was not measured in the present study, so the potential influence of abdominal thermoreceptors upon vasomotor control also needs to be investigated. Mean arterial pressure

(MAP) was also not measured in the present study, and it is possible that the ingestion of 1.5°C fluid could have elicited a pressor response, which in turn could have influenced LSR. However, MAP-mediated changes in LSR with cold water ingestion in the present study seem very unlikely. A pressor response with oral water ingestion is only observed in individuals with autonomic failure and to a lesser extent the elderly, but not in healthy young participants (12); and even when this pressor response is observed, it is not fluid temperature-dependent (12).

CONCLUSION

The serial ingestion of 1.5°C and 50°C fluid elicited significant suppressions and elevations, respectively, in local sweat rate at all sites (i.e. forehead, forearm and upper back) relative to a thermoneutral trial (37°C fluid ingestion) despite no differences in core and skin temperatures between fluid temperatures throughout. In a second study, these LSR responses following 1.5°C and 50°C fluid ingestion during exercise without any differences in core and skin temperature were replicated when fluid was delivered directly to the stomach, bypassing the mouth and esophagus, using a nasogastric tube. However, almost identical LSR responses were observed when 1.5°C and 50°C fluid was swilled in the mouth only. Collectively, these data suggest that thermoreceptors modulating sudomotor output independently of core and skin temperatures probably reside in the abdominal area, but not the mouth.

ACKNOWLEDGEMENTS

The authors would like to thank the participants for volunteering for the study. This research was supported by a Natural Sciences and Engineering Research Council (NSERC) of Canada Discovery Grant (holder: Ollie Jay #386143-2010). Mr. Morris is supported by a University of Ottawa Master's Scholarship. Mr. Bain and Mr. Cramer are each supported by a

NSERC PhD Scholarship. We would like to thank Ms. Nicole Lesperance, Ms. Zuzana Novak and Mr. Nicholas Ravanelli for their assistance during various stages of data collection.

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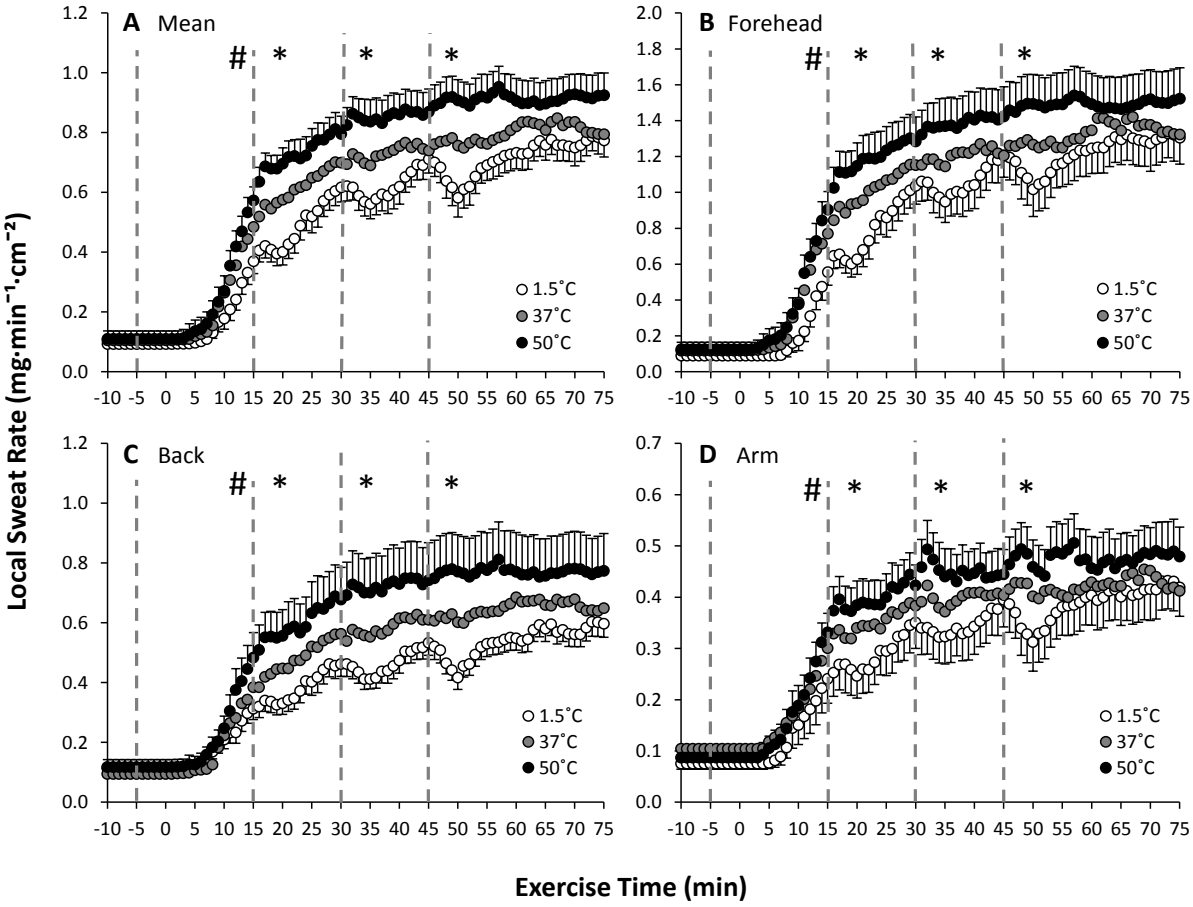

Figure 1. Mean local sweat rate (LSR) after the ingestion of 1.5°C (open circles), 37°C (grey circles), and 50°C (black circles) fluid before, and during exercise. Dashed lines denote when fluids were ingested. Values given are the grand mean (Panel A) of the following three locations: forehead (Panel B), upper back (Panel C), and forearm (Panel D). * denotes where 1.5°C<37°C<50°C, # denotes where 1.5°C<50°C ($p<0.05$). Error bars indicate standard error.

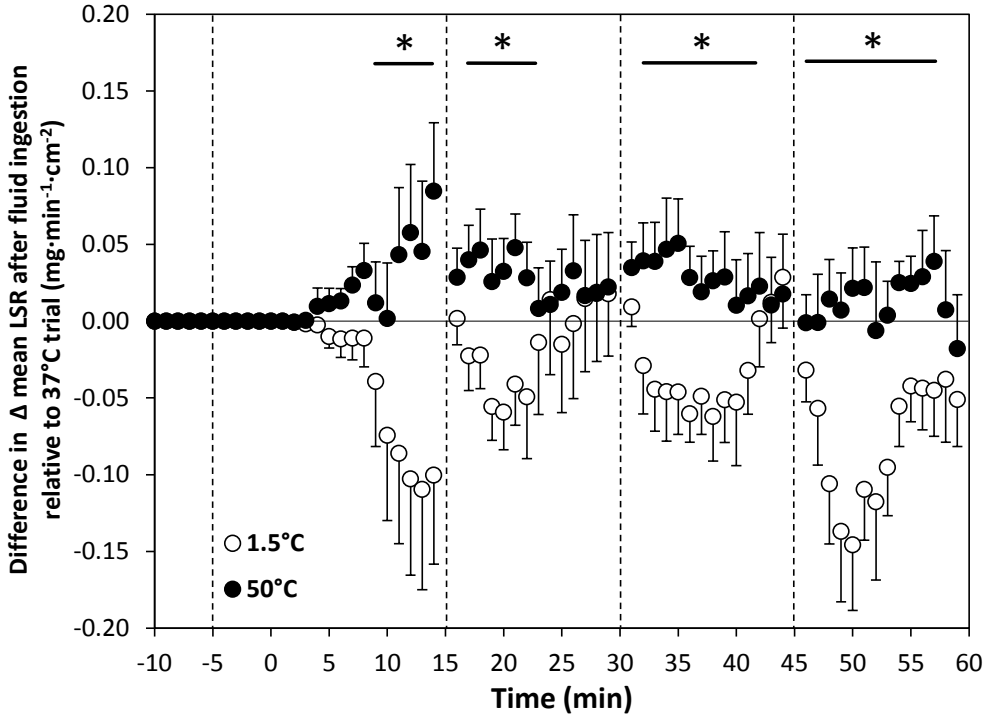

Figure 2. The difference in the change in mean LSR following the ingestion of 1.5°C (open circles) and 50°C (black circles) fluid relative to any changes in mean LSR observed during the thermoneutral 37°C fluid control trial. Dashed lines denote when fluids were ingested. * denote time points that 1.5°C<50°C. Error bars indicate standard error.

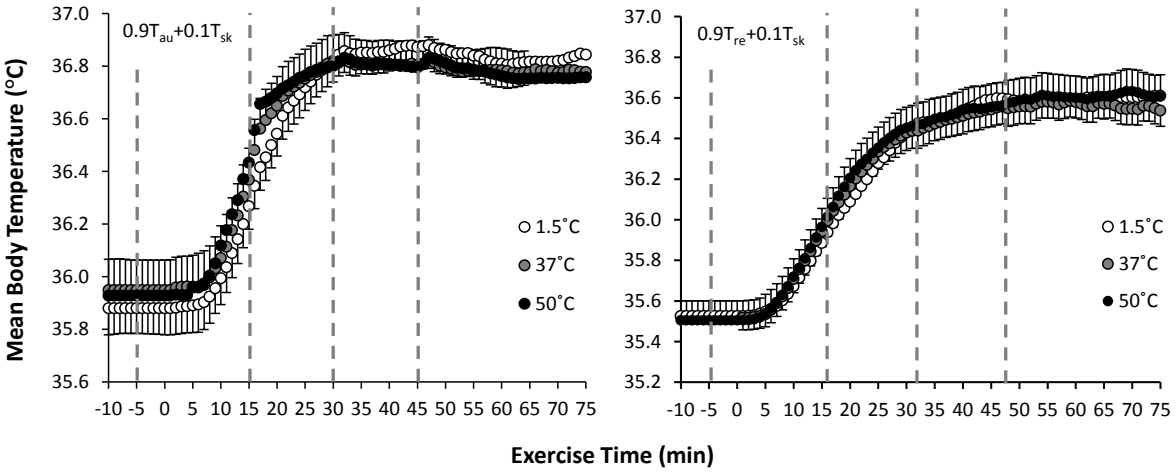

Figure 3. Mean body temperatures using a 0.9/0.1 weighting of "core" to "skin" temperatures using aural canal temperature (T_{au}) (left panel) and rectal temperature (T_{re}) (right panel) as an indication of the body "core", following the ingestion of 1.5°C (open circles), 37°C (grey circles), and 50°C (black circles) fluid before and during exercise. Dashed lines denote when fluids were ingested. Error bars indicate standard error.

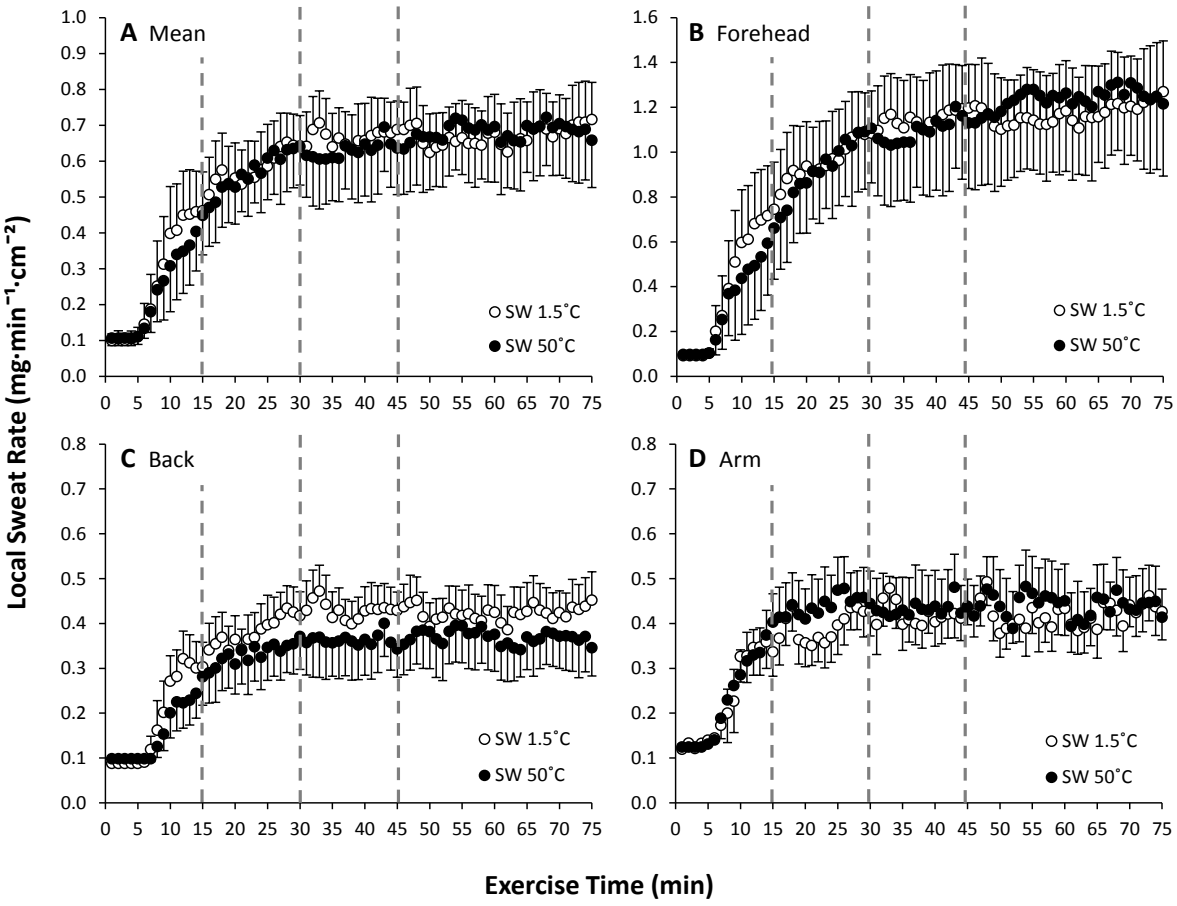

Figure 4. Mean local sweat rate (LSR) after mouth-swilling (SW trials) 1.5°C (open circles) and 50°C (black circles) fluid during exercise. Dashed lines denote when mouth-swills were administered. Values given are the grand mean (Panel A) of the following three locations: forehead (Panel B), upper back (Panel C), and forearm (Panel D). Error bars indicate standard error.

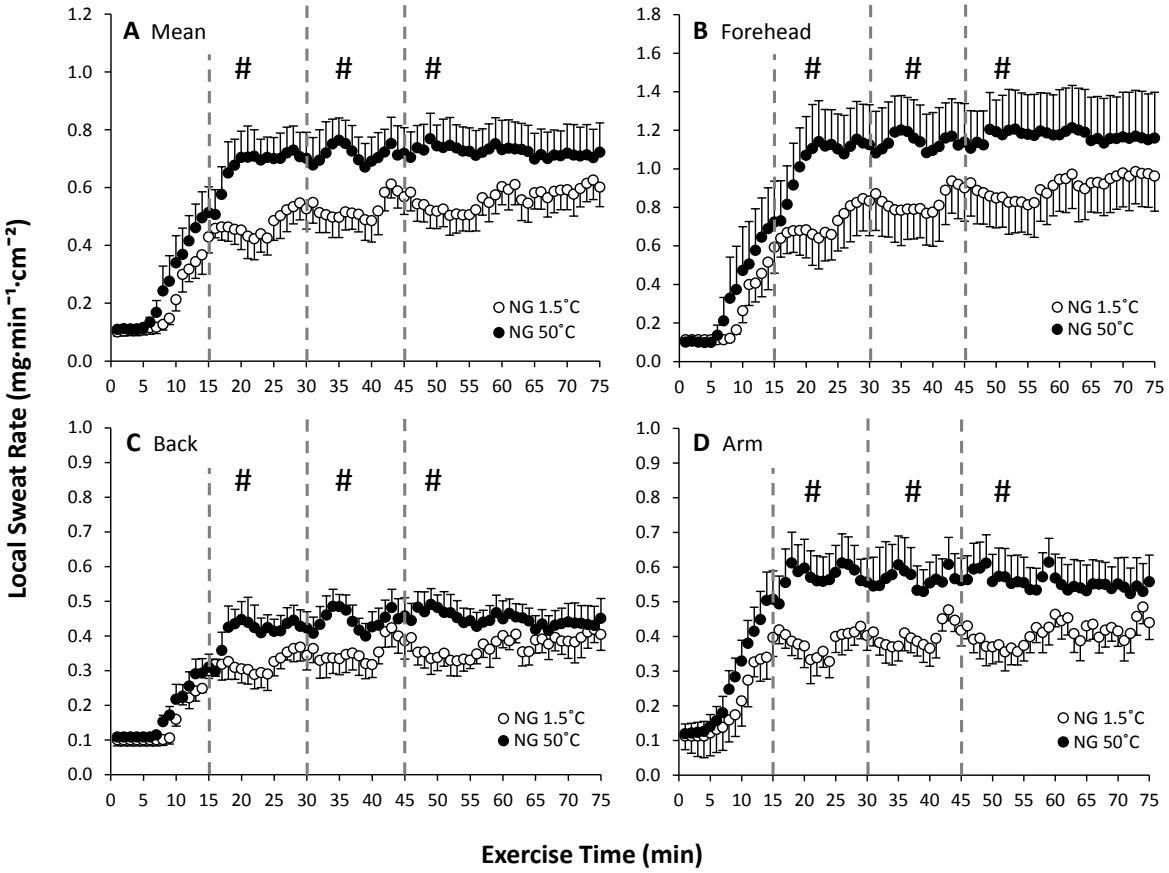

Figure 5. Absolute local sweat rate (LSR) after the ingestion of 1.5°C (open circles) and 50°C (black circles) fluid through a nasogastric tube (NG trials) during exercise. Dashed lines denote when fluids were ingested. Values given are the mean (Panel A) of the following three locations: forehead (Panel B), upper back (Panel C), and forearm (Panel D). # denotes where 1.5°C<50°C. Error bars indicate standard error.

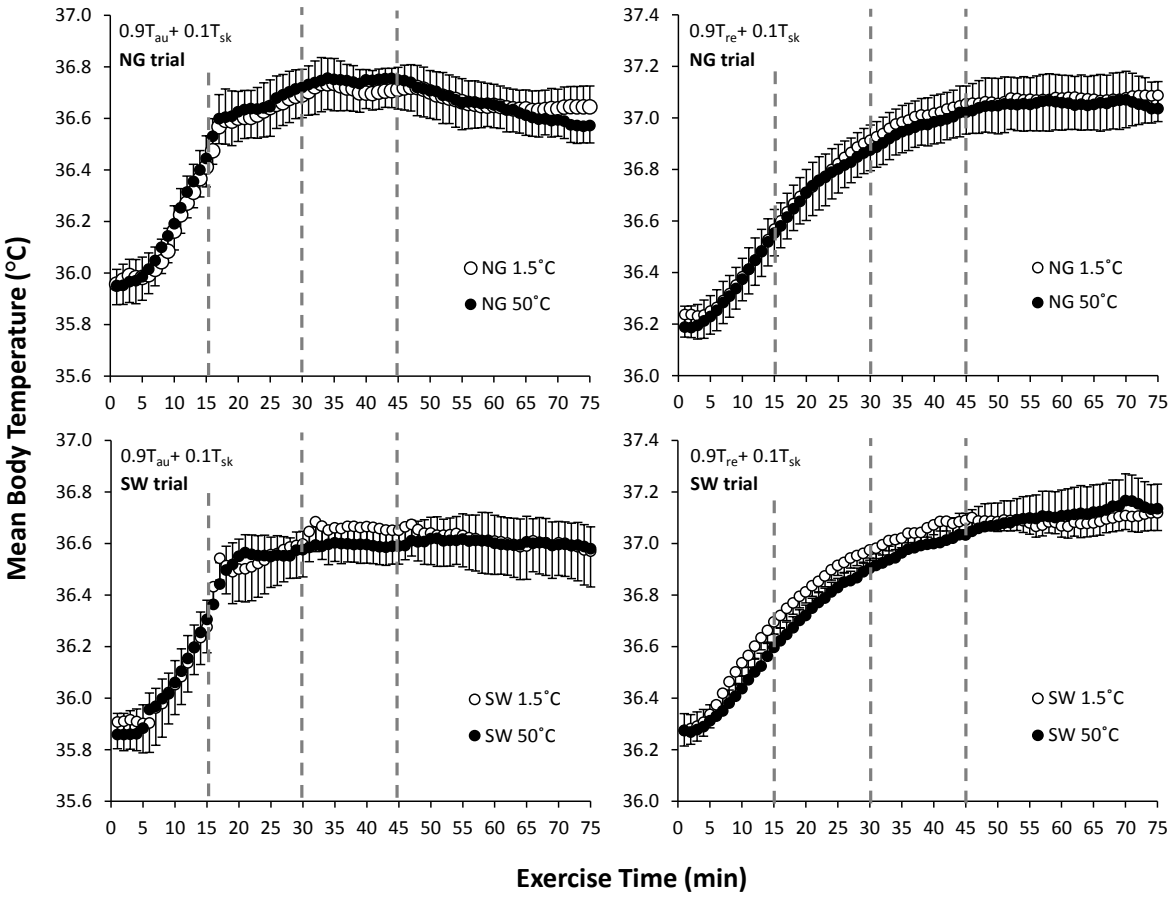

Figure 6. Mean body temperatures using a 0.9/0.1 weighting of "core" to "skin" temperatures using aural canal temperature (T_{au}) (left panels) and rectal temperature (T_{re}) (right panels) as an indication of the body "core", with 1.5°C (open circles) and 50°C (black circles) fluid during the NG trials (top row) and SW trials (bottom row). Dashed lines denote when fluids were ingested/administered. Error bars indicate standard error.

APPENDIX B: RESEARCH ETHICS BOARD APPROVAL

File Number: H06-10-03 **Date (mm/dd/yyyy):** 06/10/2013

Université d'Ottawa University of Ottawa
Bureau d'éthique et d'intégrité de la recherche Office of Research Ethics and Integrity

Ethics Approval Notice

Health Sciences and Science REB

Principal Investigator / Supervisor / Co-investigator(s) / Student(s)

First Name	Last Name	Affiliation	Role
Ollie	Jay	Health Sciences / Human Kinetics	Principal Investigator
Matthew	Cramer	Health Sciences / Human Kinetics	Research Assistant
Nathan	Morris	Health Sciences / Human Kinetics	Research Assistant

File Number: H06-10-03

Type of Project: Professor

Title: The Influence of the Temperature of Ingested Drinks on Human/Environmental Heat Exchange in Exercising Adults

Renewal Date (mm/dd/yyyy)	Expiry Date (mm/dd/yyyy)	Approval Type
07/29/2013	07/28/2014	Ia

(Ia: Approval, Ib: Approval for initial stage only)

Special Conditions / Comments:
N/A

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Bureau d'éthique et d'intégrité de la recherche Office of Research Ethics and Integrity

This is to confirm that the University of Ottawa Research Ethics Board identified above, which operates in accordance with the Tri-Council Policy Statement and other applicable laws and regulations in Ontario, has examined and approved the application for ethical approval for the above named research project as of the Ethics Approval Date indicated for the period above and subject to the conditions listed the section above entitled "Special Conditions / Comments".

During the course of the study the protocol may not be modified without prior written approval from the REB except when necessary to remove subjects from immediate endangerment or when the modification(s) pertain to only administrative or logistical components of the study (e.g. change of telephone number). Investigators must also promptly alert the REB of any changes which increase the risk to participant(s), any changes which considerably affect the conduct of the project, all unanticipated and harmful events that occur, and new information that may negatively affect the conduct of the project and safety of the participant(s). Modifications to the project, information/consent documentation, and/or recruitment documentation, should be submitted to this office for approval using the "Modification to research project" form available at: http://www.research.uottawa.ca/ethics/forms.html

Please submit an annual status report to the Protocol Officer 4 weeks before the above-referenced expiry date to either close the file or request a renewal of ethics approval. This document can be found at: http://www.research.uottawa.ca/ethics/forms.html

If you have any questions, please do not hesitate to contact the Ethics Office at extension 5387 or by e-mail at: ethics@uOttawa.ca.

Signature:

Germain Zongo
Protocol Officer for Ethics in Research
For Daniel Lagarec, Chair of the Sciences and Health Sciences REB

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APPENDIX C: INFORMATION AND CONSENT FORM

Université d'Ottawa • University of Ottawa
Faculté des sciences de la santé
École des sciences de l'activité physique
Faculy of Health Sciences
School of Human Kinetics

Background information and consent form

The Influence of the Temperature of Ingested Drinks on Human/Environmental Heat Exchange in Exercising Adults

Principal Investigator:
Dr. Ollie Jay,
Assistant Professor,

Background

During exercise in the heat, the body employs thermoregulatory (autonomic) responses that attenuate rises in body core temperature. To facilitate these responses, behavioral techniques have been proposed and employed which pronounce heat loss and reduce thermal strain during exercise. One of the most common of those techniques is the ingestion of cold fluids. Recently, Lee and Shirreffs (2007) investigated the effects of fluid temperature on thermoregulatory responses (i.e. sweating, elevations in skin temperature and subsequent elevations in body core temperature) during exercise. The authors noted that the ingestion of 1 L of a 10°C fluid relative to 1 L of a 50°C fluid produced lower rectal and skin temperatures and a correspondingly lower sweat output. However, upon further scrutiny of their data, rational heat balance analysis actually suggests a greater heat storage with ingestion of 10ºC relative to the 50°C fluid, by virtue of a lower sweat output and skin temperature. This suggests that core temperature with the ingestion of cold fluids when measured away from the stomach (the primary heat exchange location with cold fluids) may actually be greater; and the reduction in sweat output with cold fluid ingestion is disproportionate relative to the sweat rate required to attain heat balance. The question therefore remains, will the ingestion of a cold fluid reduce all body temperature elevations during exercise through means of conduction (direct cooling between two mediums), or will the local stomach cooling of the ingested fluid produce thermoregulatory shifts opposing the body's need to eliminate heat which exceed the conductive cooling capacity of the fluid?

Purpose

The purpose of the present study is to examine the effect of drink temperature upon thermoregulatory control. Changes in core body temperature, heat loss responses (i.e. sweating, skin temperature, skin blood flow) and sweating efficiency (i.e. the amount of sweat actually evaporated from the body relative to the amount of sweat produced) will be measured during 75-min of

exercise while ingesting four 250 ml aliquots of water at either 1.5°C, 10°C, 37°C, or 50°C. It is hypothesized that cold fluid ingestion will attenuate the body's natural defences against heat gain (i.e. sweating and skin temperature) and as such, the ingestion of a warmer fluid relative to a cold will provide a more effective means for reducing body temperature elevations during exercise.

Subject profile

To be a participant you must be a healthy (no history of respiratory, metabolic, cardiovascular, blood pressure disease, or of diabetes and not currently on any medication related to these conditions) male adult, aged between 18 and 39 years. If you agree to participate in this study, you will be required to participate in one preliminary session and three experimental sessions to be conducted on different days and separated by a minimum of 48 hours.

Preliminary session

Both the preliminary session and the experimental sessions will take place in at the Thermal Ergonomics Laboratory (E028), Lees Ave campus at the University of Ottawa. The time involvement will be approximately 45 min to 1 hour for the preliminary session. During the preliminary session, we will review all procedures with you. In addition, you will be introduced to all of the equipment and measuring devices that we will be using for the experimental sessions. We will give you the opportunity to read the Background and Informed consent document. If you agree to participate in the study, we will ask you to sign the informed consent below and complete a *Physical Activity Readiness Questionnaire (Par-Q)* and an *American Heart Association/American College of Sports Medicine Health/Fitness Facility Pre-participation Screening Questionnaire*. These questionnaires are standard questionnaires that have been developed to help us evaluate your readiness for exercise and are also used to assist us evaluate your general physical health and level of physical activity. Thereafter, we will complete some basic measurements including height, mass. Following these measures, you will be asked to perform a maximum oxygen consumption test on a cycle ergometer where upon you will be required to exercise until exhaustion. This will consist of pedalling at a cadence of 80 rpm while the resistance is increased by 20 watts every minute until you can no longer maintain the required cadence (8-12 min). We will also assess your body composition by using underwater weighing. You will be asked to wear a bathing suit, enter the tank and situate yourself on the hanging chair. You will be asked to immerse yourself completely under water for 5 seconds. Once the measurement is completed you will be given a few minutes to relax after which you will be asked to perform the same steps again. Five trials will be done in order to obtain accurate results.

Experimental session

The study will consist of 4 experimental sessions. Each session will last approximately 2 to 3 hours. Upon arrival to the laboratory, you will change into athletic clothing (shorts and shoes). The experimental sessions will begin with an instrumentation period. Once all the equipment and probes (see description below) are in place and functioning, you will be weighed and then enter the lab regulated at ~25ºC and ~20% relative humidity; and remain seated at rest for baseline data to be collected for 30 minutes.

At the end of this period, you will be asked to cycle on an upright cycle ergometer (Velotron) inside the climatic chamber for 90 minutes under the same environmental conditions as the baseline measurements. The exercise intensity for each session will be ~55% of your VO_{2max}.

In the first of the experimental trials, you will be asked to swill 50 ml of water at temperatures of 1.5ºC, 37ºC or 50ºC for two minutes, in random order, starting at 30 minutes of exercise and again every 10 minutes until the end of exercise, so that each water temperature will be swilled twice. In the second trial, the same protocol will be followed, but rather than swill the water in your mouth, the 50 ml of water will be introduced into your stomach with the use of a nasogastric tube (description of insertion below). In the last two trials, you will be asked to alternate between swilling 50 ml of 37ºC of plain water and 50 ml of 37ºC water containing a concentration of 1.5% menthol for one of the trials and 0.0015% capsaicin in the other. Menthol and capsaicin are the chemicals responsible for the "coolness" taste in commercial chewing gum and toothpaste, and the "spicy" taste in food, respectively.

In preparation for the experimental trials, you will be asked to abstain from alcohol, caffeine and severe or prolonged physical activities for 24 hours prior to all sessions. It is highly recommended that you avoid eating a heavy meal for at least two hours before the trial.

The following instruments will be used to monitor and record your physiological response during the experimental trial:

Metabolic data: In order to measure metabolic heat production you will be equipped with a mouth piece and nose clip and will breath through the mouth piece for the duration of exercise (Vmax® Encore Metabolic Cart).

***Esophageal probe*:** In order to monitor central body temperature, the researcher will insert a flexible oesophageal temperature probe (2 mm in diameter) will be inserted through one of your nostrils, during which time you will be asked to swallow sips of water. The tip of the probe, once fully inserted in your esophagus (swallowing tube), will rest at the level of the heart. There can be mild discomfort and mild gagging reflex from swallowing the probe. However, this sensation soon passes (5-10 seconds). **Please note that the probe will be inserted by Dr. Ollie Jay, Nathan Morris, or Matthew Cramer, who have been legally authorized to do so and who have been approved by the University of Ottawa Office of Risk Management to have the necessary skills and training to perform these insertions properly.*

***Rectal probe*:** You will be asked to insert a flexible probe though the anus into the rectum (10-12 cm). Proper instruction will be given to you on the placement of the rectal probe. A marker is placed on the rectal probe using sterile surgical tape. The subject inserts the probe until the tape reaches the anal surface. The insertion of the rectal probe may cause some mild discomfort and minor irritation; however, this sensation soon passes. This probe provides the researcher with an indication of the amount of heat stored in your body. You should be aware that there is some minimal risk associated with the insertion of a rectal probe. With the insertion, there is a risk of perforation of the rectum, and may cause some discomfort and minor irritation. However, proper instruction will be given to you on the placement of the rectal probe to ensure your safety and comfort. You will be responsible for the insertion of this probe.

***Tympanic probe*:** The researcher will insert a probe into your ear canal. The probe will be pushed gently until it touches the tympanic membrane. At this point, you will sense a slight discomfort and the probe will then be retracted slightly. The probe will be secured in its position by packing the ear with cotton balls held in place with surgical tape. The auditory canal temperature will be used as an index of brain and core temperature. **Please note that the probe will be inserted by Dr. Ollie Nathan Morris, or Matthew Cramer, who have been legally authorized to do so and who have been approved by the University of Ottawa Office of Risk Management to have the necessary skills and training to perform these insertions properly.*

***Skin temperature probes*:** Twelve skin probes will be taped to the skin surface (on the forehead, shoulder, chest, upper right back, abdomen, lower back, bicep, back of the hand, front of the thigh, back of the thigh, back of the calf and front of the calf) with hypoallergenic tape. These probes give an indication of skin temperature and heat loss from the skin surface. Some hair may need to be shaved (by the use of disposable razors) in order to secure the probes adequately to the skin surface. Some discomfort may be experienced upon removing the tape.

Nasogastric tube: In order to introduce water into the stomach while bypassing the mouth, the researcher will insert a flexible nasogastric tube (~2 mm in diameter), in an identical manner as the esophageal probe, through one of your nostrils, during which time you will be asked to swallow sips of water. The tip of the probe, once fully inserted, will enter into the superior segment of your stomach. There can be mild discomfort and mild gagging reflex from swallowing the probe, but these feelings of discomfort are mitigated with the use of a 2% lidocaine lubrication jelly. Furthermore, this initial sensation soon passes (5-10 seconds). **Please note that the probe will be inserted by Dr. Ollie Jay, Nathan Morris, or Matthew Cramer, who have been legally authorized to do so and who have been approved by the University of Ottawa Office of Risk Management to have the necessary skills and training to perform these insertions properly.*

***Sweat capsule*:** Up to three small plastic capsules will be taped to the back of the shoulder (upper back), chest, forearm and/or forehead. This capsule picks up humidity from the skin and provides a measurement of local sweat rate.

***Whole-body sweat rate*:** You will be weighed on a platform scale (Combics 2, Sartorius, Canada) immediately before the start of exercise and immediately after exercise has stopped.

***Heart rate*:** Heart rate will be monitored by a strap placed around the chest (Polar Vantage heart rate monitor).

Risks and discomforts

In the event of a health related emergency, our research staff is trained in CPR and we have emergency phones located in the laboratory for immediate contact with University emergency response (University Protection Office).

Physical activity: There are some minor physical risks associated with any form of exercise. There is essentially no major risk for young, healthy, active people while performing the submaximal exercises. Some effects of maximal exercise testing are nausea, dizziness, fainting, abnormal blood pressure, chest pain and leg cramps. For the maximal and experimental exercise sessions, the 'Guidelines for Graded Exercise Testing and Exercise Prescription' (by the American College of Sports Medicine) indicate that for men under 40 years of age, with no symptoms or risk factors for cardiovascular disease, the presence of a physician during the test is not required. The incidence of cardiac arrest during maximal exercise tests is 1 in 10000 tests. Participants may stop at any time during these tests. All tests will be conducted under standardized conditions for human exercise experiments as laid out by the Canadian Society for Exercise Physiology and the American College of Sports Medicine.

Temperature probes and nasogastric tubes: Perforation of the esophagus, aural or nasal cavities, as well as the rectum can occur during insertion of the nasogastric tube, and esophageal and rectal probes (potentially causing inflammation and infection). Perforation of the esophagus or oral or nasal cavities, as well as the rectum is very rare and no such incident has ever occurred in a laboratory the principal investigator has worked in. The risk of transmission of infectious disease is negligible as each subject has his own sterile probes and nasogastric tube that will be disposed of once all tests have been completed.

Elevation of core body temperature: There are certain risks that accompany a mark elevation in core temperature associated with exercise-induced dehydration. These include: headache, extreme weakness, dizziness, nausea, hyperventilation, hypotension, confusion, diarrhoea, vomiting and loss of consciousness. During all experimental protocols, you will be under close examination by the research assistant. Further, core body temperatures will be monitored continuously during the experimental trials, and exercise will be terminated if you reach 39.5°C esophageal temperature. Additionally, during the experimental protocols, a circulated cold water bath will be prepared and available if needed to rapidly cool you. If you become light headed or dizzy, exercise will be terminated and a mat will be readily available in an adjacent room maintained at a comfortable

ambient temperature where you will be laid in the supine position, cooled with cold towels, and given a commercially available sports drink (Gatorade) in order to rehydrate and maintain blood sugar.

Headaches associated with ingesting cold water: You may experience a mild headache from the ingestion of the cold water (sphenopalatine ganglioneuralgia) which should pass shortly after ingestion (3 to 5-min).

An emergency first aid kit is readily available if needed for all laboratory session. A qualified person (Mr. Nathan Morris) will be on hand to administer treatment if required.

Benefits of Participating

While participating in this study you will gain knowledge of your aerobic fitness and body composition. You will also learn about the research process and the knowledge acquired during the experimental sessions may be shared upon request.

Anonymity and Confidentiality

All raw data will be stored using alphanumeric coding system as such, no one will be able to identify you as your name will not appear on these files. Data will be kept in Montpetit Hall, Room 372, in locked file cabinets and only the researchers directly involved in this study will have access to your data.

No records bearing your name will leave the institution. You are encouraged to request and discuss the results of the experimental trials at any time. The results of the preliminary session (aerobic fitness and body composition) will be available to you upon completion of the study.

The data collected in this study will be published in scientific journals. The data will kept for a period of 5 years post-publication and will subsequently be destroyed by the physical resources service of the University of Ottawa.

For the entire duration of the study, it is fully understood that you may refuse to participate or withdraw from the study at any time, without question. You may also withdraw from participating when you are in the thermal chamber or at any point during either the exercise or recovery period.

INFORMED CONSENT OF PARTICIPANT

Research involving human subject require written consent of the participants.

I, _____, hereby volunteer to participate as a subject in the study entitled **"The Influence of the Temperature of Ingested Drinks on Human/Environmental Heat Exchange in Exercising Adults"**. I have read the information presented in the above background information and I had the opportunity to ask questions to the investigators. I understand that my participation in this study, or indeed any research, may involve risks that are currently unforeseen.

I recognize that there will be no direct benefit to me from my participation in this study (besides receiving an aerobic fitness and body composition evaluation).

I understand that if I have any questions regarding the study, I may contact Dr. Ollie Jay at . If I have any questions with regards to the ethical conduct of this study, I may contact the Protocol Officer for Ethics in Research, University of Ottawa, Tabaret Hall, 550 Cumberland Street, Room 159, Ottawa, ON K1N 6N5, tel.: 613-562-5841, email: ethics@uottawa.ca .

I have been given a copy of this Background Letter and Consent Form for me to keep.

Signature of participant: _____ Date: _____

Signature of Researcher: _____ Date: _____