The influence of local skin temperature on the sweat glands maximum ion reabsorption rate

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Abstract

Purpose

Changes in mean skin temperature (T_{sk}) have been shown to modify the maximum rate of sweat ions reabsorption. This study aims to extend this knowledge by investigating if modifications could also be caused by local T_{sk}.

Methods

The influence of local T_{sk} on the sweat glands maximum ion reabsorption rates was investigated in 10 healthy volunteers (3 female and 7 male; 20.8 ± 1.2 yrs, 60.4 ± 7.7 kg, 169.4 ± 10.4 cm) during passive heating (water perfused suit and lower leg water immersion). In two separate trials, in a randomised order, one forearm was always manipulated to 33˚C (Neutral), whilst the other was manipulated to either 30˚C (Cool) or 36˚C (Warm) using water perfused patches. Oesophageal temperature (T_{es}), forearm T_{sk}, sweat rate (SR), galvanic skin conductance (GSC) and salivary aldosterone concentrations were measured. The sweat glands maximum ion reabsorption rates were identified using the ΔSR threshold for an increasing ΔGSC.

Results

Thermal (T_{es} and body temperature (T_b)) and non-thermal responses (aldosterone) were similar across all conditions ($p>0.05$). A temperature dependent response for the sweat glands maximum ion reabsorption rates was evident between 30˚C (0.18 ± 0.10 mg/cm$^{2/}$min) and 36˚C (0.28 ± 0.14 mg/cm^2/min, $d = 0.88$, $p<0.05$), but not for 33˚C (0.22 ± 0.12 mg/cm^2/min), $d =0.44$ and $d=0.36$, $p>0.05$).

Conclusion

The data indicates that small variations in local T_{sk} may not affect the sweat glands maximum ion reabsorption rates but when the local T_{sk} increases by >6˚C, ion reabsorption rates also increase.

Keywords: sweat ion regulation, sweat glands, skin temperature, aldosterone

Abbreviations

ANOVA analysis of variance

CFTR cystic fibrosis transmembrane channels

Cl^- Chloride

CVC cutaneous vascular conductance (%)

ENaC epithelial sodium channel

GSC galvanic skin conductance (μS)

HASG heat-activated sweat glands

HR heart rate (bpm)

K+ potassium

MAP mean arterial pressure (mmHG)

Na^+ sodium

SGO sweat gland output

SR sweat rate ($mg/cm^2/min$)

T_b body temperature (˚C)

T_{es} Oesophageal temperature (˚C)

T_{sk} skin temperature (˚C)

$\dot{V}O_{2max}$ maximum oxygen uptake (ml/kg/min)

Introduction

Sweating is an essential physiological function for maintaining thermal homeostasis and the content of sweated ions important for maintaining epidermal barrier homeostasis and antimicrobial function of the skin. Despite rapid developments in biosensor technology

104 that aim to determine sweat rate and its ion concentration (Gao et al. 2018), our knowledge
105 of sweat ion regulation is limited. It is well established that when eccrine sweat glands are
106 stimulated, the secretory coil, located at the base of the sweat gland, forms an isotonic fluid
107 and as sweat traverses the straight duct ion reabsorption occurs; resulting in a hypotonic
108 sweat released on the skin surface (Sato 1977; Shamsuddin et al. 2005b). It has been
109 suggested that with an increasing sweat rate (SR) the secretion rate increases proportionally
110 faster than the reabsorption rate, resulting in a linear relationship between SR and sweat
111 Na^+ concentration (Buono et al. 2008). Whilst this may be the case there are numerous
112 studies providing evidence that the reabsorption rate can be enhanced, most notably
113 following heat acclimation whereby a reduction in sweated ions occurs despite an increased
114 sweat production (Buono et al. 2007; Amano et al. 2016). Furthermore, the maximum rate
115 of ion reabsorption has been shown to differ between habitually trained and sedentary
116 individuals and is also known to vary across the body (Amano et al. 2017). What regulates
117 eccrine sweat glands ion reabsorption remains unknown.

118 A regulatory mechanism that has been previously reported to affect the maximum
119 ion reabsorption rate is T_{sk}. The influence of T_{sk} on sweat production is well established
120 (Nadel et al. 1971) but its impact on sweated ions is somewhat contradictory with studies
121 reporting an influential role (Johnson et al. 1944; Robinson et al. 1985) and others reporting
122 little or no effect (Bulmer and Forwell 1956). These earlier studies were primarily focused
123 on the concentration of sweated ions but more recently Shamsuddin *et al.* (2005a)
124 investigated the reabsorption of these ions, that ultimately affects the sweated ion
125 concentration. By changing the ambient conditions (15˚C or 25˚C), Shamsuddin et al.
126 (2005a) manipulated mean T_{sk} by approximately 3˚C during an exercising protocol. The
127 result of which was a significantly lower ion reabsorption rate at a lower compared to higher
128 mean T_{sk} (0.21 ± 0.04 vs. 0.52 ± 0.06 mg/cm^2/min, respectively). The inhibition of the

129 sweat glands ion reabsorption capacity was associated with the temperature dependency of
130 epithelial sodium channel (ENaC) excitability and open probability (Chraïbi and
131 Horisberger 2003). However, they did not report the local T_{sk} where the ion reabsorption
132 rates were measured and also only focused on the effects of low T_{sk} (<31˚C) on the sweat
133 glands maximum ion reabsorption rates. Therefore, it is still unknown whether the
134 maximum ion reabsorption rates are proportional to local changes in T_{sk} systematically.
135 It is also necessary to determine the influence of local T_{sk} on the sweat glands
136 maximum ion reabsorption rates as there is cumulative evidence for regional differences in
137 ion reabsorption rates, with the torso generally having a higher reabsorption rate than the
138 extremities (Amano et al. 2017; Gerrett et al. 2018a). However these regional differences
139 were also mirrored by regional differences in local T_{sk}. It would be advantageous then to
140 investigate the influence of local T_{sk} on the sweat glands ions reabsorption rate within
141 temperatures ranges expected whilst sweating.
142 Shamsuddin et al. (2005a) speculated that higher T_{sk} might enhance the responsive
143 ability of the sweat glands to a given concentration of circulating aldosterone; a hormone
144 considered to be a primary effector of sodium exchange at the sweat gland (Hegarty and
145 Harvey 1998; Harvey and Higgins 2000). Aldosterone concentrations and other water
146 regulatory hormones such as atrial natriuretic peptide, vasopressin and renin are higher
147 during exercise and are considered to be exercise intensity dependent (Convertino et al.
148 1983; Freund et al. 1991; Yoshida et al. 2006). Shamsuddin et al. (2005a) employed an
149 exercise protocol (cycling at 60% $\dot{V}O_{2max}$) sufficient enough for the release of these
150 hormones; although their influence on sweat glands ion reabsorption is inconclusive
151 (Yoshida et al. 2006; Hew-Butler et al. 2010, 2014). As such it is important to investigate
152 the role of T_{sk} on the sweat glands maximum ion reabsorption rates when hormonal
153 responses associated with exercise are isolated.

In order to determine the role of local T_{sk} on the sweat glands ion reabsorption rates we aimed to manipulate local forearm T_{sk} to elicit a cool (30˚C), neutral (33˚C) and warm (36˚C) T_{sk}, with the latter reflecting typical T_{sk} experienced during heat stress on the sweat glands ion reabsorption. In order to minimise any non-thermoregulatory mechanisms affecting sweat glands ion reabsorption rates the influence of local T_{sk} on ion reabsorption during a passive heating protocol was selected. We hypothesized that eccrine sweat glands maximum ion reabsorption rates are temperature dependent, with maximum ion reabsorption occurring at a higher sweat rate (SR) with a higher local T_{sk} (36˚C) compared to neutral (33˚C) and cool T_{sk} (30˚C).

Methods

Participants were informed about the study purpose and procedures prior to providing verbal and written consent. The Human Subjects Committee of the Graduate School of Human Development and Environment at Kobe University (Japan) approved the study (report no. 259), which conforms to the standards set out by the Declaration of Helsinki (except for registration in a database).

Participants

Ten young healthy recreationally active participants (3 female and 7 male; 20.8 ± 1.2 yrs, 60.4 ± 7.7 kg, 169.4 ± 10.4 cm) were recruited for this study during winter in Japan and thus were considered unacclimated. Both males and females were recruited, as our previous research indicated no sex related differences in ion reabsorption rates (Amano et al. 2017). Participants were asked to refrain from consuming high sodium foods, caffeine or alcohol and to avoid any strenuous exercise 24 hours preceding the trials. For the experimental trials they were instructed to record their food and beverage intake during the

preceding 24 hours and asked to replicate this for each trial. To promote euhydration, participants were instructed to consume 500 ml of water 1-2 hours prior to the experiments. Upon arrival to the laboratory, participants provided a urine sample and hydration status was checked by a handheld refractometer (Atago Co.Ltd, Tokyo, Japan). All participants met the criteria for adequate hydration (USG <1.025) except two participants both on one occasion each. In these cases the participants were required to consume a further 300ml prior to the start of the experiments. All participants were non-smokers and were not taking any medications. Menstrual cycle phase per se was not controlled for in the female participants but each female was tested within the same stage of their cycle.

Experimental trials

Participants visited the laboratory twice for the manipulation of local forearm T_{sk}. Each trial was separated by at least 48 hours. All experimental trials were conducted in a climatic chamber (SR-3000; Nagano Science, Osaka, Japan) controlled at 27°C, 50% relative humidity, with minimal air movement. All tests were completed at the same time of day (± 1 hr), at least 2 hours after their last meal. During all experimental trials, participants wore standardized shorts and females also wore a sports bra underneath a water-perfused suit (Allen-Vanguard, Ottawa, Canada) to help elevate the thermal load and in an attempt to maintain a similar T_{sk} across the body.

Prior to each experiment participants provided a urine sample for the analysis of aldosterone. Prior to entering the experimental chamber participant self-inserted an oesophageal thermometer, as an index of core temperature (T_{es}), to the distance of one-fourth of standing height from the external nares (Mekjavic and Rempel 1990). After entering the chamber participants donned a water-perfused suit that covered the body except face, forearms, hands and lower legs (from above the knee). Participants then rested

204 in a semi supine position for approximately 60mins whilst the measuring instruments were
205 attached (detailed below) and local T_{sk} of the forearm was manipulated and remained stable
206 at the desired local T_{sk} (detailed below). During the instrumentation preparation phase,
207 water at 34˚C was passed through the suit at a flow rate of 750 ml/min to maintain a stable
208 resting mean T_{sk}. Following instrumentation, baseline data were recorded for 5 minutes and
209 a salivary aldosterone sample collected. Participants were then heated exogenously for 45
210 minutes separated into two phases. The first phase involved submerging the lower legs into
211 a water bath set at 42˚C and increasing the water temperature inside the suit to 40°C for 20
212 minutes. For the remaining 25 minutes, the lower leg water bath temperature was increased
213 to 43˚C and the temperature of the water inside the suit was increased to 45˚C.
214 During all experiments, one forearm was controlled to elicit a neutral T_{sk} of 33˚C
215 (Neutral), whilst the other forearm was either manipulated to elicit a cool T_{sk} of 30˚C (Cool)
216 or a warm temperature of 36˚C (Warm) in a balanced order. For clarity, in the rest of the
217 document the phrase *Neutral-Cool* will be used to indicate when one forearm was
218 manipulated to be Neutral and the other Cool, and *Neutral-Warm* when one forearm was
219 manipulated to be Neutral and the other Warm. Sweat rate on bilateral forearms are
220 comparable (Buono et al. 2011; Kenefick et al. 2012; Smith and Havenith 2012) and thus
221 we assume asymmetry between the left and right arm and use the mean of the two neutral
222 conditions. To manipulate local forearm T_{sk}, custom-made water perfused patches were
223 wrapped around the left and right ventral forearm during each experiment. The water
224 perfused patches consisted of tubes (inside diameter=2mm, outside diameter =4mm)
225 running in a circular motion with no gaps between tubes. A small hole positioned in the
226 centre of the patch allowed for entrance and exit of the sweat capsule tubes. Both patches
227 were of equal surface area (16x13cm) and water was supplied to each patch at a flow rate
228 of 250 ml/min (Variable-flow Chemical Transfer Pump, Fisher Scientific™). The patches

were connected to two separate water baths (Taitec Therminder DX-10 and SP12, Taitec Corporation, Saitama, Japan) where the water temperature was altered to achieve the desired local T_{sk}. The water temperature used varied for each participant but was approximately 29.4 ± 3.2˚C for Neutral, 15.8 ± 5.8˚C for Cool and 40.2 ± 2.2˚C for Warm conditions. To control the cold-water temperature an immersion cooler (Taitec Cool pipe 300LF, Taitec Corporation, Saitama, Japan) was used concomitantly with the water bath.

The water patches were positioned onto the forearms for a minimum of 30 minutes prior to the start of the test, whereby local T_{sk} was altered until the desired temperatures were reached. Local T_{sk} was maintained at the desired temperature for a minimum of 5 minutes prior to the start of the experiment. If the local T_{sk} of the forearms during passive heating increased or decreased by more than 1˚C of the target local T_{sk}, the temperature of the water bath was altered.

During the final minute of each experiment, a salivary aldosterone sample was collected. This was then followed by the removal of the forearm skin patches and all instruments so that the number of heat-activated sweat glands (HASG) using the starch-iodine technique (Inoue 1996) could be measured. Once these samples were collected the passive heating protocol was completed.

Measurements

For the determination of mean skin temperature (mean T_{sk}), 4 sites (chest, forearm, thigh and calf) were measured. In addition, local T_{sk} underneath the skin patch at the left and right forearm was measured at two locations adjacent to the sweat capsule and averaged. Both T_{es} and T_{sk} were measured using copper-constantan thermocouples (Inui Engineering, Higashi Osaka, Japan). The tip of the oesophageal thermometer was covered with silicon and the skin thermocouples were uncovered and attached to the skin using

Medipore tape. Mean T_{sk} and mean body temperature (T_b) were calculated using the following respective formula (Stolwijk and Hardy 1966; Gagge and Nishi 2011);

$$T_b = (0.8 \times T_{es}) + (0.2 \times \text{mean } T_{sk})$$

$$\text{Mean } T_{sk} = (\text{chest} \times 0.34) + (\text{calf} \times 0.18) + (\text{thigh} \times 0.33) + (\text{forearm} \times 0.15)$$

For the calculation of mean T_{sk} the forearm T_{sk}, was based on the average of both the left and right forearm T_{sk}.

SR was measured using the ventilated capsule method on the right and left mid-ventral forearm. Dry nitrogen gas was flushed (500ml/min) through the apparatus approximately 1 hour prior to each experiment to ensure stable readings. Each capsule (3.14 cm^2) was affixed to the skin using double-sided tape at least 30 minutes prior to data collection. The temperature and humidity of the air flowing out of the capsule was measured using a capacitance hygrometer (HMP50; Vaisala, Helsinki, Finland). Two Ag/AgCl electrodes (Vitrode J, Nihon Kohden, Tokyo, Japan) for measuring GSC were attached either side of the sweat capsules, approximately 3cm apart (MP100 and GSC100C; Biopac, Goleta CA, USA). GSC is expressed as a change from baseline (ΔGSC), recorded during the 5 minute resting phase prior to heating. Cutaneous vascular conductance (CVC) was estimated by measuring skin blood flow on each forearm using laser-Doppler velocimetry (ALF21; Advanced, Tokyo, Japan). CVC was calculated as a percentage of the baseline value recorded during the resting phase prior to heating. T_{es}, local T_{sk}, SkBf, SR and GSC were recorded every second by a data logger (MX100; Yokogawa, Tokyo, Japan) and 1 min averaged calculated. Heart rate and arterial blood pressure were continuously measured on the left middle finger using a Finometer (Finometer; Finapres Medical Systems, Amsterdam, The Netherlands); mean arterial pressure (MAP) was subsequently calculated and averaged over 5-min periods.

The number of HASG was determined using the starch-iodine technique (Inoue 1996). Briefly, the residual sweat on the skin was first wiped away, followed by placing a small amount of iodine onto the skin with a cotton guaze. Excess iodine was removed by blotting the area with tissue paper. Starch paper attached to a small wooden block was then held in place over the measurement area for approximately 3 sec. The iodine was transferred from the HASG to the paper as indicated by a small dot. The same investigator counted the dots within a defined area ($1cm^2$). The sweat gland output per gland (SGO) at the respective site was calculated by dividing the SR (averaged from the last 5 minutes of the experiment) by the number of HASG.

Salivary and urine aldosterone was collected using Salivettes™ (Sarstedt, Newton, NC, USA) whereby a plain cotton swab was inserted into the mouth and chewed for 60 sec. The cotton swab was then returned into the Salivette™ tube and spun at 4000RPM for 10 minutes. Samples were then frozen at -30˚C until analysis. After thawing, salivary aldosterone (pg/ml) levels were quantified by competitive ELISA (LDN, GmbH & Co.KG, Germany). The sensitivity of the assay for aldosterone was 14pg/ml and the inter- and intra-assay coefficient of variation were between 3.9-7.5% and 9.4-9.7%, respectively.

Data analysis

As described in our previous experiments (Amano et al. 2016, 2017; Gerrett et al. 2018a), the maximum reabsorption rate of the sweat glands was obtained by plotting ΔGSC against ΔSR. By plotting this relationship, it is possible to identify three distinct phases; representing different stages of sweat production. In the first phase, there is an increase in ΔGSC but no change in ΔSR, which represents the isosmotic precursor sweat production in the proximal secretory coil. Such changes in ΔGSC and no changes in ΔSR are frequently utilised to identify pre-secretory sweat gland activity (Thomas and Korr 1957; Darrow

1964; Machado-Moreira et al. 2009; Gerrett et al. 2018b). In the second phase, an increased ΔSR without an increase in ΔGSC can be observed. As ΔGSC is influenced by both the amount of sweat produced as well as the electrolyte concentration the fact that ΔSR increases but there is no change in ΔGSC represents reabsorption of sweated ions in the sweat duct. Once the rate of sweat ion secretion exceeds its reabsorption limit in the duct then the third phase occurs where there is a proportional increase in ΔGSC with increasing ΔSR. The point at which the 2nd and 3rd phase intersect is used to identify the maximum rate of sweat glands ion reabsorption. In the present study, the thresholds were determined using segmented regression analysis on GraphPad Prism (version 7) software.

To investigate the effect of local T_{sk} on local maximum ion reabsorption, local SR, local ΔGSC and local CVC, a one-way repeated measure ANOVA with condition (Warm, Neutral and Cool) was carried out. The relation between temperature and ion reabsorption rates were analysed with correlation analysis; using normalized data to reduced individual effects. Correlation coefficients were considered as strong (≥ 0.60), moderate (0.40–0.59), and weak (0.20–0.39) (Cohen 1977).

A two-way ANOVA was used to assess the remaining thermophysiological parameters (T_{es}, T_b and mean and local T_{sk}) and cardiovascular responses (CVC, a one-way repeated measure ANOVA) to determine any differences between conditions (Neutral-Cool vs. Neutral-Warm) and over time. When any significant effects were observed, post hoc comparison using the Bonferroni test were carried out. All data were checked for sphericity and normality (Shapiro-Wilk test). As ANOVA's are fairly robust to violations of normality, if the data were approximately normal then the data was assessed with parametric data analysis. If the data violated this assumption substantially then a Friedman's test was performed (comparing Neutral, Cool and Warm data); this was the case for the following sets of data: local forearm, chest and thigh T_{sk} and SR. All data were

analysed using GraphPad Prism (version 7). Effect sizes (Cohen's d) were calculated for the local maximum ion reabsorption rates with the following criteria; an effect size of <0.20 is classified as 'trivial', 0.21–0.49 as 'small', 0.50–079 as 'moderate' and >0.80 as a 'large' effect. Values are means and standard deviations (±SD) and statistical significance was set at $p<0.05$.

Results

Local skin temperature

The local forearms T_{sk} are illustrated in Figure 1. Forearm T_{sk} during Warm (36.4 ± 0.4˚C) were higher ($p<0.05$) than Neutral (33.5 ± 0.7˚C) and the Cool (30.5 ± 1.0˚C) conditions ($p<0.05$). The latter was also lower than both the Neutral conditions ($p<0.05$). The local T_{sk} on the Neutral arm was not affected by the temperature on the experimental arm (Neutral-Cool; 33.6 ± 0.7˚C and Neutral-Warm; 33.7 ± 0.7˚C, $p>0.05$). Forearm T_{sk} increased over time in all conditions ($p<0.05$) and was significantly higher than baseline after 40, 25 and 30 mins until the end of the experiment for Warm, both Neutral and Cool conditions, respectively. The increases from baseline to the end of passive heating were however similar between conditions (Warm; +0.9 ± 0.4˚C, Neutral +1.2 ± 0.5˚C, Cool; +1.7 ± 0.8˚C, no interaction effect, $p>0.05$).

Maximum ion reabsorption

The maximum ion reabsorption thresholds, as indicated by the ΔSR threshold for an increasing ΔGSC, are illustrated in Figure 2. There is a significant pattern for a temperature dependent response for maximum ion reabsorption threshold (Warm; 0.28 ± 0.14 mg/cm^2/min, Neutral; 0.22 ± 0.12 mg/cm^2/min, Cool; 0.18 ± 0.10 mg/cm$^{2/}$min, $p<0.05$), with differences noted between Warm and Cool only (d = 0.88, p=0.024). There

were small effect sizes for the maximum ion reabsorptions between Neutral and Warm (d = 0.44, p>0.05) and Neutral and Cool (d = 0.36, p>0.05). A weak, significant relation exits between local T_{sk} and the ΔSR threshold for maximum ion reabsorption (r^2=0.36, p<0.05)

Thermo-physiological measurements

T_{es}, T_b and mean T_{sk} are illustrated in Figure 3A-C. T_{es} were similar between conditions (Neutral-Warm; 37.1 ± 0.3˚C and Neutral-Cool 37.2 ± 0.3˚C, p>0.05) and both gradually increased over time (p<0.05), although the increase was similar between conditions (ΔTes: Neutral-Warm; 0.92 ± 0.2˚C and Neutral-Cool 0.77 ± 0.1˚C p>0.05). T_b were similar between conditions (Neutral-Warm; 36.9 ± 0.3˚C and Neutral-Cool 36.8 ± 0.3˚C, p>0.05) and both gradually increased over time (p<0.05), although the increases were similar between conditions (ΔTes: Neutral-Warm; 1.4 ± 0.03˚C and Neutral-Cool 1.3 ± 0.02˚C p>0.05).

Mean T_{sk} was higher during the Neutral-Warm (35.7 ± 0.6˚C) compared to Neutral-Cool (34.9 ± 1.0˚C) conditions (p<0.05) and did increase over time (p<0.05), although the increase was similar between conditions (Δmean T_{sk}: Neutral-Warm; 3.3 ± 0.5˚C and Neutral-Cool 3.2 ± 0.3˚C; no interaction effect, p>0.05). Local T_{sk} of the chest, thigh and calf were similar between conditions (Neutral-Warm; 35.4 ± 5.6˚C, 33.4 ± 5.2˚C, 42.0 ± 7.0˚C and Neutral-Cool: 35.3 ± 5.5˚C, 33.2 ± 5.3˚C, 41.9 ± 7.0˚C, p<0.05, respectively). They all increased over time (p<0.05) although the increase was similar between conditions (no interaction effect, p>0.05).

Sweating responses

SR and ΔGSC measured at the forearms whilst local forearm T_{sk} were maintained at a Warm (36˚C), Neutral (33˚C) or Cool (30˚C) temperatures are illustrated in Figure 4.

SR was higher during both Warm (0.43 ± 0.26 mg/cm^2/min) and the Neutral conditions (Neutral-Warm; 0.40 ± 0.3 mg/cm^2/min, and Neutral-Cool 0.40 ± 0.2 mg/cm^2/min) than Cool (0.35 ± 0.2 mg/cm^2/min). SR increased over time in all conditions ($p<0.05$) and was significantly higher than baseline from 30 mins until the end of passive heating. The increases from baseline to the end of passive heating were similar between conditions (Warm; +0.62 ± 0.24 mg/cm^2/min, Neutral +0.61 ± 0.23 mg/cm^2/min, Cool; +0.54 ± 0.15 mg/cm^2/min, no interaction effect, $p>0.05$).

ΔGSC was not significantly different between Warm (10.3 ± 11.5 μS), Neutral (10.1 ± 9.8 μS), or Cool (8.6 ±11.3 μS) conditions ($p>0.05$). ΔGSC increased from baseline after 10 mins for all conditions ($p<0.05$). There was no interaction effect as the increases from baseline to the end of passive heating were similar between conditions (Warm; 18.1 ± 14.6 μS, Neutral; 19.4 ± 12.8 μS, Cool; 19.35 ± 14.1 μS, $p>0.05$).

The HASG was similar between conditions: Warm; 107 ± 20 gland/cm^2, Neutral; 120 ± 24 gland/cm^2, Cool; 115 ± 18 gland/cm^2 ($p>0.05$). The SGO was similar between conditions: Warm; 6.9 ± 2.7 μg/gland/min, Neutral; 5.6 ± 1.7 μg/gland/min, Cool; 5.3 ± 1.1 μg/gland/min, $p>0.05$).

The T_{es} threshold for SR was not significantly different between conditions (Warm; 36.72 ± 0.4˚C, $p>0.05$, Neutral; 36.92 ± 0.4˚C, Cool; 37.08 ± 0.3˚C). The slope was also not significantly different (Warm; 0.74 ± 0.4, Neutral; 1.00 ± 0.3, Cool; 1.13 ± 0.5, $p>0.05$).

Urine and Salivary Aldosterone

Urine aldosterone concentrations were similar between conditions (Neutral-Warm; 5.03 ± 2.6 μg/L and Neutral-Cool; 5.37 ± 2.5 μg/L, $p>0.05$). The urine specific gravity was also similar between conditions (Neutral-Warm; 1.021 ± 0.01 and Neutral-Cool; 1.021 ± 0.01, $p>0.05$). Salivary Aldosterone samples were similar between conditions and over

time (pre vs. post); there was also no interaction effect (Neutral-Warm-pre; 122.2 ± 40.5 pg/ml, Neutral-Warm-post; 116.1 ± 43.0 pg/ml, and Neutral-Cool-pre; 113.7 ± 28.9 pg/ml and Neutral-Cool post; 115.8 ± 41.7 pg/ml, $p>0.05$).

Cardiovascular measurements

HR, MAP, CVC are presented in Table 1. HR's were similar between conditions (Neutral-Warm; 78.0 ± 8 bpm and Neutral-Cool 79.0 ± 9 bpm, $p>0.05$) and both gradually increased over time ($p<0.05$) and the increase was similar between conditions (ΔHR: Neutral-Warm; 30.0 ± 2bpm and Neutral-Cool 31.2 ± 2.bpm, $p>0.05$). MAP were similar between conditions (Neutral-Warm; 99.2 ± 2 mmHg and Neutral-Cool 99.1 ± 2 mmHg, $p>0.05$) and did not increase over time in either condition ($p>0.05$).

Discussion

The aim of the present study was to investigate the influence of local T_{sk} on the eccrine sweat glands maximum ion reabsorption rates during passive heating. We hypothesised that the sweat glands maximum ion reabsorption rates would occur at a higher sweat rate (SR) with a higher local T_{sk} (36˚C) compared to neutral (33˚C) and cool T_{sk} (30˚C). The data indicates that there is a temperature dependent response, which was most prominent when local T_{sk} differed by 6˚C (30˚C vs. 36˚C) whilst not when local T_{sk} differed by only 3˚C (30˚C vs. 33˚C and 33˚C vs. 36˚C). In practical terms, a higher ΔSR for maximum ion reabsorption means that a more dilute sweat would be secreted onto the skin surface for a given SR, which may be advantageous when sweat rate is high to prevent the excess loss of ions. By selecting a passive heating protocol, we ensured that the values were obtained in controlled conditions to eliminate any potential effect from non-thermal control mechanisms, such as aldosterone, and other potential thermal controllers such as core and

428 body temperature. Mean T_{sk} was significantly different by only approximately 0.6˚C
429 between conditions, which is likely due to differences in local T_{sk} manipulations as all other
430 measured local skin site temperatures were similar between conditions.
431
432 *Thermal mechanism*

433 We hypothesized that the maximum sweat gland ion reabsorption rate would be
434 affected by local T_{sk} as previous studies, in vivo and in vitro, have reported a temperature
435 dependency of Na^+ channels excitability and ion reabsorption regulation (Ruff 1999;
436 Chraïbi and Horisberger 2003; Shamsuddin et al. 2005a). The reabsorption of NaCl is
437 primarily driven by the movement of Na^+ down a steep concentration gradient that is
438 generated by the Na^+/K^+ pump via ENaC (Bovell 2015). In vitro studies utilising a wide
439 temperature range (19˚C and 37˚C) have demonstrated the temperature dependency of Na^+
440 channels excitability; where 30% and 93% of the channels were excitable at these
441 respective temperatures (Ruff 1999). However, the percentages of excitable channels
442 became less prominent when comparing 31˚C and 37˚C (85% vs. 93%, respectively), which
443 are closer to the physiological temperatures experienced whilst sweating. Chraïbi and
444 Horisberger (2003) more recently showed that ENaC open probability was greater at lower
445 (15ºC) compared to higher temperatures (24-30ºC). Indeed other studies showing
446 temperature dependency of ENaC, and cystic fibrosis transmembrane channels (CFTR) that
447 are responsible for the reabsorption of Cl^- and interacts with ENaC (Reddy and Quinton
448 2003), show the effects of temperature are more prominent outside the physiological ranges
449 examined in the present study. Our data indicates that when T_{sk} is within appropriate
450 physiological ranges of 30-33-36˚C, a T_{sk} difference of ~3˚C is an insufficient temperature
451 stimulus to affect the maximum rate of ion reabsorption but differences of ~6˚C may elicit
452 changes of approximately 0.1mg/cm^2/min. Whilst the inter and intra-individual variability

453 is high for sweating responses, the ventilated sweat capsule technique is highly reliable and
454 accurate and thus we are confident that our method is sensitive to detect these differences.
455 Whilst GSC is more variable, the values themselves are less meaningful but the pattern of
456 the response in relation to SR is important for detecting the sweat glands ion reabsorption
457 rates.

458 Contrasting to our findings, Shamsuddin et al. (2005a) reported differences in ion
459 reabsorption rates when mean T_{sk} was clamped at ~31˚C and ~28˚C, a differences of 3˚C,
460 during dynamic exercise. These findings, alongside the in vitro studies mentioned earlier
461 may indicate that cooler temperatures (<30˚C) have stronger effects on ion regulation.
462 Alternatively, smaller differences in T_{sk} may affect ion reabsorption rates during dynamic
463 exercise when hormonal mediators are likely in effect.

464 Core temperature (T_c), as indicated by T_{es}, in our present study and by Shamsuddin
465 et al. (2005a) were not significantly different between the two conditions; thereby
466 eliminating the role of T_{es} as a controlling mechanism. However, that is not to say that T_c
467 per se will not influence the sweat glands maximum ion reabsorption rates in other
468 conditions outside the realms of the studies under discussion. Temperature stimulation has
469 been deemed an important regulatory mechanism for sudomotor activity. We reported no
470 significant differences between conditions in the T_{es} threshold for the onset of neither
471 sweating, nor the slope, or the SGO in this study. Indeed, the relative importance of T_c on
472 SR compared to mean T_{sk} is well known (Nadel et al. 1971) and both Na^+ secretion and
473 Na^+ reabsorption increase linearly with increasing SR (Buono et al. 2008). As SR increases
474 it has been suggested that there becomes insufficient time for sweat ion reabsorption to
475 occur and hence a maximum reabsorption rate is reached. The SR for maximum ion
476 reabsorption occurred between 0.18 and 0.28 mg/cm^2/min across the 3 temperature ranges,

477 which typically occurred within 15 minutes of passive heating. As can be seen in left panel
478 of Figure 4, the SR response over time is similar between conditions, as also confirmed by
479 a non-significant interaction effect (condition x time). It seems therefore that if any thermal
480 controls exist over the sweat glands maximum ion reabsorption rates, it requires a stronger
481 thermal input (e.g. from mean T_{sk} and/or T_c) than from small changes ($\leq$3˚C) in local T_{sk}.
482 It would be interesting to determine the minimum change in local T_{sk} that would affect the
483 sweat glands maximum ion reabsorption rate. The role of T_c on SR and ion reabsorption
484 seems likely, but certainly warrants clarification, as to does the role of mean and local T_{sk}
485 under varying T_c responses.

486 Previous studies have reported higher reabsorption rates on the torso compared to the
487 extremities but have been unable to determine any contributing mechanism as regional
488 differences in local T_{sk} existed (Inoue et al. 1998; Amano et al. 2017). The findings of the
489 present study provide important information to help elucidate why these regional
490 differences occur and variations in local T_{sk} can be ruled out, as regional differences were
491 less than ~2˚C. Instead, we hypothesize that structural differences in the sweat glands
492 across the body may account for the regional differences reported in aforementioned
493 studies.

494 *Non-thermal mechanism*

495 Regulation of ion loss is predominated in the literature by renal function despite
496 potential large fluid and ion losses from eccrine sweat glands during exercise and/or heat
497 exposure. Although structurally and functionally different to the kidneys, the influence of
498 various water regulating hormones (arginine vasopressin, aldosterone and atrial natriuretic
499 peptide) on sweat output have been investigated (Kirby and Convertino 1986; Hew-Butler
500 et al. 2010, 2014). The precise mechanism for the regulation of the ion reabsorption is
501 unknown but it is hypothesized that aldosterone, an important hormone in renal sodium

502 regulation, plays a role. Acting on mineralocorticoid receptors, aldosterone increases
503 intracellular calcium, which regulates epithelial Na^+ and K^+ channels, reportedly by
504 increasing either the permeability of the membrane to Na^+, increasing active transport of
505 Na^+ out of the cell and/or increasing the energy available to the Na^+/K^+ pump (Hegarty and
506 Harvey 1998; Harvey and Higgins 2000).

507 By using a passive heating protocol, our study aimed to reduce non-thermal
508 mediators, such as water-regulatory hormones (e.g. aldosterone, vasopressin and plasma
509 renin activity) that are released during exercise (Convertino et al. 1983; Freund et al. 1991;
510 Yoshida et al. 2006). We confirmed similar aldosterone concentrations between our two
511 conditions, both before and after passive heating. In addition, hydration statuses were
512 similar between both conditions so to rule out any potential effects of hydration on
513 circulating hormones. Previous research by Shamsuddin et al. (2005a) reported a role of
514 mean T_{sk} on ion reabsorption, but this occurred during dynamic exercise (cycling at 60%
515 $\dot{V}O_{2max}$) where aldosterone concentrations would have been elevated, as demonstrated in
516 our previous research comparing passive heating and cycling at 60% $\dot{V}O_{2max}$ (Gerrett et al.
517 2018a). Shamsuddin et al. (2005a), speculated that higher T_{sk} might enhance the
518 responsiveness of the sweat glands to a given aldosterone concentration. In the present
519 study however, aldosterone concentrations remained unchanged but local T_{sk} differed and
520 we hypothesize that small differences (≤3˚C) in T_{sk} may only affect ion reabsorption rates
521 when aldosterone (or other water regulatory hormones) are elevated. Further support is
522 provided from our previous research where higher maximum ion reabsorption rates were
523 reported during moderate intensity exercise (supine cycling at 60% $\dot{V}O_{2max}$) compared to
524 passive heating (lower leg submersion 43˚C water), despite a lower mean $T_{sk.}$ We attributed
525 those differences partially to the elevated salivary aldosterone concentrations during
526 exercise compared to passive heating. To date no studies have determined the effect of

varying T_{sk} and/or T_c on aldosterone concentrations during exercise but it certainly warrants investigation.

Perspective

Sodium chloride plays an important role in the formation of sweat within the secretory coil. Why we do not, or cannot, reabsorb all the sodium chloride ions in the reabsorptive duct is not clear; it may play a role in the evaporative potential of sweat on the skin surface, it may aid skin barrier function and protection. Yet there is an adaptive response as seen with heat acclimation (Buono et al. 2008; Amano et al. 2016). We know from cases of cystic fibrosis that preventing an excess loss of these ions is an important regulatory mechanism yet, it is poorly understood and often overlooked in favour of its thermoregulatory role. The regulation of ion reabsorption is a fundamental research question that requires further consideration.

Furthermore, in recent years there has been a drive to produce non-invasive techniques to inform us about the human condition, using sweat as the medium. As a result, the development of sensors to continuously measure sweat content is a rapidly growing field in biomedical engineering but our knowledge of sweat ion regulation is limited and this fundamental study adds to our knowledge. We provide further insight into the methodological considerations for future studies in this area. In particular, our research group has been accumulating consistent evidence of regional differences in ion reabsorption across the body and the current study suggest these regional differences may not have been due to differences in T_{sk} but rather structural or regulatory differences at the level of the sweat gland across the body. If T_{sk} is expected to vary considerably then knowing the T_{sk} is important. Consistent sensor placement in the same locations will allow for better comparisons between conditions and from different studies.

Conclusion

Local T_{sk} within physiological ranges of 30-33-36˚C only influenced the sweat glands maximum ion reabsorption rates during a passive heating protocol when local T_{sk} differed by 6˚C (30˚C vs. 36˚C), whilst no differences were observed when local T_{sk} differed by 3˚C (30˚C vs. 33˚C and 33˚C vs. 36˚C). These findings were observed when all other potential thermal and non-thermal controlling mechanisms were similar between conditions. Information from the literature and data from our study indicate that thermal controllers may exist but most probably from stronger thermal stimulus, such as mean T_{sk} and/or T_c, compared to smaller changes (≤3˚C) in local T_{sk}. We provide important insights for previous studies that have reported regional differences in maximum ion reabsorption rates but have been unable to confirm such differences in the presence of differing local T_{sk}. The data provides useful information for furthering our understanding of sweat gland ion reabsorption and potential controlling mechanisms. The application of which may be useful in the fields of thermoregulation, hypo/hypernatremia, dermatology and biosensor technology research.

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Figures and Tables

Table 1: Cardiovascular responses measured continuously and averaged over 5 minutes during the passive heating protocol. There were no differences in heart rate (HR) or mean arterial pressure (MAP) between the Neutral-Warm and Neutral-Cool conditions ($p>0.05$). There were no differences in CVC between both Neutral (33˚C) conditions (Neutral-Warm and Neutral-Cool), Cool (30˚C), or Warm (36˚C) conditions ($p>0.05$). All variables increased over time but there was no interaction effect ($p>0.05$). Values are based on 5-min averages and expressed as mean ± SD for 10 participants.

		BL	5	10	15	20	25	30	35	40	45
HR (bpm)	Neutral-Warm	61.3 ± 8.7	65.5 ± 6.9	67.9 ± 8.4	72.7 ± 8.8	77.0 ± 7.1	78.2 ± 9.4	79.2 ± 10.8	82.5 ± 11.3	86.9 ± 10.7	89.9 ± 10.6
	Neutral-Cool	61.9 ± 8.1	65.6 ± 8.7	67.0 ± 9.9	72.2 ± 7.1	78.4 ± 7.0	79.7 ± 7.7	81.1 ± 8.1	84.3 ± 8.0	88.6 ± 8.1	91.4 ± 8.9
MAP (mmHg)	Neutral-Warm	93.9 ± 6.8	98.9 ± 10.6	99.1 ± 8.8	99.8 ± 9.6	101.5 ± 9.6	100.4 ± 9.9	97.4 ± 6.8	99.7 ± 8.5	99.4 ± 6.4	97.3 ± 6.8
	Neutral-Cool	95.1 ± 7.1	98.3 ± 7.6	99.7 ± 9.7	99.2 ± 8.5	96.8 ± 8.7	97.8 ± 8.7	98.9 ± 8.9	99.9 ± 8.5	99.5 ± 8.5	101.5 ± 7.4
CVC (%)	Warm	100.0 ± 0.0	143.0 ± 97.2	205.0 ± 158.2	351.5 ± 291.0	480.2 ± 310.8	578.0 ± 304.9	620.5 ± 301.7	643.4 ± 288.7	648.8 ± 298.5	655.0 ± 310.1
	Neutral	100.0 ± 0.0	120.5 ± 42.7	159.8 ± 59.9	252.4 ± 129.5	384.7 ± 163.	460.8 ± 173.0	524.7 ± 170.3	601.7 ± 200.7	645.6 ± 212.3	663.6 ± 232.8
	Cool	100.0 ± 0.0	101.4 ± 19.1	120.4 ± 35.0	165.4 ± 51.9	224.9 ± 79.4	281.6 ± 74.5	357.1 ±105.6	464.5 ±114.0	590.9 ± 180.9	645.5 ± 215.7

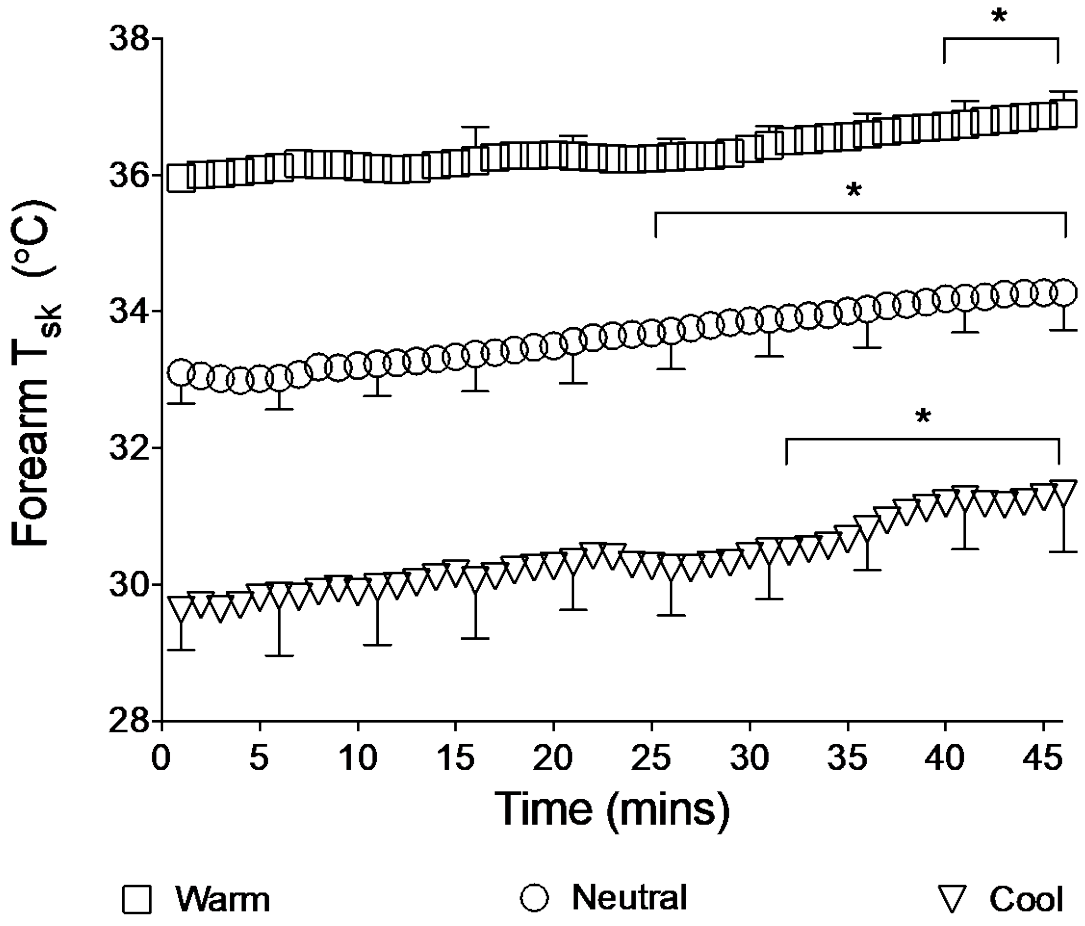
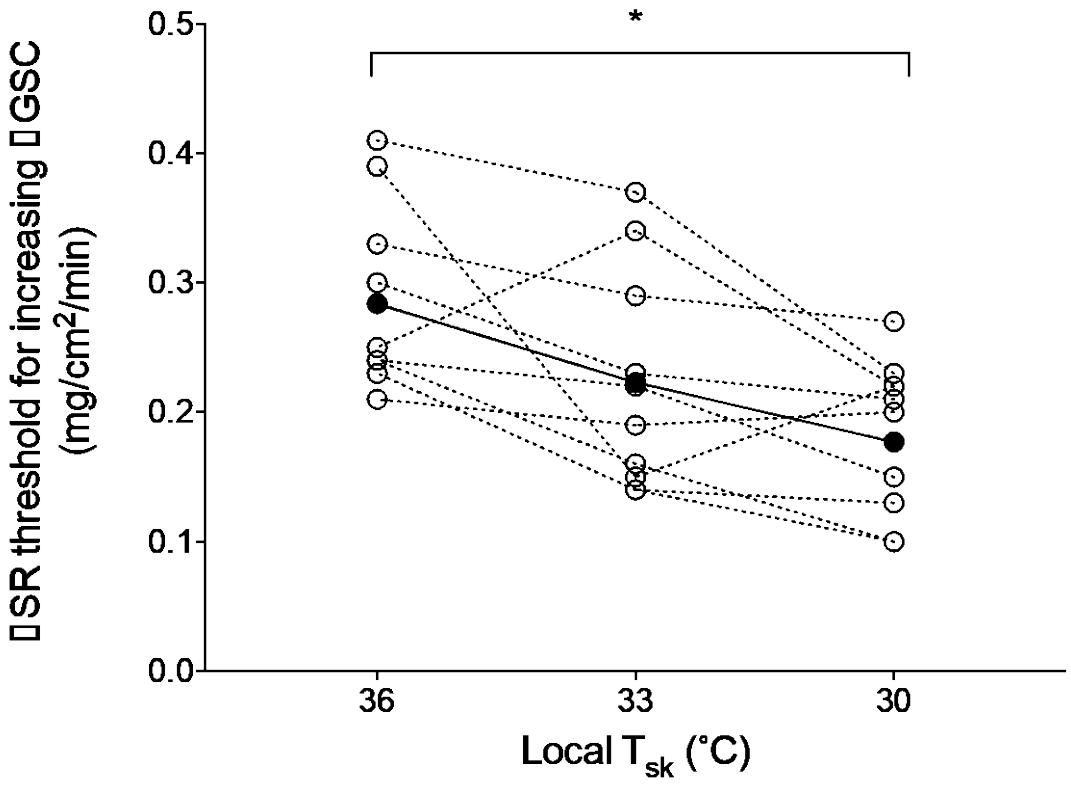
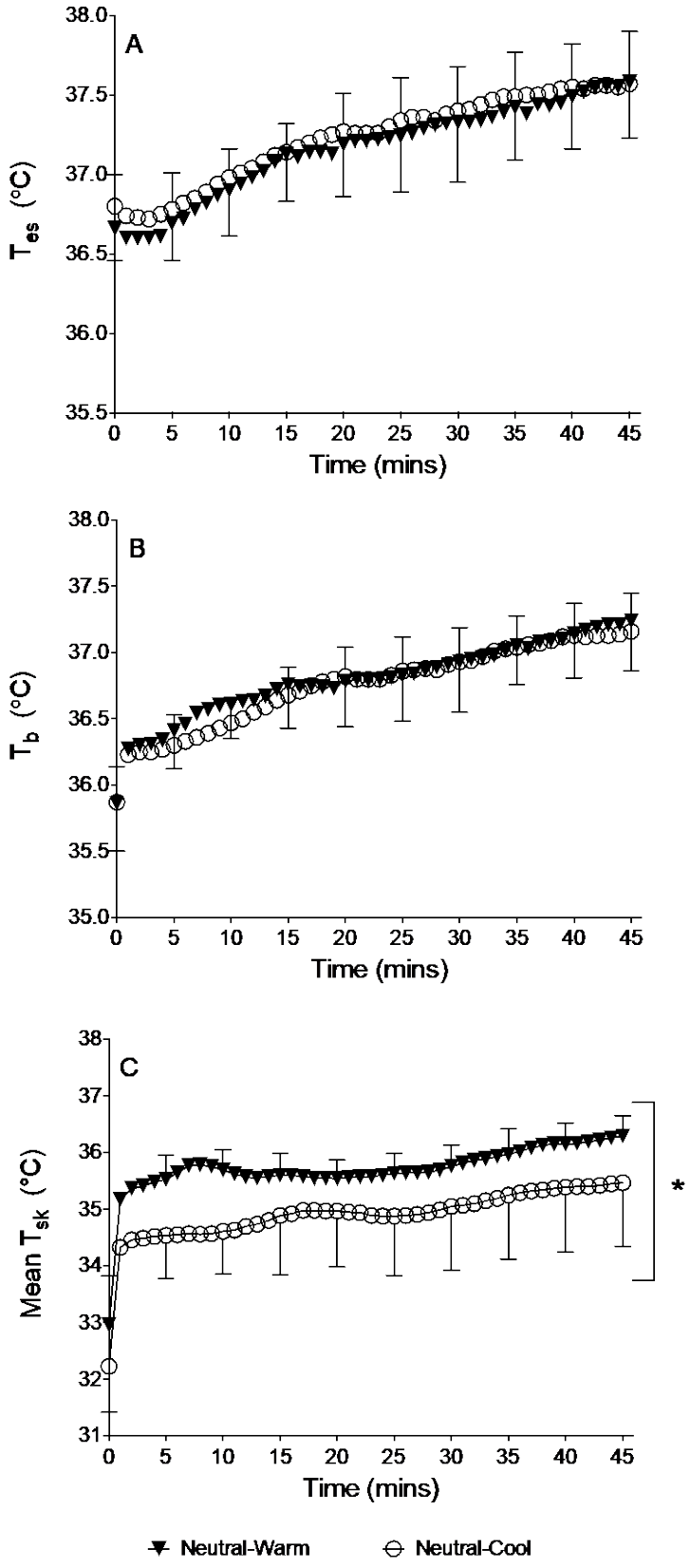


Figure 1: Local forearm skin temperature (T_{sk}) measured whilst manipulating local T_{sk} to 36˚C (Warm), 33˚C (Neutral) and 30˚C (Cool) during a 45-minute passive heating protocol. Forearm T_{sk} was higher during Warm compared to both Neutral and Cool condition and Neutral was also higher than the Cool condition ($p<0.05$). Forearm T_{sk} increased over time in all conditions ($p<0.05$) and * indicates when forearm T_{sk} was higher than baseline from each condition. The increases were similar between conditions; hence there was no interaction effect between condition and time ($p>0.05$). Values are based on 1-min averages and expressed as mean ± SD for 10 participants. To aid clarity error bars are provided at 5-min intervals.



688

689 **Figure 2:** The relation between local T_{sk} and the ΔSR threshold for an increasing ΔGSC.
690 The solid line and black cycle represent the mean data (n=10), whilst the dashed line and
691 empty circles are the individual responses. Warm was significantly higher than Cool (*
692 indicates p>0.05)



693

694 **Figure 3:** Esophageal temperature (T_{es}) (A), body temperature (T_b) (B) and mean skin
695 temperature (T_{sk}) (C) during the 45-minute passive heating protocol when local forearms
696 skin temperature were stimulated to be Neutral and Warm or Neutral and Cool. There were

no significant differences found between conditions for T_{es} or T_b and whilst they both increased over time there was no interaction effect ($p>0.05$). Mean T_{sk} was higher during the Neutral-Warm compared to Neutral-Cool conditions (*$p<0.05$) likely due to differences in local forearm T_{sk} as all other skin sites were not different ($p>0.05$). Mean T_{sk} increased over time ($p<0.05$) but this was similar between conditions ($p>0.05$). Values based on 1-min averages and are expressed as mean ± SD for 10 participants. To aid clarity error bars are provided at 5-min intervals.

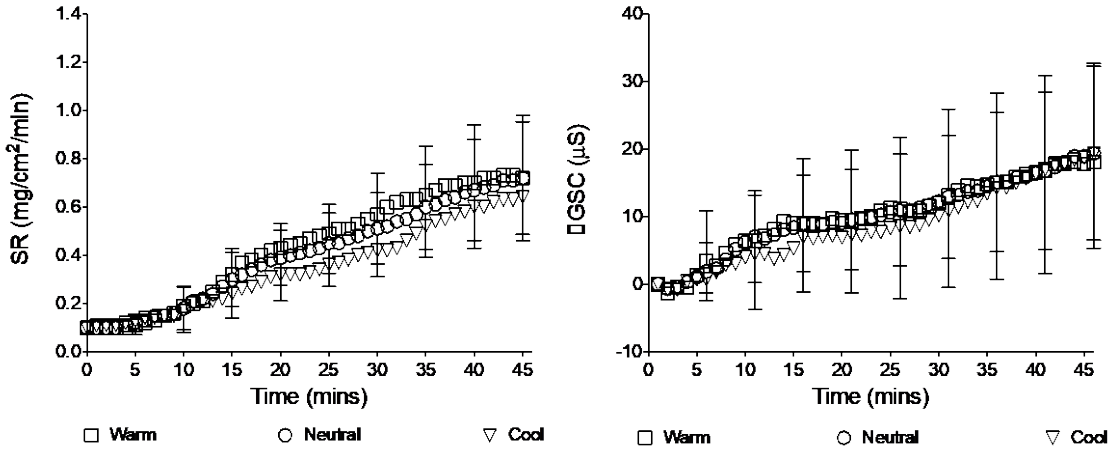


Figure 4: Local sweat rate (SR) (left) and ΔGSC (right) measured whilst stimulating local T_{sk} at 36˚C (Warm), 33˚C (Neutral) and 30˚C (Cool) during a 45-minute passive heating protocol. SR was significantly different between all conditions ($p<0.05$) and whilst SR increases over time the increase was similar between conditions (no interaction effect, $p>0.05$). There were no differences in ΔGSC between conditions ($p>0.05$). Whilst ΔGSC increased over time the increase was similar between conditions (no interaction effect, $p>0.05$). Values are based on 1-min averages and expressed as mean ± SD for 10 participants. To aid clarity error bars are provided at 5-min intervals.